Health-promoting Properties of Fruit and Vegetables
To
Joanne, Oscar and Alice –
for your patience and love
Health-promoting Properties of Fruit and Vegetables

Edited by
Leon A. Terry
of
Cranfield University, UK
## Contents

**Contributors** vii  
**About the Editor** ix  
**Acknowledgements** x  

1. **Introduction**  
   *Leon A. Terry and A. Keith Thompson*  

2. **Alliums [Onion, Garlic, Leek and Shallot]**  
   *Gemma A. Chope, Katherine Cools and Leon A. Terry*  

3. **Avocado**  
   *Marjolaine D. Meyer, Sandra Landahl, Manuela Donetti and Leon A. Terry*  

4. **Blueberry and Cranberry**  
   *Charles F. Forney and Wilhelmina Kalt*  

5. **Brassicas**  
   *Peter Glen Walley and Vicky Buchanan-Wollaston*  

6. **Citrus [Orange, Lemon, Mandarin, Grapefruit, Lime and Other Citrus Fruits]**  
   *Amarat H. Simonne and Mark A. Ritenour*  

7. **Cucurbits [Cucumber, Melon, Pumpkin and Squash]**  
   *D. Mark Hodges and Gene E. Lester*  

8. **Exotics [Litchi, Longan, Rambutan, Pomegranate, Mangosteen, Kiwifruit, Passion Fruit, Persimmon, Carambola]**  
   *Nettra Somboonkaew and Leon A. Terry*  


9 Grape
   Pierre-Louis Teissedre and Christian Chervin

10 Leafy Vegetables and Salads
   Peter M.A. Toivonen and D. Mark Hodges

11 Pome Fruit
   Chris B. Watkins and Rui Hai Liu

12 Potato and Other Root Crops
   Anne Pihlanto

13 Prunus
   Ariel R. Vicente, George A. Manganaris, Luis Cisneros-Zevallos and Carlos H. Crisosto

14 Ribes and Rubus [Blackberry, Currants and Raspberry, etc.]
   Jordi Giné Bordonaba and Leon A. Terry

15 Strawberry
   Jordi Giné Bordonaba and Leon A. Terry

16 Tomato and Other Solanaceous Fruits
   Amarat H. Simonne, Cecilia do Nascimento Nunes and Jeffrey K. Brecht

17 Tropical Fruit [Banana, Pineapple, Papaya and Mango]
   Thiruchelvam Thanaraj and Leon A. Terry

18 Methodologies for Extraction, Isolation, Characterization and Quantification of Bioactive Compounds
   Katherine Cools, Ariel Vicente and Leon A. Terry

19 Methodologies for Evaluating In Vitro and In Vivo Activities of Bioactive Compounds
   Paul J. Thornalley, Mingzhan Xue and Naila Rabbani
Contributors

Jeffrey K. Brecht, Horticultural Sciences Department, Institute of Food and Agricultural Sciences, University of Florida, POB 110690, Gainesville, FL 32611-0690, USA. E-mail: jkbrecht@ufl.edu

Vicky Buchanan-Wollaston, School of Life Sciences, Wellesbourne Campus, The University of Warwick, Wellesbourne, Warwick CV35 9EF, UK. E-mail: vicky.b-wollaston@warwick.ac.uk

Christian Chervin, INP/ENSAT, GBF, BP 32607, Université de Toulouse, 31326 Castanet-Tolosan, France. E-mail: chervin@ensat.fr

Gemma A. Chope, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: g.a.chope@cranfield.ac.uk

Luis Cisneros-Zevallos, Department of Horticultural Sciences, Food Science Program, Horticulture/Forest Science Building, Texas A&M University, College Station, Texas 77843-2133, USA. E-mail: lcisnero@ag.tamu.edu

Katherine Cools, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: k.cools.s06@cranfield.ac.uk

Carlos H. Crisosto, Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, California 95616, USA. E-mail: chcrisosto@ucdavis.edu

Manuela Donetti, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: m.donetti.s06@cranfield.ac.uk

Charles F. Forney, Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main Street, Kentville, Nova Scotia B4N 1J5, Canada. E-mail: Charles.Forney@agr.gc.ca

Jordi Giné Bordonaba, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: j.ginebordonaba.s05@cranfield.ac.uk

D. Mark Hodges, Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, Kentville, Nova Scotia, Canada. E-mail: Mark.Hodges@agr.gc.ca

Wilhelmina Kalt, Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main Street, Kentville, Nova Scotia B4N 1J5, Canada. E-mail: Wilhelmina.Kalt@agr.gc.ca

Sandra Landahl, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: s.landahl@cranfield.ac.uk
Contributors

Gene E. Lester, Food Quality Laboratory, USDA Agricultural Research Service, 10300 Baltimore Avenue, Building 002, Room 103, Beltsville, MD 20705-2350, USA. E-mail: Gene.Lester@ARS.USDA.GOV

Rui Hai Liu, Department of Food Science, Cornell University, New York 14853, USA. E-mail: rl23@cornell.edu

George A. Manganaris, Department of Agricultural Production and Food Science and Technology, Cyprus University of Technology, Athinon & Anexartisias 57 Corner, 3603 Lemesos, Cyprus. E-mail: george.manganaris@cut.ac.cy

Marjolaine D. Meyer, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: marjomeyer@hotmail.fr

Cecilia do Nascimento Nunes, Food Quality Laboratory, College of Human and Social Sciences, University of South Florida Polytechnic, 4100 South Frontage Road, Building 100, Suite 112, Lakeland, Florida 33815, USA. E-mail: mariecicilia@poly.usf.edu

Anne Pihlanto, MTT, Biotechnology and Food Research, 1600 Jokioinen, Finland. E-mail: anne.pihlanto@mtt.fi

Naila Rabbani, Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, Coventry, UK. E-mail: N.Rabbani@warwick.ac.uk

Mark A. Ritenour, Indian River Research and Education Center, Institute of Food and Agricultural Sciences, 2199 S. Rock Road Fort Pierce, FL 34945-3138, USA. E-mail: ritenour@ufl.edu

Amarat H. Simonne, Family, Youth and Community Sciences Department, Institute of Food and Agricultural Sciences, 3025 McCarty Hall, PO Box 110310, University of Florida, Gainesville, FL 32611-0310, USA. E-mail: asim@ufl.edu

Nettra Somboonkaew, Postharvest and Processing Research and Development Office, Department of Agriculture, Chatuchak, Bangkok 10900, Thailand. E-mail: nettra_s@yahoo.com

Pierre-Louis Teissedre, Faculté d’Oenologie - ISVV, Université Victor Segalen Bordeaux 2, UMR 1219 Oenologie, 210 Chemin de Leyssotte, CS 50008, 33882 Villenave d’Ornon Cedex, France. E-mail: pierrelouis.teissedre@u-bordeaux2.fr

Leon A. Terry, Head of Plant Science Laboratory, Head of Food Security and Environmental Health, Vincent Building, Cranfield University, Bedfordshire, MK43 0AL, UK. E-mail: l.a.terry@cranfield.ac.uk

Thiruchelvam Thanaraj, Plant Science Laboratory, Vincent Building, Cranfield University, Bedfordshire MK43 0AL, UK. E-mail: tthanaraj29@gmail.com

A. Keith Thompson, Plant Science Laboratory, Cranfield University, Bedfordshire, UK. E-mail: keiththompson28@yahoo.com

Paul J. Thornalley, Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, Coventry, UK. E-mail: P.J.Thornalley@warwick.ac.uk

Peter M.A. Toivonen, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada. E-mail: Peter.Toivonen@agr.gc.ca

Ariel R. Vicente, Facultad de Ciencias Agrarias y Forestales, University of La Plata, Calle 60 y 119 s/n, CP 1900 La Plata, Argentina. E-mail: arielvicente@quimica.unlp.edu.ar

Peter Glen Walley, School of Life Sciences, Wellesbourne Campus, The University of Warwick, Wellesbourne, Warwick CV35 9EF, UK. E-mail: Peter.G.Walley@warwick.ac.uk

Chris B. Watkins, Department of Horticulture, Cornell University, New York 14853, USA. E-mail: cbw3@cornell.edu

Mingzhan Xue, Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, Coventry, UK. E-mail: Mingzhan.Xue@warwick.ac.uk
About the Editor

Leon A. Terry, BSc (Hons) ARCS MSc PhD (Reader in Plant Science at Cranfield University, UK) received his BSc from Imperial College, London, and both his MSc and PhD from Cranfield University. Dr Terry established the Plant Science Laboratory at Cranfield University in 2001, which is now one of the largest groups dedicated to research, consultancy and education in postharvest science of fresh produce in the EU. As well as being Head of the Plant Science Laboratory, Dr Terry currently heads Food Security and Environmental Health at Cranfield University, with responsibility over all staff and students in the department.
The Editor is grateful for the contributions from all authors. Without their knowledge and expertise, the completion of this book would have been impossible.

All current and past staff and students of the Plant Science Laboratory at Cranfield University (UK) are thanked for their enthusiasm for all things postharvest.
1 Introduction

Leon A. Terry and A. Keith Thompson

1.1 Introduction

Fruit and vegetables have been an important constituent of the human diet from time immemorial. Indeed, some 3500 years ago, when the children of Israel were wandering in the desert from Egypt to Canaan, they were reported to complain to Moses, 'And wherefore have ye made us to come up out of Egypt... it is no place of seed or of fig or of vine or of pomegranate...' (Numbers 20:5) and 'We remember the fish, which we did eat in Egypt freely; the cucumbers, and the melons, and the leeks, and the onions, and the garlick' (Numbers 11:5). Hippocrates (c.460-370 BC) later wrote 'Let food be thy medicine and medicine be thy food'.

Fruit and vegetables are doubtless eaten for their unique taste and flavour, yet their health and healing properties have been known for centuries. In the 18th century a French pharmacist, Antoine-Augustin Parmentier, demonstrated, for several years by his own diet, that all the nutrients required to sustain a healthy life were found in potatoes (Block, 2008). Yet potatoes are specifically excluded from the modern '5-a-day' fruit and vegetable recommendations. The Chinese have long used plant parts, including fruit and vegetables, to prevent or control diseases. In the West, specific plants have been used; for example, in 1753 a Royal Naval surgeon (James Lind) showed that scurvy could be prevented with citrus juice. However, it was not until 1932 that Albert Szent-Györgyi identified vitamin C as the curative chemical in the juice, for which he received a Nobel Prize. However, Carr and Frei (1999) suggest that the amount of vitamin C required to prevent scurvy is not sufficient to protect against other chronic diseases and have argued that requirements should be increased to take these effects into account.

The type of fruit or vegetable consumed is influenced, among other things, by availability, weather, culture, price and promotion. With the introduction of modern postharvest storage technologies and greater efficiencies in international trade, the availability and thus opportunity for consumers to eat more fresh produce of greater diversity has increased dramatically. Improvements in breeding, crop management, transport, storage and packaging have resulted in seasonality becoming less important, such that the opportunity for consumers to choose from a large array of different fresh produce types and thus expose their bodies to a greater diversity of phytochemicals is welcome.

Much myth surrounds the supposed health-giving properties of fresh fruit and vegetables. For instance, the term 'fresh' is ill defined. The notion that a stored product is universally less healthy is false. Most consumers
eat products that have been stored for variable periods. This fact is often overlooked, and indeed the effect of storage on health-promoting properties of fruit and vegetables has been overshadowed largely by the drive towards reducing waste and maintaining eating quality and storage life. With more urbanization, the greater reliance on logistics and supply chain management will serve to make stored fruit and vegetables more accessible, yet only to those who can afford it.

The incidence of chronic non-communicable diseases, namely coronary heart disease, diabetes, hypertension and obesity, has become a major public health problem in developing countries, and these diseases are expected to account for about 70% of deaths by 2020 (WHO, 2002). However, deaths from chronic non-communicable diseases will escalate with the greater consumption of animal-based food products, but will decrease with higher consumption of fruit and vegetables. It is clear that these worrying trends are now starting to be reflected in the emerging economies.

The increased dietary intake of antioxidant vitamins (vitamins A, C and E) and fibre from vegetables and fruit is highly recommended in order to reduce the risk of cardiovascular disease, stroke and cancer (mouth, oesophagus, stomach and colon). Fruit is generally a good source of dietary antioxidants such as vitamins, phenolic compounds and carotenoids (β-carotene, lycopene, lutein and zeaxanthin). Fruit antioxidants play an important role in reducing the risk of degenerative diseases, in particular cardiovascular diseases, diabetes and several types of cancer. The antioxidant activity of fruit is also believed to reduce the progress of senescence. However, antioxidant capacity varies with genetic differences, harvest maturity, harvesting season, postharvest storage and processing. The antioxidant activities of fruit like apples and berries have been studied extensively, since they are rich in polyphenolics and ascorbic acid (see Chapters 11, 14 and 15 of this volume). Generally, most research on elucidating the health-promoting effects of fruit and vegetables has been conducted on products that are important to Western consumers. Fruits that are consumed more commonly in the developing world are not as well studied, even though they can make up a larger proportion of the global diet.

The contribution of fruit and vegetables to human health has been increasingly recognized. However, low fruit and vegetable intake was identified as an important risk factor for chronic diseases in the WHO World Health Report 2002. Overall, it was estimated that up to 2.7 million lives potentially could be saved each year if fruit and vegetable consumption was sufficiently increased (Keller and Tukuitonga, 2007). In 1988 the ‘5-a-day’ portion of fruit and vegetables campaign was initiated in the USA, and thereafter in Europe and elsewhere. A portion was defined as 80 g of fruit (or vegetable) or 150 ml of unsweetened fruit juice, although juice should contribute only one portion. The WCRF/AICR (1997) recommended that the inclusion of 400–800 g/day of a variety of fruit and vegetables should be included in the diet to reduce the risk of cancer. In their subsequent review, the WCRF/AICR (2007) still recommended to include ‘at least 5 portions/servings (at least 400 g per day) of a variety of non-starchy vegetables and of fruit each day’, but this consumption might not be effective in reducing the risk of all cancers. However, in a UK survey more than 60% of adults had intakes below the recommended level of 400 g/day (Ashfield-Watt et al., 2003) and the situation has not radically improved. Although the ‘5-a-day’ message is flawed in that it equates or assumes that one portion by weight of one product is as ‘valuable’ as another, the ‘5-a-day’ recommendation can only be a generalization to give a simple indication and message to consumers to eat more fruit and vegetables. It is likely that the body mass, gender, ethnicity and age of the person affect their requirements, as does the percentage of each portion that consists of peel and other parts that are not eaten.

A study in the USA showed that the concentration of flavonoids in fruit and vegetables consumed by the public was highly variable. Hamly et al. (2006) suggested that this was most likely due to different cultivars, local growing conditions and processing methods. There is a range of phytochemicals in individual species and cultivars of fruit and vegetables; for example, the bioactive
phytochemicals in citrus fruit include limonoids, vitamin C, \( \beta \)-carotene, flavonoids, folic acid and dietary fibre (see Chapter 6 of this volume). Taking phenolics as an example, Tomás-Barberán and Gil (2008) showed that the intake of phenolics from selected fruit and vegetables, depended on the method of preparation and the parts that were consumed (Table 1.1).

Besides the chemicals required for human nutrition, fruit and vegetables contain what have been referred to as phytochemicals. Of the thousands of phytochemicals (known and unknown) in fruit and vegetables, only a limited number have been evaluated in terms of their effects on human health. Rice-Evans and Miller (1995), reviewing the literature on antioxidants, found that there was a strong case that they could contribute to the prevention or delaying of the onset of cancer and heart disease. Silalahi (2002) also found strong evidence that there was a low risk of degenerative diseases, cardiovascular disease, hypertension, cataract, stroke and cancers in people with a high intake of fruit and vegetables.

The effect of different phytochemicals on human health is influenced not only by the identity and abundance of a single target analyte or cluster of chemicals, but also by the intake, bioavailability and metabolism of these bioactive compounds in the individual. The abundance and profile of bioactive compounds is affected by many factors; yet many of the main features that influence health-promoting properties often have been overlooked or disregarded in most epidemiological studies to date. Fruit and vegetables increasingly are sourced from different locations with varied soil type and climate. In addition, fruit and vegetables are harvested at different horticultural maturities and may be stored and transported for long distances before they are sold and then eventually eaten. Fresh produce continues to undergo physiological, mechanical and biochemical changes after harvest and it is these temporal and even spatial changes, as defined by genotype and the way the product is grown, harvested, stored, processed or cooked, that should be taken into account in any epidemiological study, as all these changes and factors can impact on intake, bioavailability and the interaction between the phytochemical(s) and the individual. For instance, postharvest changes can be influenced by implementing appropriate (or inappropriate) technologies, such as controlling temperature, relative humidity, gaseous composition and so on, and these are known to have profound effects on phytochemicals. Many fruit and vegetables are processed or cooked before being consumed and often are not eaten in isolation; that is, they are commonly eaten with other foodstuffs in combination (i.e. in a meal).

Classification of phytochemicals is complicated. Jaganath and Crozier (2008) defined phytochemicals as ‘bioactive non-nutrient plant compounds that have been linked to reductions in the risk of major chronic diseases’. They classified them into four major groups: nitrogen-containing alkaloids, phenolics and polyphenolics, sulfur-containing compounds, and terpenoids. The premise of this book is not, however, to reassess this classification or to expand upon it, but rather

<table>
<thead>
<tr>
<th>Fresh fruit or vegetable</th>
<th>Phenolic antioxidant intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peach</td>
<td>110 mg not peeled for cv. Snow King</td>
</tr>
<tr>
<td>Grape cv. Napoleon</td>
<td>150 mg with seeds and peel</td>
</tr>
<tr>
<td>Orange cv. Navel</td>
<td>400 mg with part of albedo</td>
</tr>
<tr>
<td>Lettuce</td>
<td>300 mg cv. Lollo Rosso leaving external leaves</td>
</tr>
<tr>
<td>Spinach</td>
<td>120 mg steam cooked</td>
</tr>
</tbody>
</table>
to classify the health-promoting properties of fruit and vegetables on a species-specific basis. Consequently, each chapter provides a review on the current understanding of the identity, abundance, variance, bioavailability and efficacy of individual bioactive phytochemicals specific to that fresh produce type(s) and describes the measures and research needs that will be required in the future to preserve ‘bioactive life’.

References


2 Alliums
[Onion, Garlic, Leek and Shallot]

Gemma A. Chope, Katherine Cools and Leon A. Terry

2.1 Introduction

The Allium genus is very large and consists of many wild edible species; however, only a small selection is cultivated commercially and these include onion (Allium cepa L), garlic (A. sativum L), leek (A. ampeloprasum leek group) and shallot (A. cepa Aggregatum group). Garlic and leek are part of the subgenus Allium, which comprises 37 species in 15 sections, while onion and shallot fall into the subgenus Cepa, which has 30 species in five sections (Fritsch and Keusgen, 2006). In South-east (SE) Asia, species such as Chinese chives (A. tuberosum Rottl.) and Rakkyo (A. chinense G. Don), which are often used for pickles, are cultivated commercially (Brewster, 1994). The Japanese bunching onion (A. fistulosum L.), also known as the spring onion, salad onion, Welsh onion or scallion, is also historically from eastern Asia but is now cultivated and consumed in numerous countries (Brewster, 1994). Onion production extends from the tropics to temperate regions, in countries ranging from the equator to Scandinavia and South Africa. Onion plants are sensitive to photoperiod; therefore, a very wide range of cultivars exists, from ‘short day’ to ‘very long day’ types, which are adapted to cover all latitudes. Therefore, onions grown in one zone may not be readily transferable to another (Currah et al., unpublished data).

Shallots are genetically very close to the common onion and are used in SE Asia as a substitute for onion. Onions are the most commercially important member of the Allium genus, with a worldwide production of almost 64.5 million tonnes (Mt), followed by garlic (15.5 Mt) (FAOSTAT, 2008). Shallots are less commercially important, with a worldwide production of green onions, including shallot, of more than 3.5 Mt.

Allium vegetables have been cultivated and eaten for centuries by many cultures all over the world. Many health benefits historically have been attributed to the consumption of Allium vegetables since the Egyptians in 1550 BC (El-Bayoumy et al., 2006). Since then, their health-promoting properties, which have been shown or suggested, include anticancer, antimicrobial, antiplatelet, antithrombotic, antihyperlipidaemic, antihypertensive, antiasthmatic and immunostimulatory (Block, 2005; Corzo-Martinez et al., 2007).

2.2 Identity and Role of Bioactives

2.2.1 Organic acids

The literature on the organic acid content of onion bulbs is very limited. Malic and citric acid, determined by gas chromatography (GC)
prior to storage, were found to be the major organic acids in onion bulbs cv. Sentinel, comprising 2.8 and 1.8% dry weight (DW) (c.280 and 180 mg/100 g fresh weight (FW)), respectively. Both fumaric and succinic acids were also present; however, levels were quoted as very low, with no data given (Salama et al., 1990). Benkeblia and Varoquaux (2003) also agreed that succinic and fumaric acids were found at very low concentrations, of 0.2 and 0.75 mg/100 g FW, respectively. Both malic and citric acid concentrations were lower than those stated by Salama et al. (1990), at 102 and 20 mg/100 g FW, and an additional organic acid, namely oxalic acid, was measured at a concentration higher than citric acid: 37 mg/100 g FW (Benkeblia and Varoquaux, 2003). More recently, an extended range of organic acids, including tartaric and glutamic acids, has been measured, using HPLC, in six onion cultivars from Tenerife stored for 1 week at 20–25°C. Glutamic acid was found in much higher concentrations than malic and citric acids, at 192–433 mg/100 g FW, whereas tartaric acid was c.8.9–25.2 mg/100 g FW (Rodriguez Galdón et al., 2008). Discrepancies between these published results may be due to variations between cultivars and growing conditions, combined with the use of different techniques to extract and measure the organic acids. Salama et al. (1990) used 80% ethanol (v/v) for extraction and GC coupled with flame ionization detection (FID) for analysis, whereas Benkeblia and Varoquaux (2003) and Rodríguez Galdón et al. (2008) used water to extract and as a mobile phase for HPLC analysis coupled to a diode array detector (DAD). Differences between the two latter studies could be explained, as only Rodríguez Galdón et al. (2008) used acidified water for both the extraction and HPLC mobile phase, which would have helped to preserve antioxidants.

Ascorbic acid is the most abundant vitamin in onion bulbs, with concentrations around 1 mg/g DW (Breu, 1996). According to Gorinstein et al. (2008), ascorbic acid content (measured by the cupric-reducing antioxidant capacity (CUPRAC) assay) was 735, 1994 and 1385 µg/g DW in garlic, red onion and white onion, respectively (cultivar and storage time not stated).

### 2.2.2 Phenolics

Phenolics are secondary metabolites characterized by hydroxylated aromatic rings which include flavonoids, hydroxycinnamic acids and phenolic acids (Veliglu et al., 1998). Phenolics have been found to contribute to the antioxidant properties of onions and have been correlated positively with radical scavenging activity and antioxidant activity (Nuutila et al., 2003). The phenolic acids, ferulic, gallic and protocatechuic acid, were highest in red onions at 21.4, 263 and 138 µg/g DW, respectively, and were more concentrated in the outer scales (Prakash et al., 2007). These recent data support work from the early 20th century on the antifungal properties of onion skin. It was found that red onion skins contained protocatechuic acid and catechol (not found in the outer scales of brown onions) and were toxic to the fungus, Colletotrichum circinans, responsible for the disease, smudge (Walker et al., 1929; Link and Walker, 1933). The literature on the phenolic acid content of leek is scarce, but Schmidtlein and Herrmann (1975) quoted that the most abundant phenolic acids were ferulic acid and p-coumaric acid.

The total phenolic content of Allium species extracted using various solvents and analysed using the standard Folin–Ciocalteu reagent has been investigated. Lin and Tang (2007) reported that the total phenolic concentration of red and white onions (cultivars not stated) were, respectively, c.310 and 216.7 mg gallic acid equivalent (GAE)/100 g FW, as determined using lyophilized onion powder dissolved in deionized water. Garlic and shallot both contained c.55 mg GAE/100 g FW, as determined using a mixture of juice filtrate and hexane extract, which was then hydrolysed in acidified 50% methanol (v/v) (Leelarungrayub et al., 2006). Leek contained the lowest concentration of phenolics at 27.7 mg GAE/100 g FW, extracted from lyophilized powder with 80% methanol (v/v) (Marinova et al., 2005). Discrepancies exist between values reported in the literature; these are possibly due to variations in cultivar, growing site, tissue choice and extraction method. Nuutila et al. (2003) agreed that red onion (cultivar not stated) had a higher total phenolic content.
than yellow onion, but the concentrations in the scales were much lower than those reported by Lin and Tang (2007), at c.20.8 and 15.5 mg GAE/100 g FW, respectively. However, Nuutila et al. (2003) investigated spatial differences in total phenolic content and found that concentrations (GAE) in skins from red and yellow onions (cultivars not stated) were 38- and 17-fold higher than in the bulb scales, respectively. Similarly, garlic total phenolic concentration was 2.3-fold higher in the skin than the bulb, and leek concentrations were 1.3-fold higher in the leaves than the stem. The increased levels of total phenolics in the outer layers of these Alliums may be due to their UV-B exposure. Onion tissue perceives UV-B as a stress signal, which results in enhanced synthesis of enzymes, such as phenylalanine ammonia-lyase (PAL) and chalcone synthases, that catalyse the biosynthesis of phenolic compounds (Mogren et al., 2006). Onion scales (second or fourth) cv. Mansang irradiated with white fluorescent light (3000 lux, 25°C) had 1.2- to 1.6-fold higher quercetin and isorhamnetin glucoside concentrations, after just 24 h, than those held in the dark (Lee et al., 2008b).

**2.2.3 Flavonoids**

Flavonoids consist of two subgroups, flavonols and anthocyanins (Leighton et al., 1992), which are thought to protect against cancer and cardiovascular disease by inhibiting tumour growth and microbial cells (Griffiths et al., 2002). In the main, this link has been shown only by *in vitro* assays. The major flavonoids found in onion are quercetin, kaempferol and isorhamnetin, which can exist as aglycons (quercetin) or as sugar conjugates (glycosides), with 25 different flavonol derivatives characterized in onion bulbs to date (Slimestad et al., 2007). The most abundant flavonol, quercetin, and its glycosides are found mainly in the outer scales of the onion bulbs, specifically in the abaxial epidermis (Hirota et al., 1998). Unlike those in onion, leek-derived flavonols are comprised mainly of kaempferol derivatives, of which Fatorusso et al. (2001) identified and isolated five: three known and two previously unknown.

Quercetin in the bulb scales of onion cv. Sherpa was mainly in the form of quercetin 3,4-diglucoside (4.68 mg/g FW) and quercetin 4-glucoside (2.87 mg/g FW), whereas in the skin it was comprised mainly of quercetin aglycon (10.29 mg/g FW) (Figs 2.1 and 2.2; Downes et al., 2009, 2010). In rats, the bioavailability of quercetin glucosides was found to be 50% that of quercetin aglycon, possibly due to the hydrophilic sugar group hindering passive diffusion across the intestine lining (Sclabert and Williamson, 2000; Wiczkowski et al., 2003). Onion skin is not consumed, although recent work by Roldán et al. (2008) showed that onion by-products could be processed into a paste, followed by mild pasteurization to form a stable product with high antioxidant capacity for possible use as a food ingredient.

The pigment in red onions is predominantly due to anthocyanins, which are comprised mainly of cyanidin derivatives, although peonidin, delphinidin, petunidin and 5-carboxypyranocyanidin derivatives also exist (Fig. 2.3; Slimestad et al., 2007).

Lee et al. (2008b) investigated the effect of baking (5 min), boiling (5 min), frying (2 min), microwaving (1 min), sautéing (3 min) and steaming (5 min) on onion cv. Tubo flavonoid content extracted using 80% aqueous ethanol (v/v). The largest percentage loss (32.8%) of total flavonoids occurred after frying, whereas steaming and microwaving caused the least total flavonoid reduction at 5.7 and 4.4%, and baking resulted in a 1.1% increase, although this was not significant.

**2.2.4 Organosulfur and organoselenium compounds**

Onions and other Allium vegetables are eaten for their unique taste and the medicinal properties of their flavour compounds (Griffiths et al., 2002). The majority of the compounds that contribute to flavour and taste are secondary metabolites whose biosynthesis involves the metabolism of cysteine and glutathione, which are essential pathways for uptake of sulfur and detoxification (Jones et al., 2004). In intact Alliums, the major organosulfur compounds are γ-glutamyl-S-allyl-L-cysteines.
Fig. 2.1. HPLC-DAD chromatographic profile of major flavonols from the fleshy bulb scales of onion cv. Wellington (Downes et al., 2010). 1, quercetin 3,4-diglucoside; 2, isorhamnetin 3,4-diglucoside; 3, quercetin 3-glucoside; 4, quercetin 4-glucoside; 5, isorhamnetin 4-glucoside.

and S-allyl-L-cysteine sulfoxides (ACSOs). In an intact cell, the enzyme alliinase [EC 4.4.1.4] is located in the vacuole, and the ACSOs in the cytoplasm. When the tissue is disrupted (for example, during mastication or homogenization), alliinase hydrolyses the ACSOs, yielding pyruvate, ammonia and transient and unstable sulfenic acids (Uddin and Mac Tavish, 2003), which then condense spontaneously in pairs to form volatile thiosulfimates that contribute to perceived flavour (Briggs and Goldman, 2002). Four different ACSOs are present in Alliums; S-2-propenyl (alliin), S-1-propenyl-(isoalliin, or 1-PrenCSO), S-methyl-(methiin, or MCSO) and S-propyl-(propiin, or PSCO) L-cysteine S-oxides (Fig. 2.4). The composition and concentration of these compounds are responsible, in part, for imparting the characteristic taste and odour of individual Alliums. Methiin is found in garlic, onion, leek and shallot (Fritsch and Keusgen, 2006). The predominant ACSO in garlic is alliin, while in onion and shallot isoalliin predominates. Isoalliin gives rise to the lachrymatory factor, thiopropanal S-oxide, via an enzyme known as lachrymatory factor synthase (Imai et al., 2002). Shallot has a higher relative propiin content (more than 10%) and lower isoalliin than other onion-type Alliums and, in general, leek and shallot have relatively low amounts of ACSOs. Allicin is a thiosulfinate formed from alliin, and many of the reputed health benefits of garlic are attributed to this compound (Cavagnaro et al., 2007). The thiosulfimates are themselves unstable and decompose rapidly into a variety of strong-smelling volatile sulfur compounds, such as polysulfides, cepaenes/ajoenes and zwiebelanes. Cepaenes and ajoenes are α-sulfinyl disulfides and zwiebelanes are cyclic S-S compounds. The compounds produced as a result of this reaction are highly dependent on conditions such as: the initial concentration and ratio of sulfenic acids, pH,
temperature, polarity of extraction solvent, etc. For example, in garlic, ajoenes (Fig. 2.4) are formed in ethanolic extracts and dithiins are formed in oil extracts (Keusgen, 2002), and diallyl disulfide is formed during steam distillations (Brewster, 2008).

The organosulfur compounds are the main active antimicrobial, antifungal and antibacterial agents in Allium vegetables (Corzo-Martinez et al., 2007). Ajoene is a stable rearrangement product of allicin, which is reputed to demonstrate a range of biological activities such as antithrombotic, antimicrobial, antifungal and anticancer (Hunter et al., 2008). It is thought that the organosulfur compounds produced by Alliums have a role in chemical defence against grazing animals and some fungi and bacteria (Brewster, 2008). The organosulfur compounds and their γ-glutamyl derivatives contribute a significant amount to the dry weight of Allium plants, constituting between 1 and 5%; therefore, it is also likely that these compounds play a role in nitrogen, sulfur and carbon turnover, storage and transport.

Organoselenium compounds also exist in the Alliums, which are analogous to the organosulfur compounds in that selenium is substituted for sulfur in these molecules. Selenium can be incorporated in the plant metabolism in place of sulfur where it is available in the growing medium. The major organoselenium compound in onion bulbs is γ-glutamyl-Se-methyl selenocysteine, whereas in onion leaves it is Se-methyl selenocysteine (Arnault and Auger, 2006). Selenium can be incorporated further into Allium chemistry when Alliums are grown in a selenium-rich environment (Wróbel et al., 2004; Block, 2005). The major organoselenium compound in selenium-enriched onion and garlic is Se-methyl selenocysteine, accompanied by other compounds including γ-glutamyl-Se-methyl selenocysteine, selenocysteine and selenomethionine (Arnault and Auger, 2006). Selenosulfur compounds have been reported to have greater
Fig. 2.3. HPLC-DAD chromatographic profile and chemical structure of major anthocyanins from the skin of onion cv. Red Baron (Downes et al., 2009). 1, cyanidin 3-(malonoyl)-glucose-5-glucose; 2, cyanidin 3-glucose; 3, cyanidin 3-laminariboside; 4, cyanidin 3-(3"-malonoylglucoside); 5, peonidin 3-glucose; 6, cyanidin 3-(3"-acetoyl)glucoside; 7, cyanidin 3-(6"-malonoylglucoside); 8, cyanidin 3-(6"-malonoyl-laminariboside); 9, peonidin 3-(malonoyl)glucoside; 10, cyanidin 3-(malonoyl)(acetoyl)glucoside.

anticancer activity than their sulfurous counterparts demonstrated by in vivo studies on mice and rats (El-Bayoumy et al., 2006). Se-methyl selenocysteine is unstable in water extracts at room temperature, but its stability can be maintained by freeze-drying (Arnault and Auger, 2006).

2.2.5 Fructans

Fructans are the principal storage carbohydrates in Alliums. In a study of 60 vegetables, onion, garlic, shallot and leek were among the top six in terms of fructan concentration (a range of 1.8-17.4 g/100 g FW edible portion) (Muir et al., 2007). These polysaccharides are not digested in the upper intestine and are a source of energy for bacteria-producing β-fructosidases in the caeco-colon. Fructan has a reported prebiotic effect, whereby it is believed to promote proliferation of beneficial bacteria like Bifidobacteria (Bielecka et al., 2002) and Lactobacilli (probiotic bacteria), which in turn results in a decrease in the population of potentially harmful bacteria (Roberford, 2007). The beneficial colonic microbiota produce short-chain fatty acids (e.g. lactate and butyrate) which lower pH, thus favouring increased absorption of mineral cations (such as Ca and Mg) from the gut into the bloodstream. Changes in colonic bacteria may reduce carcinogen activation in the colon and stimulate the immune system. Animal studies have also shown benefits for glucose metabolism (increased insulin secretion and changes to hormone metabolism) (Brewster, 2008). It has been suggested that fructans with a higher degree of polymerization (such as those present in garlic and leek) are less likely to induce undesirable gastrointestinal side effects (Muir et al., 2007). Fructan profiles (Fig. 2.5) vary with cultivar for onion, garlic,
Fig. 2.4. Structures of some of the widely studied organosulfur compounds found in *Allium* species.

Fig. 2.5. HPLC-evaporative light scattering detector (ELSD) chromatographic profile of non-structural carbohydrates from the fleshy bulb scales of onion cv. Wellington.
leek and shallot; however, there are characteristic patterns for each species, with onion and shallot containing fructo-oligosaccharides with a degree of polymerization (DP) of up to c.18, garlic containing high concentrations of high DP fructans, and leek containing high DP fructans as well as smaller fructo-oligosaccharides (Ernst et al., 1998).

### 2.2.6 Saponins

*Allium* vegetables are a source of steroid saponins (Carotenuto *et al.*, 1999; Lanzotti, 2006). Steroid saponins have haemolytic, antiparasitic and antifungal activities, and a bitter taste. Steroidal saponins can be divided into two groups: the spirostanol glycosides and the furostanol glycosides. Sapogenins are the aglycones of the saponins. β-Chlorogenin is a characteristic steroid sapogenin of garlic and is bioavailable *in vivo*. Plant saponins are thought to prevent the absorption of cholesterol in the intestine (Amagase, 2006).

### 2.3 Chemopreventive Activity and Bioavailability

Many of the studies conducted on the potential health benefits of *Allium* vegetables are made on known doses of a particular purified chemical or concentrated extract. Therefore, the benefits of dietary intake of the fresh or cooked product depend on the bioavailability of the bioactive constituents, and whether the required concentration for clinical effectiveness can be reached through consumption alone rather than with dietary supplements (Powolny and Singh, 2008). Early studies suggest that organosulfur compounds are readily available, but further investigation is required. It has been suggested that chewing garlic, as is traditional practice for folk medicine, could increase the bioavailability of the active ingredients (Borrelli and Izzo, 2008). The bioavailability of the allyl thiosulfimates found in garlic can be assessed by measuring the amount of allyl methyl sulfide on the breath following consumption of garlic preparations (Lawson and Gardner, 2005).

#### 2.3.1 Onion

Many studies have been conducted since the turn of the century to help identify the mechanisms by which onion intake can help sustain or improve human health (Table 2.1). These investigations involved mainly human studies which took into account the individual’s lifestyle choices, such as diet, body mass index (BMI), education, smoking and alcohol intake, to reduce bias. These human studies, plus those using animal models and cell culture, rarely take into account onion genotype, which accounts for huge variability in concentrations of sulfur compounds and antioxidant capacity. Of the authors listed in Table 2.1, most specified a region in which the onions were sourced but did not state the cultivar. Apart from cultivar, onion biochemistry varies with postharvest treatment, i.e. fresh, cured or stored, and these details are also rarely specified.

**Cancer studies**

Phenolics have been found to contribute to the antioxidant properties of onions and have been correlated positively with radical scavenging activity and antioxidant activity contributing to anticarcinogenic actions (Nuutila *et al.*, 2003). Alkyl sulfides and diallyl disulfides have also been suggested to protect against cancer by the metabolism of carcinogenic compounds (Griffiths *et al.*, 2002). A comprehensive review on the mode of action of these organosulfur compounds in cancer chemoprevention/chemotherapy found the mechanisms were based on interactions with cellular proteins, DNA or oxidative stressors. Cell death is caused by apoptosis or inhabitation of proliferation. *Allium* vegetables can prevent cancers such as prostate, skin, lung, etc., showing that topical application is not necessary, but systemic effects occur. Some organosulfur compounds have selective activity against cancer cells and can modulate drug resistance of cancer cells (Scherer *et al.*, 2009). Specifically, diallyl disulfide induces apoptosis in human colon cancer cells (COLO 205) by a mechanism associated with an increase in the production of reactive oxygen species (ROS) (Yang *et al.*, 2009).
Table 2.1. Health-promoting action of *Allium cepa*.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer</td>
<td>Reduced risk of colorectal, laryngeal, ovarian, oral cavity and oesophagus cancer</td>
<td>Human</td>
<td>At least seven portions of onion per week</td>
<td>Fresh/cooked</td>
<td>Galeone <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Reduced risk of stomach cancer</td>
<td>Human</td>
<td>'High' onion intake. High/low cut-off point based on the median distribution of the controls</td>
<td>Fresh/cooked</td>
<td>Setiawan <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Antiproliferation of HL-60 leukaemia cells and induction of differentiation into granulocytic lineage</td>
<td>HL-60 cell cultures</td>
<td>20 µg/ml Oil</td>
<td>Seki <em>et al.</em> (2000)</td>
<td></td>
</tr>
<tr>
<td>Anticancer</td>
<td>Decreased CYP 2E1 activity which metabolizes low molecular weight carcinogens and nitrosamines</td>
<td>Rat</td>
<td>200 g/kg diet for 9 days Powder</td>
<td>Teyssier <em>et al.</em> (2001)</td>
<td></td>
</tr>
<tr>
<td>Anticancer</td>
<td>The antioxidant effect of disulfides and thiols found in onion oil caused an inverse correlation between antioxidant enzymes and lipid peroxidation in nicotine-treated rats</td>
<td>Rat</td>
<td>100 mg/kg body weight Oil</td>
<td>Helen <em>et al.</em> (2000)</td>
<td></td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Reduced cholesterol in the liver of high-fat-fed rats resulting in decreased glutamic pyruvate transaminase activity, indicating improved liver function</td>
<td>Rat</td>
<td>50 g/kg diet (+ 40 g/kg lard and 10 g/kg cholesterol) Powder</td>
<td>Lee <em>et al.</em> (2008a)</td>
<td></td>
</tr>
<tr>
<td>Antithrombotic</td>
<td>Antiplatelet, antithrombosis and thrombolytic activity. Mechanism inconclusive as no correlation was found between thrombosis and quercetin content</td>
<td>Mouse and rat</td>
<td>Thrombosis experiment: mouse oral administration 3.85 ml/kg Juice</td>
<td>Yamada <em>et al.</em> (2004)</td>
<td></td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>Mechanism may involve inhibition of arachidonic acid, thromboxane A2 (TXA2) synthase and TXA2/PGH2 receptor blockage decreasing TXA2 production, which is a potent platelet aggregator</td>
<td>Rat</td>
<td>0.1–1 g/ml Aqueous extraction reduced to powder Juice</td>
<td>Moon <em>et al.</em> (2000)</td>
<td></td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Decrease in urea and creatinine, markers of renal function, in alloxan-induced diabetic rats. In addition, markers for hepatic dysfunction were reduced in onion-fed diabetic rats, as well as a significant decrease in blood glucose levels</td>
<td>Rat</td>
<td>1 ml onion juice/100 g body weight/day Juice</td>
<td>El-Demerdash <em>et al.</em> (2005)</td>
<td></td>
</tr>
</tbody>
</table>

Note: *From onion cvs. Kitamiko27, Toyohira, Kitawase3, Tsukisappu, K83211, 2935A, Superkitamomiji, CS3-12, Tsukiko22 and Rantaro.*
**Cardiovascular disease**

Cardiovascular diseases can be caused by many factors, including increased blood cholesterol and triglycerol levels, increased blood platelets and homocysteine levels, leading to heart disease and clotting, hypertension, diabetes and obesity (Corzo-Martínez et al., 2007). An Italian case-control study consisting of 760 patients with non-fatal acute myocardial infarction (MI), and 682 controls, found that decreased risk of MI was associated with increased intake of onion but not garlic (Galeone et al., 2009). Moon et al. (2000) studied the effect of aqueous onion extract on animal models and suggested that it inhibited the production of thromboxane A2 (TXA2), an eicosanoid platelet aggregating agent. TXA2 is synthesized from membrane phospholipids, which are converted into arachidonic acid (AA) by phospholipase A2 (PLA2) and then to TXA2. Onion was found to lower the concentration of intracellular Ca2+ ([Ca2+]i) and, as PLA2 is [Ca2+]i dependent; this might lead to the inhibition of PLA2, reducing the downstream production of AA and TXA2. However, a second pathway involved in the production of TXA2 via cyclooxygenase (COX) was not affected by aqueous onion extract; therefore, it was suggested that onion might also block the TXA2 receptor: Since the anti-aggregatory effect of flavonoids has not been replicated in vivo, it is generally considered that organosulfur compounds are responsible for the antiplatelet effect of onion (Griffiths et al., 2002). However, although onions are well known for their positive health benefits, it has been demonstrated in rats that toxic sulfide compounds may be formed from S-allyl-l-cysteine sulfoxides and, after chronic ingestion, these can cause haemolytic anaemia (Munday and Manns, 1994).

**Antibiotic effects**

Onions contain an antifungal peptide, allicepin, which is distinct from the antimicrobial peptide (Ace-AMP1) contained in the onion seed. Allicepin is a chitinase and is active against a variety of fungi, including Botrytis cinerea, Fusarium oxysporum and Mycosphaerella arachidicola (Wang and Ng, 2004). Onion extracts have been found to inhibit oral bacteria, as well as many yeast species and Gram-positives (Griffiths et al., 2002). Proteins, saponins, phenolics and ACSOs are thought to contribute to the antimicrobial activity. Ramos et al. (2006) investigated the antibacterial effects of yellow onion skin compounds and found that quercetin had a mild inhibitory effect on multi-resistant Staphylococcus aureus (MRSA) and Helicobacter pylori. However, the most potent antibacterial compound was a previously unknown quercetin-derived oxidation product, 3-(quercetin-8-yl)-2,3-epoxyflavanone, which showed high antibacterial activity against two strains of MRSA (MRSA#5 and MRSACOL) and H. pylori and increased activity in the presence of β-lactam (part of the structure of several antibiotic families). ACSOs are broken down enzymatically into thiosulfinates and their derivatives, which posses a –S(O)–S– group that disrupts the essential proteins of microorganisms by reacting with –SH groups (Kyung and Lee, 2001).

**Metabolic diseases**

Diabetes increases levels of urea and creatinine in the blood, leading to renal dysfunction. Treatment of alloxin-induced diabetic rats with onion decreased the levels of urea in the plasma by 16% compared with the untreated group (El-Demerdash et al., 2005). Diabetic rats also had higher levels of liver enzymes in the bloodstream; however, these were reduced significantly in those fed with onion. It was suggested that onion was able to reduce the leakage of liver enzymes into the bloodstream by inhibiting liver damage. This was supported by the high bilirubin concentrations found in the alloxin-induced diabetic rats, thought to be due to reduced liver uptake; those fed an onion diet had significantly reduced bilirubin levels.

**Other beneficial effects**

Moon et al. (2000) found that aqueous onion extracts had no effect on COX activity; however, compounds isolated from onion, such as thiosulfoxides and cepaenes, have been shown to inhibit sheep seminal microsomal COX and porcine leukocyte 5-lipogenase.
activity, resulting in anti-inflammatory and antiasthmatic effects (Wagner et al., 1990). Quercetin has been shown to have antiasthmatic properties in guinea pigs treated with aerosolized ovalbumin to stimulate specific airway resistance and immediate- and late-phase asthmatic response. Quercetin was found to inhibit these responses as well as leukocyte recruitment at a similar level to the potent anti-inflammatory drug, dexamethasone (Jung et al., 2007).

The cell walls (dietary fibre) of onions consist mostly of cellulose and non-cellulosic polysaccharides such as pectin and xyloglucan. Sun-Waterhouse et al. (2008) found that dietary fibre had a protective effect on ascorbic acid in vitro. It was suggested that the polygalacturonic acid portion of pectin might form a complex with ascorbic acid via calcium ions or a complex with multivalent metal ions, catalysts for the oxidation of ascorbic acid.

2.3.2 Garlic

The therapeutic and medicinal values of garlic have been reviewed by Keusgen (2002). Therefore, studies published in the past 5–10 years are mainly considered here. The bioactive components depend on the method of preparation, and Tripathi (2009) reviewed the effects of various garlic preparations on extract composition. For example, the major sulfur compound present in raw garlic and garlic powder is alliin, while allicin is the major sulfur compound in crushed garlic. Solvent-extracted garlic and garlic oils contain mainly allyl and methyl sulfides (Rahman, 2007). Allicin from garlic is unstable and is metabolized rapidly by both blood and liver cells, and therefore is not present for long periods in the body. In addition, allicin is not formed in the acidic conditions of the stomach, as the enzyme alliinase is inactivated at the low pH (> pH 3), but is converted by the liver to other compounds such as diallyl disulfide (DADS) (Amagase, 2006). There is a paucity of information on the bioavailability of the bioactive compounds in garlic. Examples of various studies demonstrating the health-promoting properties of garlic are summarized in Table 2.2.

Cancer studies

Epidemiological evidence suggests that dietary consumption of Allium vegetables is correlated inversely with the occurrence of colorectal, laryngeal, ovarian, oral, esophageal, prostate, stomach and renal cell cancers (Galeone et al., 2006; Stan et al., 2008; Chan et al., 2009). A population-based case–control study conducted in Shanghai showed that men who consumed more than 10 g of Allium vegetables per day had a reduced risk of prostate cancer compared with those who consumed less than 2.2 g/day. Among individual Allium vegetables, garlic had a pronounced effect on the reduction of prostate cancer risk (Hsing et al., 2002).

The anticancer effect of garlic is thought to be due partly to the following pathway – enzymes such as glutathione transferases (phase 2) inactivate the carcinogenic intermediates that are activated by cytochrome p450-dependent monoxygenases (phase 1). The sulfur compounds can both inhibit phase 1 enzymes and increase expression of phase 2 enzymes. They can also halt cell cycle progression in neoplastic cells. There are also reports of organosulfur compound-mediated apoptosis in cancer cell lines. Diallyl sulfide, diallyl disulfide and diallyl trisulfide are compounds found in garlic and have been shown to induce apoptosis in T98G and U87MG cells. These cells are human glioblastoma cells and are the most malignant type among primary brain tumours. The effect is brought about by a mechanism involving the production of ROS, which are thought to signal the activation of stress kinases and cysteine proteases (Das et al., 2007). Compounds present in garlic have long been reputed to have a selective antiproliferative effect on tumour cells, mediated in a variety of ways including inhibition of metabolism, inhibition of DNA adduct formation, free-radical scavenging, antiproliferative activities and induction of apoptosis. Other modes of action include histone modification and inhibition of angiogenesis. For in-depth reviews on the anticancer effects of garlic, see Shukla and Kalra (2007) for in vivo, in vitro and epidemiological studies and Powolny and Singh (2008) for mechanisms of action.
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombotic</td>
<td>Antiplatelet activity</td>
<td>Human blood</td>
<td>30 μl garlic juice/ml blood</td>
<td>Cavagnaro et al. (2007)</td>
</tr>
<tr>
<td>Antithrombotic</td>
<td>Protect against gonadotoxic and spermicidal effects of Cd poisoning</td>
<td>Rats</td>
<td>0.5–1.0 ml aqueous extract/100 g body weight/day</td>
<td>Ola-Mudathir et al. (2008)</td>
</tr>
<tr>
<td>Antidiabetic, cardiovascular effects</td>
<td>Reduced serum triglycerides and reduced serum fructosamine levels</td>
<td>Human study, patients with type 2 diabetes mellitus</td>
<td>Time release garlic powder tablets, 600 mg/day for 4 weeks</td>
<td>Sobenin et al. (2008)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Improved glucose tolerance and decreased fasting blood glucose</td>
<td>Fructose-fed male albino Wistar rats</td>
<td>Daily injection of aqueous garlic extract for 8 weeks</td>
<td>Jalal et al. (2007)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Decreased serum glucose, triglycerides and total cholesterol, and increased serum insulin levels</td>
<td>Streptozotocin-induced diabetic male Wistar rats</td>
<td>Daily oral administration of 0.25 or 0.5 g ethanolic extract of Iranian garlic/kg body weight for 14 days</td>
<td>Eidi et al. (2006)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Growth inhibition of cancer cells</td>
<td>Human promyelocytic leukaemia cell line (HL-60)</td>
<td>Ethanolic extract</td>
<td>Nishida et al. (2008)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Not stated</td>
<td>Population based case-control study in Shanghai</td>
<td>Allium vegetables</td>
<td>Hsing et al. (2002)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Reduction in size and number of colorectal adenomas</td>
<td>Human subjects with colorectal adenomas</td>
<td>High dose 2.4 ml AGL/day; low dose 0.16 ml/day for 12 months.</td>
<td>Tanaka et al. (2006)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Reduced incidence of papilloma-bearing mice and decreased size and number of papillomas</td>
<td>Female ‘Swiss albino mice’ with DMBA-induced skin carcinoma</td>
<td>0.5 ml of c.10.4 mg/ml aqueous garlic paste/day orally for 8 weeks</td>
<td>Das and Saha (2009)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Prolonged bleeding and thrombin time, enhanced anticoagulation factor activity</td>
<td>Sprague–Dawleys rats</td>
<td>5–50 mg garlic oil/kg body weight</td>
<td>Chan et al. (2007)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Reduced blood pressure in hypertensive rats</td>
<td>Sprague–Dawleys rats with/without two-kidney, one-clip induced hypertension</td>
<td>500 mg aqueous garlic extract/kg body weight/day for 2 weeks as 0.5 ml intraperitoneal injection</td>
<td>Al-Qattan et al. (2006)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Reduced the rise in total cholesterol and LDL cholesterol, accompanied by decrease in plasma fibrinogen in cholesterol-fed rats</td>
<td>Wistar rats fed on diets +/- cholesterol</td>
<td>25 mg lyophilized garlic/kg body weight</td>
<td>Jastrzebski et al. (2007)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Reduced systolic blood pressure in subjects with marginal hypertension</td>
<td>Human study (n = 25)</td>
<td>2500 mg garlic powder/day</td>
<td>Mousa and Mousa (2007)</td>
</tr>
</tbody>
</table>
Carbohydrate disease

Extracts of garlic have been shown to have an antihypertensive effect, and also to inhibit platelet aggregation in vitro. However, others have shown raw garlic and extracts of garlic (garlic powder tablets and aged garlic powder) to have no effect on the plasma low-density lipoprotein concentrations in patients with moderate hypercholesterolaemia, with the study lasting 6 months (Gardner et al., 2007). The authors recommend that more studies with a wider range of dosage levels and situations are carried out. Ried et al. (2008) conducted a systematic review and meta-analysis of the currently available data, considering only those studies that included a placebo control and garlic only supplements, and concluded that garlic supplementation reduced both systolic and diastolic blood pressure in hypertensive adults, and that a high starting blood pressure was a significant predictor of a positive effect. The benefit for patients in this group was likened to the hypotensive effects of conventional drugs.

Garlic extract has been shown to double nitric oxide (NO) production in vitro by human umbilical vein endothelial cells (Mousa and Mousa, 2007). Nitrous oxide is a well-known vasodilator, and the hypotensive effects of garlic have been attributed, in part, to this effect. Benavides et al. (2007) hypothesized that hydrogen sulfide mediated the vasodilatory effect of garlic. They showed that the garlic-derived compounds, DADS (diallyl disulfide) and DATS (diallyl trisulfide), were converted to the endogenous cardioprotective vascular cell signalling molecule hydrogen sulfide by human red blood cells in vitro. It is likely that a combination of these effects occurs.

Antibiotic effects

The antibacterial activity of garlic extract has been shown to decline in concert with the decline in concentration of allicin. Therefore, it has been concluded that allicin is responsible for most of the antibacterial activity of garlic, although there is a difference in the chemical and biological halflives of the extract, indicating that other compounds or breakdown products of allicin also contribute (Fujisawa et al., 2008; Portz et al., 2008). Aqueous fresh garlic extract is effective against Candida albicans (Low et al., 2008).

Metabolic diseases

Garlic has been reputed to have an antidiabetic effect and has been used traditionally in Indian medicine for treatment of this condition. Liu et al. (2007) have reviewed research concerning the effect of garlic in the treatment of diabetes. They conclude that there is evidence for the antidiabetic effect of garlic, but the studies have been undertaken mainly on animals with drug- or fructose-induced diabetes (Eidi et al., 2006; Jalal et al., 2007), with results comparable to those obtained by administration of commonly used antidiabetic drugs. A study has also shown a beneficial effect of garlic powder in human subjects with diabetes (Sobenin et al., 2008), although the component of garlic responsible for the effect is yet to be identified.

Negative effects

When garlic is consumed to an excessive degree, it can cause unpleasant odours on both the breath and the skin, and has been reported to cause allergic reactions. Allicin has been identified as the major irritant in raw garlic, and the oil-soluble components are generally more toxic than the water-soluble ones. Aged garlic extract is left for up to 20 months, by which time most of the harsh, odorous, irritating compounds are metabolized into naturally stable sulfur compounds (Rahman, 2007). Direct contact of the skin with raw garlic can cause contact dermatitis (Borrelli et al., 2007). High dietary intake of garlic has been implicated in interactions with drugs used to control blood pressure (e.g. warfarin) and diabetes. These interactions are thought to be due to the antiplatelet and hypoglycaemic activity of garlic and have not been proven (Borrelli et al., 2007).

2.3.3 Leek and shallot

There have been few studies into the anticarcinogenic properties of leek in isolation,
although population-based studies involving the category of ‘Allium vegetable intake’ have found inverse links between such intake and risk of stomach, colon, oesophagus, breast and prostate cancer (Sengupta et al., 2004). Leeks contain both spirostanol-type saponins and a rare cholestanol-type saponin not found in onion, garlic or shallot (Lanzotti, 2005). There is little information on the health-benefiting properties of cholestanol-type saponins, although the spirostanol saponins have been linked with cholesterol-lowering and antitumour effects (Lanzotti, 2005). Leeks contain antifungal chitinases (isoforms APC-Dr, -D and -F) that are structurally distinct from those of other Alliums, yet all share a high proportion of cysteine residues. The chitinase of onion showed antifungal activity, determined using bioassays against F. oxysporum and B. allii, yet the chitinase of leek did not (Wang and Ng, 2004). Many antifungal compounds found in Alliums are also bioactives.

Shallot has an antioxidant activity similar to that reported in fresh garlic, and this is highest when extracted using hexane as opposed to water or pressing (Leelarungrayub et al., 2006). Shallot is the only Allium to contain the flavonoid isoliquiritigenin (ISL). ISL is a potent antioxidant and has been shown to inhibit cell proliferation in HepG2 (human liver cancer cells) and A549 (human lung cancer cells) (Kuo et al., 2005). Further in vitro studies showed that ISL caused apoptosis in human prostate cancer cells and reduced metastatic potential. Aqueous extracts of shallot were compared with aqueous garlic extracts for hypoglycaemic properties. Shallot was found to improve intraperitoneal glucose tolerance and reduced, to a greater degree than garlic extract, the fasting insulin resistance index (FIRI) in Wistar rats fed a high-fructose diet for 8 weeks (Jalal et al., 2007). Shallot bulbs contain a novel antifungal peptide, ascalin, which is similar to chitinases from other Allium species (>30 kDa), but much smaller at only 9.5 kDa. Ascalin not only inhibited B. cinerea mycelial growth, but also inhibited human immunodeficiency virus type 1 (HIV-1) reverse transcriptase activity at a very low IC$_{50}$ of 10 μM (normal range 100–300 μM) (Wang and Ng, 2002).

2.4 Effect of Preharvest and Postharvest Continuum

Preharvest treatment and conditions in the field, including nutrition, temperature during the growing season, crop maturity at harvest and the harvesting process, can affect the quality of field vegetables. In addition, many biochemical and physical changes occur in stored fresh produce, including changes in sensory quality perception by the consumer and variations in the concentration of bioactive compounds. A better understanding of how changes in health-promoting compounds vary with preharvest factors and postharvest storage conditions and time will allow optimization of the health-promoting properties in these products.

2.4.1 Onion

Preharvest consideration must include cultivar selection, as the health-benefiting properties of onions are not uniform throughout the vast array of cultivars available. As already mentioned, red onions tend to have a higher antioxidant capacity due to the presence of anthocyanins, although this does not always hold true. Vägen and Slimestad (2008) analysed 15 cultivars of red, brown and low pungency onion varieties for flavonol content using HPLC and total antioxidants using trolox equivalent antioxidant capacity (TEAC). Six individual flavonols were measured and their total concentrations ranged from 35 to 159 mg/100 g FW, for cvs. Domenica Super sweet and Powell Brown, respectively. Total antioxidant capacity ranged from 72 to 509 μmol/100 g FW, for cvs. Colossus and Powell Brown, respectively.

The Department of Crop Science in Sweden has published a great deal of literature on the effects of preharvest factors on quercetin content. Mogren et al. (2007) investigated the effect of low N (72 kg/ha) compared to levels similar to those used by many commercial growers, of 80 kg/ha extra. The authors found that low N had no deleterious effects on the quercetin glycoside concentrations of onion cvs. Barito and Summit F$_1$ as
determined immediately after harvest and after 5 months storage at 1°C. Quercetin concentrations in the fleshy scales also were not affected by the application method (harrowing or rotary cultivation), the type of N fertilizer (organic or non-organic) (Mogren et al., 2008) or lifting time (Mogren et al., 2007). Atmospheric temperature was found to have no clear effect on quercetin, although global radiation had a significant effect, especially during August, when irradiation levels were 4937 W/m² in 2005 and 6059 W/m² in 2002, with corresponding quercetin concentrations of 175 and 564 mg/kg FW, respectively (Mogren et al., 2006).

Although onion skin is currently not consumed, there is increasing research into how it can be utilized as a food additive. Anthocyanin content of onion bulb skin was influenced by curing temperature in onion cv. Red Baron. Immediately after curing, onions cured at 28°C contained less than half the cyanidin 3-(3’-malonoylglucoside), cyanidin 3-(6’-malonoyl-l-aminaribioside) and peonidin 3-(malonoyl)glucoside than those cured at 20 or 24°C (Downes et al., 2009). It has been suggested that anthocyanins do not remain stable throughout storage. Red onions cv. Tropea stored for 2 and 4 weeks at 5°C and 30% RH showed less cyanidin 3-(6’-malonylglucoside) degradation in the whole bulb than those stored at 25°C and 66% RH, or 30°C and 50% RH; however, after 6 weeks the cyanidin 3-(6’-malonylglucoside) content had reduced to c.8 mg/kg FW for all treatments (Gennaro et al., 2002).

The literature on changes in the quercetin concentrations of the fleshy scales during curing is conflicting. Field curing (mean 16.7°C) of onion cvs. Barito and Summit F1 increased levels of quercetin glycosides significantly in the onion fleshy scales from 10 to 40 mg/100g FW (Mogren et al., 2006). Other authors (Price et al., 1997) have reported that curing onions cv. Cross Bow for 10 days at 28°C reduced flavonol concentrations in the flesh, due mainly to reductions in quercetin monoglycosides. However, Downes et al. (2010) found quercetin and isorhamnetin glucoside concentrations increased during curing at 28°C for 6 weeks in onions cv. Sherpa. Quercetin biosynthesis has been linked to UV-B radiation, with exposure resulting in an increase in soluble flavonoids (Mogren et al., 2006). As Mogren et al. (2006) cured their onions in the field, they may have been exposed to more UV-B radiation, causing an increase in quercetin levels, whereas artificial curing usually takes place in the dark. Price et al. (1997) did not detail their specific method of curing. The role of UV-B radiation in quercetin biosynthesis was also discussed by Hirota et al. (1998), who found higher concentrations of quercetins in the outer and top section of onion cv. Takanishiki bulbs, where light exposure was at a maximum.

Controlled atmosphere (CA) is used to extend the storage life of onions. Onions cv. Hysam held in 2 kPa O₂ and 2 kPa CO₂ (80% RH) at 0.5°C for 9 weeks had decreased concentrations of three ACSOs: MCSO, PrenCSO and PCSO. Increasing CO₂ concentration to 8% resulted in a further decrease in ACSO concentration. The concentrations of MCSO and PrenCSO in onions cv. Hysam held in regular atmosphere storage (21 kPa O₂ and 0.1 kPa CO₂) increased, causing an overall increase in total ACSOs (Uddin and MacTavish, 2003). However, only one cultivar of onion was considered in this study. Chope et al. (2007) investigated the effect of CA on pyruvate concentration in three onion cvs., Renate, Ailsa Craig and SS1. The enzyme alliinase hydrolyses pyruvate from ACSOs after cell disruption and is a reliable indicator of pungency. Onion cvs. Renate and SS1 showed a 1.9- and a 1.2-fold increase in pyruvate after storage in 3.03 kPa CO₂ and 5.05 kPa O₂ at 2°C, for 230 and 81 days, respectively. Pyruvate concentration of cv. Ailsa Craig decreased 1.9-fold after 129 days CA storage. These studies suggest that consideration of cultivar choice and storage regime may provide onions with increased organosulfur compound content, and possibly greater health-benefiting properties. Higher sulfur compounds result in a more pungent onion, and with a growing market for sweet, low pungency onions, there must be a compromise between consumer taste preference and nutritional value.
2.4.2 Garlic

Garlic is stored in a number of formats and storage conditions for varying durations, and such factors are likely to affect the concentration, nature and bioactivity of endogenous health-promoting bioactives. As with many other cultivated crops, a wide range of genetic diversity exists in the form of different cultivars, which in turn results in a range of phenotypes that vary according to morphology and biochemical composition. Baghalian et al. (2005) studied 24 Iranian garlic ecotypes and found that the allicin content was significantly higher in some varieties than in others. They concluded that this difference was more likely to be due to genetic variation than to geographical origin.

Typically, garlic cloves can be stored at room temperature for c.2 months, at an intermediate temperature (15-18°C) for c.4 months, or in the cold (-1 to 0°C) for an extended period (Hughes et al., 2006). During storage of intact garlic cv. Fukuchi-howaito cloves for 150 days, the concentrations of γ-glutamyl peptides decreased and the concentrations of the ACSOs increased, with this change being most pronounced in those cloves stored at 4°C, as compared with -3 and 23°C (Ichikawa et al., 2006). This was also the case in garlic cv. Printanor bulbs (Hughes et al., 2006). Similarly, selenium compounds in aqueous garlic extracts from garlic grown on naturally selenium-rich soils were stable during storage for 1 month at 0°C, but were degraded at 4°C (Auger et al., 2004).

A hot water dip at 55°C for 10 min can control sprouting and rooting of garlic cloves during subsequent storage for 4 weeks at 10°C. This treatment did not affect the thiosulfinate concentration (measured by colorimetric assay) of garlic; however, thiosulfinates did increase during the storage period (Cantwell et al., 2003). Gamma irradiation can also be used to control sprout growth of garlic. Garlic cloves of a Korean cultivar were irradiated with 0.1 kGy and stored at 3 ± 1°C, 80 ± 5% RH, for 10 months. In both irradiated and control cloves, total sulfur content decreased after 6 months of storage (Kwon et al., 1989). If boiled for 20 min at 100°C garlic retained its bioactivity in reducing lipid levels in rats, but did not do so after 40 or 60 min of boiling (Jastrzebski et al., 2007).

There are many different garlic preparations available on the market and these have been shown to contain different concentrations of the supposed bioactive compounds, both between one another and compared with that shown on the label (Arnault et al., 2005).

The mode of preparation of the extract affects the amount and relative composition of organosulfur compounds. For example, the content of allicin decreases with extraction temperature and time, and an increased proportion of monosulfur compounds is observed with exposure to high temperatures (100–130°C) over a period of 1–3 h (Woo et al., 2007). Antioxidant activity of garlic extract increased on heating at 110–130°C for 2–3 h (Woo et al., 2007). The antimicrobial activity of garlic juice is diminished on heating and storage (Al-Waili et al., 2007).

Garlic is often dried to be taken as a supplement. The method of drying affects the allicin content of the final product. Hot air drying at moderate temperatures results in an allicin content similar to that of fresh garlic, but allicin concentration is decreased at 60°C (Ratti et al., 2007). The best method of garlic drying, in terms of allicin concentration, is to freeze dry whole cloves at 20°C. Ajoene is formed when garlic is heated (e.g. frying and sautéing) and the concentration of ajoene in garlic oil depends on the garlic’s country of origin. The concentration of ajoene in fresh garlic oil decreased by c.5-fold during 6 months storage at -20°C (Naznin et al., 2008).

2.4.3 Leek and shallot

The effects of preharvest and postharvest conditions on the health-benefiting properties of shallot are limited. As shallots are considered to be conspecific with bulb onions, changes in health-benefiting properties in response to growing and storage conditions may be similar.

Sorensen et al. (1995) investigated the effects of preharvest factors, namely, nitrogen and water supply and harvest maturity, on leek nutrition. High nitrogen supply (280 kg/ha)
increased leek nitrate concentrations to 307 mg/kg, compared with 5 mg/kg in those grown at low nitrogen supply (100 kg/ha). Leeks grown under water stress conditions (deficit of 29% below plant available water) had higher concentrations of vitamin C, protein, magnesium and manganese in both the stem and trimmed leaves. Additionally, when harvested later in October or November rather than September, vitamin C content was higher, although this was significant for only one out of two growing seasons. Results are conflicting concerning whether nitrates are beneficial or detrimental to human health. It has been postulated that, when swallowed, salivary nitrate is converted into HNO$_2$, which may nitrosate secondary amines to produce nitrosamines, some of which have been demonstrated as carcinogenic in animal studies (McKnight et al., 1999). However, more recently it has been suggested that a reduction in endogenous nitric oxide can cause cardiovascular disease such as atherosclerosis, hypertension and ischaemic heart disease, but that nitric oxide concentrations can be replaced by nitrate ingestion (Lundburg et al., 2006). Other suggested benefits of nitrate include a non-immune antimicrobial effect against ingested pathogens in the gastrointestinal (GI) tract, plus regulation of recirculation, platelet activity and GI mobility (McKnight et al., 1999). High nitrogen supply may therefore improve the health-benefiting properties of leeks by increasing nitrate concentrations, as well as improving on yield. There is limited literature on the effect of CA storage on the health-benefiting properties of leek, especially in recent years. Kurki (1979) stored leeks in air at 0°C (100% RH) and found that vitamin A content remained higher in leeks stored in CA conditions. A recent study compared the effects of heat treatment (55°C, 17.5 min) with those of the removal of 2 cm from the base on the nutritional quality of leeks tray-packaged, wrapped with 16 μm stretch film, then stored at 10°C for 7 days. Although both treatments controlled leaf growth, total thiosulfimates decreased from 0.857 to 0.305 μmol/g FW, ascorbic acid content reduced from 51.7 to 24.2 μg/g FW, total soluble phenols from 0.369 to 0.247 mg/g FW and antioxidant capacity from 49.5 to 32.9 μg/g AEAC (ascorbic acid equivalents antioxidant capacity) FW (Tsouvaltzis et al., 2007).

2.5 Conclusions

The future for research in the health-promoting properties of fruit and vegetables in general, and Allium vegetables in particular, should include detailed analysis of the concentrations and bioavailability of the suspected bioactive components and how this is affected by both pre- and postharvest factors, including cooking and processing. There should be a combination of using pure compounds in studies, and intervention studies where the whole product is consumed. Allium vegetables are rarely eaten in isolation, but form the basis of many meals; therefore, the interactions with other foodstuff should be taken into consideration. It is yet to be proven that the concentrations of individual compounds required to produce the desirable effects in vivo can feasibly be reproduced by dietary intake or supplementation.

References


3 Avocado

Marjolaine D. Meyer, Sandra Landahl, Manuela Donetti and Leon A. Terry

3.1 Introduction

Avocado fruit (Persea americana Mill.) originates from Meso-America, where it has been consumed for no less than 9000 years (Smith, 1966). Avocados are now cultivated in numerous regions at tropical and subtropical latitudes, including Mexico, Chile, Indonesia, the Dominican Republic, Brazil, Peru, the USA, Israel, South Africa, Australia, New Zealand and Spain, among others (Table 3.1). Avocado flesh is eaten raw, either alone or incorporated into salads or guacamole. The oil extracted from the pulp of the fruit is also used as a food ingredient or cooking oil, but has also been identified for its potential use in cosmetics and skin-care products (Athar and Nasir, 2005). Although relatively new in international commerce terms compared with many other fruit, avocado has gained popularity worldwide and volumes traded internationally have increased significantly in the past decades (Table 3.1), with the main importers being Europe and North America. Avocado ranks as the second most commonly consumed raw fruit in the USA (Electronic Code of the Federal Regulations, 2006).

The fruit is appreciated not only for its unique taste and flavour – it is also highly nutritious and constitutes a good supply of minerals, vitamins and fibre (Pursglove, 1968; Bergh, 1992; Table 3.2). A detailed publication by Slater et al. (1975) indicated that one half of an avocado cv. Fuerte (about 80 g edible fruit) supplied a substantial percentage of the daily nutritional needs in magnesium (13%), iron (11%), vitamin B$_6$ (pyridoxine; 15%), vitamin B$_3$ (niacin; 8%), vitamin B$_9$ (folacin; 16%) and vitamins A (12%), C (12%) and E (19%) of a child aged 7–10 years.

Avocado fruit is a naturally rich dietary source of health-beneficial bioactive substances, with reported medicinal effects toward many diseases, including prevention against cardiovascular risk and potential anticancer activity (Ding et al., 2007) (Table 3.3). These compounds include monounsaturated and polyunsaturated lipids, carotenoids, vitamins B, C and E, terpenoids, D-mannoheptulose, β-sitosterols, persenone A and B and phenols. Studies have reported on the antioxidant activity (Leong and Shui, 2002; Soong and Barlow, 2004; Bertling et al., 2007), radical suppressing (Kim, O.K. et al., 1998, 2000; Vinson et al., 2001), acetylCoA carboxylase inhibitory (Hashimura et al., 2001), antifungal (Prusky et al., 1991; Domergue et al., 2000) and chemopreventive (Lu et al., 2005; Ding et al., 2007, 2009) activities of the bioactive compounds present in the avocado fruit and its extracts. Additionally, avocado has been shown to assist in the uptake of nutrients from other foodstuffs (Unglu et al., 2005). However, the high caloric value of avocado
Table 3.1. Values in t/year for main avocado producers and importers (from FAOSTAT, 2007).

<table>
<thead>
<tr>
<th>Country</th>
<th>Production (t/year)</th>
<th>Country</th>
<th>Import (t/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexico</td>
<td>1,142,892</td>
<td>USA</td>
<td>348,858</td>
</tr>
<tr>
<td>Chile</td>
<td>250,000</td>
<td>France</td>
<td>110,632</td>
</tr>
<tr>
<td>Indonesia</td>
<td>201,635</td>
<td>Netherlands</td>
<td>63,211</td>
</tr>
<tr>
<td>Colombia</td>
<td>193,996</td>
<td>UK</td>
<td>44,526</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>183,468</td>
<td>Japan</td>
<td>26,511</td>
</tr>
<tr>
<td>USA</td>
<td>175,177</td>
<td>Canada</td>
<td>23,252</td>
</tr>
<tr>
<td>Spain</td>
<td>120,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>85,913</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>65,203</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

associated with its high fat content may dis- suade consumers. Nevertheless, contrary to popular assumptions, including avocado in the diet could be part of a successful reduced energy intake diet (Walker and O'Dea, 2001) and scientific evidence has shown that eating avocado does not compromise good weight control (Pieterse et al., 2003).

This chapter reviews the identity, abundance and effect of different bioactive compounds found in avocado fruit and presents the latest scientific evidence on the health-promoting properties of avocado fruit and its extracts.

3.2 Origin of Avocado (Persea americana Mill.)

The commercial avocado tree belongs to the large tropical family of Lauraceae and to the genus Persea. Other known members of the genus exist but have not been recognized as being commercially important. The crop originated in a large geographic area extending from the eastern and central highlands of Mexico, through Guatemala and up to the Pacific coast of Central America (Popenoe, 1920; Smith, 1966; Storey et al., 1986). The fruit was appreciated by both Mayan and Aztec civilizations, which were believed to have semi-domesticated the crop and selected for larger fruit size with improved eating quality (Smith, 1966; Storey et al., 1986). Three distinct, ecologically separate races are generally recognized – Mexican, Guatemalan and West Indian, or Lowland – based on morphological differences and their respective climatic adaptations around 90 years ago (Popenoe, 1920). The Mexican race is adapted to elevated and cool habitats with a 6–8 months winter–spring dry period (Wolstenholme and Whiley, 1999). The Guatemalan race is native to tropical highlands, with year-round cool conditions, although it can also be found in warmer subtropical areas. The West Indian race, in contrast, is adapted more to a hot and humid tropical, lowland climate with a short dry season. The flesh has a lower oil percentage than the other two types and a different flavour. It is possible that fruit properties such as ripening, quality and abscission may be related to the climatic conditions of the respective areas of origin (Praloran, 1970). The three races are compatible and their hybrids represent the varieties that dominate the international market (Scora et al., 2002). The cultivar Hass is predominantly Guatemalan with some Mexican germplasm, and is the cultivar grown most widely today. This cultivar is particularly appreciated for its postharvest qualities since it shows better storability, essential for long transit times, than other cultivars. The skin is thick and confers protection from pests and disease. Unlike many other green-skin cultivars, the ripening process is accompanied by a distinct skin colour change from green to purplish-black. The cv. Fuerte, another economically important cultivar, is a Guatemalan × Mexican hybrid showing good resistance to cold conditions, while other commercially important varieties include cvs. Ryan, Lula, Booth8,
Table 3.2. Avocado nutrient composition.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Amounts per 100 g fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>73.3</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>160</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>670</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.00</td>
</tr>
<tr>
<td>Total lipid (fat) (g)</td>
<td>14.66</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>8.53</td>
</tr>
<tr>
<td>Total sugars (g)</td>
<td>0.66</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.06</td>
</tr>
<tr>
<td>Glucose (dextrose)</td>
<td>0.37</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.12</td>
</tr>
<tr>
<td>Total dietary fibre (g)</td>
<td>6.7</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>1.58</td>
</tr>
<tr>
<td>Calcium, Ca (mg)</td>
<td>12</td>
</tr>
<tr>
<td>Magnesium, Mg (mg)</td>
<td>29</td>
</tr>
<tr>
<td>Phosphorus, P (mg)</td>
<td>152</td>
</tr>
<tr>
<td>Potassium, K (mg)</td>
<td>485</td>
</tr>
<tr>
<td>Vitamin C, total</td>
<td>10.0</td>
</tr>
<tr>
<td>ascorbic acid (mg)</td>
<td></td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>146</td>
</tr>
<tr>
<td>α-Carotene (µg)</td>
<td>24</td>
</tr>
<tr>
<td>β-Carotene (µg)</td>
<td>62</td>
</tr>
<tr>
<td>β-Cryptoxanthin (µg)</td>
<td>28</td>
</tr>
<tr>
<td>Vitamin E (α-tocopherol) (mg)</td>
<td>2.07</td>
</tr>
<tr>
<td>Lutein + zeaxanthin (µg)</td>
<td>271</td>
</tr>
<tr>
<td>Vitamin K, phylloquinone (µg)</td>
<td>21.0</td>
</tr>
<tr>
<td>Fatty acids, total saturated (g)</td>
<td>2.126</td>
</tr>
<tr>
<td>16:0</td>
<td>2.075</td>
</tr>
<tr>
<td>18:1</td>
<td>0.049</td>
</tr>
<tr>
<td>Fatty acids, total monoensaturated (g)</td>
<td>9.799</td>
</tr>
<tr>
<td>16:1 (g)</td>
<td>0.698</td>
</tr>
<tr>
<td>18:1 (g)</td>
<td>9.066</td>
</tr>
<tr>
<td>Fatty acids, total polynsaturated (g)</td>
<td>1.816</td>
</tr>
<tr>
<td>18:2 (g)</td>
<td>1.674</td>
</tr>
<tr>
<td>18:3 (g)</td>
<td>0.125</td>
</tr>
<tr>
<td>Stigmasterol (mg)</td>
<td>2</td>
</tr>
<tr>
<td>Campesterol (mg)</td>
<td>5</td>
</tr>
<tr>
<td>β-Sitosterol (mg)</td>
<td>76</td>
</tr>
</tbody>
</table>


3.3 Identity and Role of Bioactives

3.3.1 Phenolics and polyphenolic compounds

Phenolic compounds are plant secondary metabolites including a variety of compounds such as phenolic acids, flavonoids, stilbenes, coumarins and tannins. Avocado is not valued as good a source of phenolics as some other fruit (e.g. soft fruit), probably explaining why investigation into the polyphenolic profile of avocado has remained scarce. Most information on phenolic content has been carried out principally to understand better the economic significance and mechanism of the browning reaction when fruit are cut or where internal disorders occur (Golan et al., 1977; Kahn, 1983). Phenolic acids generally are found in the cell vacuole or in special tissues, and are precursors of many other phytochemicals. The concentration of these compounds and the activity of the enzyme polyphenol oxidase (PPO) have long been known to be related to the process of browning. When membrane integrity is lost, the phenols are released and oxidized to quinines by PPO (Torres et al., 1987). PPO induces the conversion of polyphenolic compounds in o-quinones, which in turn form melanins, responsible for the brown coloration (Kahn, 1983; Bower and Cutting, 1988). This process is influenced by different factors, such as concentration of H2O2 in cells, the structure of phenolic compounds and the rapidity of the main reaction (Amiot et al., 1997).

The phenolic content in avocado fruit varies according to cultivar, tissue type and ripening stage (Golan et al., 1977; Torres et al., 1987). For instance, Golan et al. (1977) reported that the mesocarp of cv. Fuerte contained significantly more phenolics than the mesocarp of the Leman variety (0.29 versus 0.03 mg/g fresh weight (FW) of chlorogenic acid equivalent, respectively). The same authors found a difference in phenolic content between the proximal and distal end of the mesocarp of
Table 3.3. Health-promoting action of avocado (*Persea americana* Mill.).

<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower cholesterol levels</td>
<td>Lower cholesterol levels in human coronary system</td>
<td>Review</td>
<td>67% monounsaturated FA found in the study presented, which compares</td>
<td>Blanched, homogenized, dissolved in methylene chloride–methanol.</td>
<td>Kaiser and Wolstenholme (1994)</td>
</tr>
<tr>
<td></td>
<td>(but also tend to lower protective HDL). FA composition showed</td>
<td></td>
<td>favourably with data for olive oil</td>
<td>Organic base-catalysed technique to obtain FAMES of cv. Hass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>seasonality</td>
<td></td>
<td></td>
<td>avocado flesh from two different sites (cool, warm)</td>
<td></td>
</tr>
<tr>
<td>Fall of c.12% total cholesterol and LDL in human plasma. Monounsaturated (MU) most important dietary FA. MUFA diet decreases oxidative stress of LDL</td>
<td>Human</td>
<td>Diets enriched with olive oil, avocado, almonds (total cholesterol consumption was const. around 310 mg/day)</td>
<td>Blood samples (enzymatic, phosphotungstic acid, GLC analysis on erythrocyte-FA)</td>
<td>Berry et al. (1995)</td>
<td></td>
</tr>
<tr>
<td>Lower cholesterol levels in human coronary system</td>
<td>Dietary phytosterol (β-sitosterol) anticholesterololaemic</td>
<td>Analyses of pooled batches of avocado flesh at different times (human)</td>
<td>Average of 76.4 mg/100 g β-sitosterol (ingestion)</td>
<td>Not specified (raw cv. Hass avocado fruit)</td>
<td>Duester (2001)</td>
</tr>
<tr>
<td>Cancer prevention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of glucose uptake</td>
<td>Inhibition of glucose uptake by the tumour cell: ‘energy starving’</td>
<td>Human tumour in experimental animals</td>
<td>Suggested: 1.7 mg/g daily for 5 days (in a drink)</td>
<td>Mannoheptulose purified from avocados</td>
<td>Board et al. (1995)</td>
</tr>
<tr>
<td>by the tumour cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of the proliferation of human prostate cancer cell lines.</td>
<td>Inhibition of the proliferation of human prostate cancer cell lines,</td>
<td>Prostate cancer cell lines in vitro</td>
<td>Androgen-independent cancer proliferation was 60% inhibited by 300 µg/ml dose. Androgen-dependent cancer: 100 µg/ml dose</td>
<td>Acetone extract of California cv. Hass avocado</td>
<td>Lu et al. (2005)</td>
</tr>
<tr>
<td>Cell cycle arrest resulting from downregulation of p27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of tumour growth</td>
<td>Targeting of multiple signalling pathways and increasing intracellular reactive oxygen leading to apoptosis</td>
<td>Human oral cell lines G&lt;sub&gt;50&lt;/sub&gt; for malignant cell line 14 µg/ml</td>
<td>Chloroform extract from California cv. Hass avocado</td>
<td>Ding et al. (2007)</td>
<td></td>
</tr>
</tbody>
</table>
| Nutraceutical / Nutraceutical | Inhibition of tumour growth | Inhibitory effect on \( \text{H}-\text{leucine} \) incorporation for protein synthesis in Ehrlich ascites tumour cells | Mice | Perseitol K\(^+\) complex (molar ratio 20:1) | Methanol extract of *Scurrula fusca* (BL.) leaves | Ishizu et al. (2002)

| Nutraceutical | Increase essential fatty acids in human breast milk | Human | Partially breast-fed (mother eats avocado c. twice a week) | Milk | Rocquelin et al. (1998)

| Excipient in cosmetics and medicine | Cosmetic: lipids act as emollient, protector, regenerator. Oleic acid is an excipient, improves bioavailability of poorly water-soluble drugs and is raw material for ointments. Linoleic and linolenic acid activate metabolic processes in the skin, promote vitamins A and E and help recovery of stratum corneum. Pharmaceutical: FAs prevent cardiovascular disease by reducing total and LDL cholesterol levels. Linoleic has antiarrhythmic effect on heart, lowers cholesterol, prevents clots in arteries. | Human | Ingestion or application on skin | Avocado oil | Rabasco Alvarez and González Rodríguez (2000)

| Antioxidant | Lowering incidence of human degenerative disease | Review | 143 mg/100 g *L*-ascorbic acid equivalent antioxidant capacity (high) | 50% aq. ethanol extract of homogenized Thai avocado flesh, ABTS free-radical decolourization assay, DPPH radical-scavenging assay, RP-HPLC | Leong and Shui (2002)

| Antioxidant | Highest antioxidant activity and phenol content in avocado leaf compared to 47 other medical plants. Treatment of respiratory infection | Correlation to traditional medicine | Equivalent ascorbic acid: 0.157 g/g, equivalent gallic acid: 0.061 g/g | Aqueous extract from Portuguese avocado leaves. Antioxidant: modified ABTS free-radical decolourization assay. Phenol content: Folin–Ciocalteu reagent | Giao et al. (2007)

(Continued)
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of osteoarthritis</td>
<td>Superior to placebo with osteoarthritis of hip and knee</td>
<td>Human</td>
<td>Ingestion of components</td>
<td>Unsaponifiable fraction of avocado oil after hydrolysis (1:2 avocado:soybean extract)</td>
<td>Curatolo and Bogduk (2001)</td>
</tr>
<tr>
<td>Treatment of osteoarthritis</td>
<td>Symptom relief for osteoarthritis patients</td>
<td>Human</td>
<td>300 mg once per day</td>
<td>Unsaponifiable fraction of avocado oil after hydrolysis (1:2 avocado:soybean extract)</td>
<td>Ameye and Chee (2006)</td>
</tr>
<tr>
<td>Allergy</td>
<td>NEG: latex-associated avocado allergy</td>
<td>Human serum</td>
<td>Serum samples were investigated for hevein-specific IgE antibodies: &gt;0.35 kU/l</td>
<td>Ingestion of raw or stewed avocados, or skin-prick test</td>
<td>Posch et al. (1999)</td>
</tr>
</tbody>
</table>

Note: FA = fatty acids; LDL = low-density lipoproteins; HDL = high-density lipoproteins; FAMEs = fatty acid methyl esters; ABTS = 2,2’-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid); DPPH = 2,2-diphenyl-1-picrylhydrazyl; RP-HPLC = reversed phase HPLC; NEG = negative effect.
the same fruit. A total phenolic content of 0.24 mg/g FW of gallic acid equivalent (GAE) was reported in the mesocarp of avocado fruit, but the cultivar was not specified (Luximon-Ramma et al., 2003). Haiyan et al. (2007), using the Folin–Ciocalteu method, measured a total phenol content of 0.50 mg/g FW (as caffeic acid) from avocado flesh, while total phenol content of the seed was sixfold greater than in the corresponding flesh.

A wide range of phenolic compounds has been measured in avocado. Previous studies have found mainly caffeic and p-coumaric acids in cv. Fuerte (Ramirez-Martinez and Luh, 1973; Golan et al., 1977) and in an unnamed variety (Prabha and Patwardhan, 1980). Ramirez-Martinez and Luh (1973) also identified, in the pulp of cv. Fuerte, epicatechin, isoflavone, chlorogenic acid, leucoanthocyanidine, p-coumarylquinic acid and, in much lower amounts, catechin, while Golan et al. (1977) reported leucoanthocyanidine and catechin. These findings were supported by Torres et al. (1987), who identified, by means of HPLC, benzoic acid derivatives (p-coumaric, ferulic, p-hydroxy, protocatechic, vanillic and syringic acids) and cinnamic derivatives (caffeic and sinapic acids) as being the main compounds. More recent investigation identified epicatechins (0.06–0.08 µg/g FW) in the edible part of avocado (Arts et al., 2000; de Pascual-Teresa et al., 2000).

The avocado seed has been found to have a phenolic content almost 80-fold higher than that in the edible portion of the fruit (Soong and Barlow, 2004). The seed is rich in polyphenolic compounds such as (+)-catechin and (-)-epicatechin, but also highly polymeric substances (Geissman and Dittmar, 1965). The peel of avocado fruit contains the flavonoids (+)-catechin and (-)-epicatechin (Terasawa et al., 2006) and anthocyanins (in purple-coloured ripe fruit). One anthocyanin in particular, cyanidin 3-O-glucoside, has been shown to be responsible for the increase in total anthocyanin concentration in the skin of avocado cv. Hass during ripening, resulting in the purpling development (Cox et al., 2004; Ashton et al., 2006). Levels of cyanidin 3-O-galactoside, another anthocyanin detected at harvest (Prabha et al., 1980; Cox et al., 2004), remained stable as skin colour changed.

Availability of phenolic compounds is influenced by their chemical structure and stability. Generally, phenolic acids are absorbed as aglycon at the gastrointestinal and stomach level (Lafay et al., 2006). The metabolic absorption of some of the main phenolics found in avocado fruit has been studied in detail. For instance, work on chlorogenic acid showed that the incorporation of this compound in the stomach could be operated by bilitranslocase or a system of unidentified anion transporters (Lafay et al., 2006). Caffeic acid, which has been reported to have antioxidant activity, is absorbed in the small intestine and a reduction in its absorption is detected when esterificated in chlorogenic acid.
acid (Oltzho et al., 2001). Ferulic acid has been suggested to have beneficial effects on cardiovascular disease, diabetes and Alzheimer’s disease (Zhao and Moghadasian, 2008), and is usually present in a bound form with carbohydrates or acids and seems to be absorbed in the gut (Adam et al., 2002; Zhao and Moghadasian, 2008). p-Coumaric acid may have protective action by acting as a free-radical scavenger and antioxidant in the eye tissues of rabbit, reducing the effect of UV-B radiation (Lodovici et al., 2008). Some classes of polyphenols can be present in their acetylated form, such as epicatechins, or glycosilated, such as anthocyanins. The presence of glycosilation seems to limit passive absorption in the small intestine (Scalbert and Williamson, 2000).

### 3.3.2 Carotenoids

Five main carotenoids are present in avocado fruit: lutein, β-cryptoxanthin, zeaxanthin, α-carotene and β-carotene. Lutein, in particular, is thought to be beneficial by reducing the risk of age-related macular degeneration (Koh et al., 2004; Richer et al., 2004) and can account for up to 70% of total carotenoids in avocado cv. Hass fruit (Lu et al., 2005). The concentration of carotenoids reported in the literature for avocado varies according to the cultivar studied: concentrations on a FW basis of 2.93 μg/g lutein, 0.11 μg/g zeaxanthin, 0.25 μg/g β-cryptoxanthin and α-carotene, and 0.60 μg/g β-carotene in ripe flesh of Californian cv. Hass harvested from January to May were found (Lu et al., 2009), while a Finnish study by Heinonen et al. (1989) reported 3.20 μg/g FW conjugated lutein and zeaxanthin, 0.38 μg/g FW β-cryptoxanthin, 0.19 μg/g FW α-carotene and 0.34 μg/g FW β-carotene in an unidentified cultivar. In cv. Nabal avocados, total carotenoids in the flesh ranged from 10 to 14 μg/g FW (Gross et al., 1973). More recently, other compounds have been identified by HPLC in Californian-grown avocado cv. Hass, namely all-trans-neoxanthin, violaxanthin, neochrome and chrysanthenaxanthin (Lu et al., 2009).

Carotenoid content in the fruit is influenced by factors such as maturity stage and time of harvest (Lu et al., 2005, 2009). For example, the content of lutein in Californian avocado cv. Hass varied from 2.32 to 3.36 μg/g FW in samples harvested in two different seasons, and from 2.67 to 3.62 μg/g in samples from the same harvest (Lu et al., 2009). In a more recent study, Lu et al. (2009) found a significant increase in carotenoid concentrations as fruit were harvested later in the year. This increase was correlated positively with that of total fat content in the fruit. Avocados additionally may vary in their carotenoid content depending on tissue type and ripening stage: the concentration of total carotenoids in the peel is greater than in the fleshy mesocarp, with lutein the most abundant carotenoid in both tissues (Gross et al., 1973; Ashton et al., 2006). The concentration of total carotenoids was found to be greatest in the dark-green flesh just under the skin after harvest and during 10 days ripening of avocado cv. Hass grown in New Zealand (Ashton et al., 2006), these results being confirmed later by Lu et al. (2009). This nutrient-rich outer section of the avocado should therefore not be discarded in order for the consumer to benefit from health properties of carotenoids. As fruit ripen, all carotenoids (lutein as well as minor carotenoid compounds, neoxanthin, violaxanthin, α-carotene) present in the peel tend to decline after harvest, while the carotenoid composition in the pulp does not change considerably during the ripening process of cv. Hass fruit (Ashton et al., 2006).

Some carotenoids can be assimilated better when ingested in combination with lipids, which stimulate the production of bile acids and increase their bioavailability (Roodenburg et al., 2000). In that sense, absorption of the carotenoids present in salads and other vegetables can be enhanced when these are consumed in combination with avocado (Unlu et al., 2005).

### 3.3.3 Phytosterols

Phytosterols (or plant sterols) have a chemical structure similar to that of cholesterol, but a different side-chain configuration. Prominent
phytosterols include β-sitosterol, campesterol and stigmasterol, which have anticholester-
aemic properties (Moghadasian and Frohlich, 1999). Dietary intake of phytosterols has been
associated with a reduction in serum choles-
terol levels, contributing to the prevention of
cardiovascular diseases, as well as potential
protection in the development of several can-
cers (Awad and Fink, 2000; Piironen et al.,

Analysis of avocado undertaken in the
past decade has provided new information
showing that this fruit represents a significant
source of dietary phytosterols, with 0.75 mg / g
FW compared with 0.20 and 0.23 mg / g FW in
grape and orange, respectively (Piironen
et al., 2003). Similarly to many other fruit, the
major sterol is β-sitosterol, being present at
0.76 mg / g in raw edible fruit (Duester, 2001).
The avocado provides more β-sitosterol than
other commonly eaten fruit such as strawber-
ries, grapes or banana and up to fourfold the
amount present in orange (Duester, 2001).
The next most abundant phytosterol
is
campesterol (0.05 mg / g FW) (Duester, 2001;
Piironen et al., 2003) and, in much lesser
amounts, stigmasterol (less than 0.03 mg /g
FW) (Duester, 2001).

3.3.4 Fatty acids

Avocado is an important oleaginous fruit
with a lipid content that can reach over 20%
FW (Mazliak, 1970; Biale and Young, 1971;
Lewis, 1978), depending on the cultivar. Gen-
erally, the oil content is lower in cultivars
from the West Indian horticultural race (from
2.5 to 8%; Hatton et al., 1964) than in fruit
from the Guatemalan (10–13%) and Mexican
(15–22%) races (Knight, 2002). The lipid frac-
tion is predominantly monounsaturated,
with oleic acid (C18:1) consistently represent-
ing the most abundant fatty acid (around 60%
of total fatty acids). Other reported fatty acids
are, in order of abundance, the saturated pal-
mitic acid (C16:0), representing 20% of total
fatty acids, and the unsaturated linoleic
(C18:2), palmitoleic (C16:1) and linolenic
(C18:3) acids, constituting c.12%, 8% and 1%
or less of total fatty acids, respectively. Trace
amounts of stearic, myristic and arachidic
acids can be found in the pulp of avocado
fruit (Ahmed and Barmore, 1980; Ozdemir
and Topuz, 2004; Vekiari et al., 2004).

Avocado ranks among the most impor-
tant natural dietary sources of food-derived
monounsaturates and essential fatty acids,
with amounts of unsaturated fatty acids up to
fivefold those of saturated fatty acids (Slater
et al., 1975; Vekiari et al., 2004). For compari-
son, in olive oil, which is recognized widely
for its health benefits, oleic acid represents
83% of total fatty acids, linoleic 7%, palmitic
6% and stearic 4% (Brown, 1975). High
dietary intake of monounsaturates has been
associated with potential cardiovascular
benefits including effects on serum lipids
(Alvizouri-Munoz et al., 1992; Colquhoun
et al., 1992; Caranza et al., 1995; Ledesma
et al., 1996; Caranza-Madrigal et al., 1997). It
has been shown that the dietary intake of avo-
cado oil increases the percentage of serum high-
density lipoprotein (HDL) cholesterol when
compared with corn or coconut oils. The
reported beneficial effects of avocado oil on
atherogenicity are comparable to those of
corn and olive oil (Kritchevsky et al., 2003).

Although the fatty acid composition
remains generally consistent, with the pre-
dominance of oleic acid followed by palmitic
and linoleic acids, the concentration of each
fatty acid varies with cultivar. For instance,
Ozdemir and Topuz (2004) found differences
in the fatty acid profile of avocado cvs. Fuerte
also reported different fatty acid contents in
avocado cvs. Ettinger, Hass and Fuerte. How-
ever, the ratios of unsaturated and polyun-
saturated to saturated fatty acids were not
statistically different for all cultivars studied.
Luza et al. (1990), when investigating the fatty
acid composition of avocado cvs. Fuerte,
Negra La Cruz and Ampolleta Grande grown
in Chile, found that cv. Ampolleta had the
greatest value of palmitic and palmitoleic
acids, while Fuerte had the lowest, and Negra
La Cruz had the lowest amount of polyunsatur-
sated fatty acids.

Fatty acid composition also varies with
harvest time. For instance, Ozdemir and Topuz
(2004) reported an increase in oleic acid and
a decrease in palmitic, palmitoleic, stearic,
linolenic and arachidic acids between November and January. In agreement with these data, Vekiari et al. (2004) also found an increase in oleic acid and a decline in palmitic, palmitoleic, but also linoleic acid content of avocado cvs. Ettinger, Fuerte and Hass sampled over the commercial harvest season. Lu et al. (2009), on the other hand, reported no changes in the fatty acid composition of Californian avocado cv. Hass sampled between January and September. So, total amounts of monounsaturated fatty acids in the fruit increase with later harvest in the year, as does lipid content.

A recent detailed study showed that the fatty acid profile of avocado cv. Hass differed with different growing regions, hence with different agricultural practices and agroenvironmental conditions, and varied in different parts of the fruit (Landahl et al., 2009). The authors investigated the spatial distribution of fatty acids in avocado cv. Hass originating from three different growing regions (namely, Peru, Chile and Spain) in three different seasons. Palmitic acid and palmitoleic acid contents were lowest in the basal part of Spanish and Chilean fruit, while in Peruvian fruit, palmitic was lowest in the apical region of the fruit. Abundance of oleic acid decreased toward the basal end in Peruvian avocado, whereas it tended to be distributed equally across the vertical axis of fruit mesocarp in Spanish and Chilean fruit. Linolenic and linoleic acid concentrations were found to be highest in the middle and basal regions of fruit, independent of origin. These findings are of nutritional importance since not all parts of the fruit provide the same amount of health-related unsaturated fatty acids.

3.3.5 Vitamins

Vitamins present in avocado fruit are the liposoluble vitamin A (present in the form of its precursor, β-carotene), vitamin E (α-tocopherol) and the water-soluble vitamin C (ascorbic acid). The amount of vitamins varies with cultivar. For instance, cv. Fuerte has been reported to have 484 IU (for 100 g) of vitamin A, 2.4 IU of vitamin E and 0.058 mg/g of vitamin C, while cv. Hass contained 740 IU, 1.6 IU and 0.1 mg/g of the respective vitamins (Slater et al., 1975). Smith et al. (1983) found a concentration of vitamin A higher in avocado fruit than in other commonly consumed fruit such as peach, apple, banana and grape. The free form of vitamin A can be toxic for the human cell if in excess, but the β-carotene form present in avocado prevents this eventuality (Bergh, 1992). The presence of γ- and α-tocopherols has been recorded at concentrations of 3.34 and 28.71 µg/g FW, respectively, in Californian cv. Hass fruit (Lu et al., 2005). Tocopherol content may vary according to cultivars and from season to season, while in cv. Hass fruit the concentration of α-tocopherol tends to increase with fruit maturity (Lu et al., 2009). The opposite was observed for cv. Fuerte (Slater et al., 1975). The season with the highest level of α-tocopherol coincided with that of highest total carotenoid content (Lu et al., 2009).

Ascorbic acid (vitamin C) is one of the most important water-soluble vitamins mainly supplemented through fruit and vegetables (Naidu, 2003). Although avocado fruit is not recognized as a good source of vitamin C, as in some others fruit (e.g. blackcurrant, see Chapter 14 of this volume), the concentration of vitamin C in the flesh has been detected between 0.058 and 0.1 mg/g, depending on the cultivar (Slater et al., 1975). Recently, Bertling et al. (2007) found ascorbic acid, as measured using the colour reaction with 2,4-dinitrophenylhydrazine (DNPH), to be the main antioxidant present in the seed (accounting for half of the seed total antioxidant activity) and the rind tissue, while ascorbic acid content was lowest in mesocarp tissues. Other important vitamins present in avocado are riboflavine (vitamin B₂), thiamine (vitamin B₁) and folacin (Slater et al., 1975).

3.3.6 Protein and ashes

Avocado pulp is a better source of protein than many other fruit, although not as protein-rich as meat, milk and some pulses. Higher amounts of free amino acids than in other fruit have been measured in avocado, the major ones being asparagine, aspartic
3.3.7 Seven-carbon sugars

The peculiarity of avocado fruit, besides its high lipid content and ripening physiology, lies in its non-structural carbohydrates composition: the avocado fruit contains large amounts of the uncommon seven-carbon (C7) sugar, \( \alpha \)-mannoheptulose, and its reduced form polyol, perseitol (Fig. 3.2). These soluble sugars have been measured in various part of the avocado, such as the leaves, shoots, trunk, roots and fruit mesocarp (Liu et al., 1999a), in equal or greater amounts to that of starch. The concentration of the C7 sugars is influenced by seasonality, with reduced concentrations of mannoheptulose present in fruit harvested later in the season (Liu et al., 1999b; Meyer and Terry, 2010). The studies available differ in the reported C7 sugar concentrations, especially for mannoheptulose, as the variation between harvest dates can be very high. Research in California has reported c.30 mg/g DM of mannoheptulose and perseitol in the mesocarp of mature unripe avocado cv. Hass harvested midseason (Liu et al., 1999b), and such results have also been found by others in the flesh of early season cv. Hass grown in Spain (Meyer and Terry, 2008, 2010). However, in another Californian study, Liu et al. (2002) recorded 10-fold less mannoheptulose and Meyer and Terry (2010) detected almost no mannoheptulose in late season fruit. As fruit ripen, amounts of C7 sugars decline substantially (Liu et al., 1999b; Meyer and Terry, 2008, 2010; Landahl et al., 2009).

Most studies have used cv. Hass as the material of investigation and have not specified the part of the mesocarp used when quantifying C7 sugars in the flesh. Hence, the available studies may differ greatly in the tissue region (basal, apical or combined regions). Bertling and Bower (2005) found spatial disparities in the distribution of these compounds among different cultivars. Specifically, mannoheptulose in cv. Hass was found to have the highest concentration in the mesocarp tissue, while in cvs. Pinkerton and Fuerte, it appeared to have the highest levels in the rind tissue. In a more recent study, Landahl et al. (2009) examined the spatial distribution of non-structural carbohydrates in avocado fruit (cv. Hass) and found a trend that perseitol concentrations were lower in the middle region. Mannoheptulose concentrations in the fruit tissue from stem end to rind were highly heterogeneous, but there was a trend toward greater concentration in the apical region, near the stem.

The mechanism(s) for biosynthesis and metabolism of heptose sugars, as well as their function in avocado fruit remain, to date, largely unknown. It has been suggested that the decline in C7 sugars as fruit ripen may

![Fig. 3.2. Structures of \( \alpha \)-mannoheptulose (left) and perseitol (right; both drawn with ChemDraw Ultra v 11.0, CambridgeSoft©).](image-url)
indicate a major role in controlling flesh softening and that these carbohydrates could act as ripening inhibitors (Liu et al., 2002). However, more recent work has shown that fruit softening could be delayed, in spite of very low mannoheptulose concentrations in the mesocarp of late season fruit (Meyer and Terry, 2010). It remains unknown whether the decline in C7 initiates ripening or whether it is an artifact of fruit softening. Cowan (2004) proposed various important potential functions for mannoheptulose activity, including protection from damage by reactive oxygen species (ROS) of certain key enzymes that are essential for fruit growth and development, recently confirmed by Bertling et al. (2007). Thus, the antioxidative properties of C7 sugars may also carry health benefits for the consumer. Mannoheptulose (Board et al., 1995) and perseitol (Ishizu et al., 2002) have been reported to have anticancer activity; mannoheptulose has been associated with an insulin secretion inhibitory effect (Ferrer et al., 1993).

3.4 Health Benefits

Most of the health benefits associated with the dietary intake of avocado have been attributable largely to its remarkable content in mono- (MUFA) and polyunsaturated (PUFA) fatty acid. Such monounsaturates have been investigated for their potential cardiovascular benefits, including effects on serum lipids (Alvizouri-Munoz et al., 1992; Colquhoun et al., 1992; Carranza et al., 1995; Carranza-Madrigal et al., 1997). The oil extracted from avocado pulp has been reported to decrease the risk of coronary heart disease (CHD), cataracts, diabetes, prostate cancer and age-related macular diseases (Bendich, 1993; Birkbeck, 2002; Semba and Dagnelie, 2003; Lu et al., 2005). In contrast, less attention has been given to other bioactive substances with potential health-enhancement properties, such as carotenoids, vitamins B, C and E, terpenoids, o-mannoheptulose, β-sisterol, perseinone A and B, phenols and phytosterols present in avocado fruit. Carotenoids are known to have antioxidant and anticarcinogenic effects, as well as other potential mechanisms in the chemoprevention of cancer (Nishino et al., 2000, 2005; Khachik et al., 2004). Lutein, the carotenoid partly responsible for the yellow-green colour of avocado, is known to have antiproliferative and antimutagenic properties (Chew et al., 1996; Kim, J.M. et al., 1998; Park et al., 1998; Kozuki et al., 2000). Tocopherols (vitamin E), found in substantial amounts in avocado fruit, have been associated with antiproliferative effects on certain types of cancer (Awad and Fink, 2000). Studies have also shown that bioactive substances present in avocado and in its extract have antioxidant (Leong and Shui, 2002; Soong and Barlow, 2004; Bertling et al., 2007), radical suppressing (Kim, O.K. et al., 1998, 2000; Vinson et al., 2001), acetylCoA carboxylase inhibitory (Hashimura et al., 2001), antifungal (Prusky et al., 1991; Domergue et al., 2000) and chemopreventive (Lu et al., 2005; Ding et al., 2007, 2009) activities. Besides containing beneficial bioactive substances, avocado has also been shown to enhance the uptake of nutrients from other food (Unlu et al., 2005).

The following sections provide scientific evidence of the effects of avocado fruit on various diseases and cancer (summarized in Table 3.3).

3.4.1 Antioxidant activity

Several studies have examined the antioxidant activity (principally hydrophilic) of avocado using different methods such as DPPH, FRAP, ORAC or ABTS’ decolourization assays. Depending on the respective study, authors found low (Luximon-Ramma et al., 2003; Garcia-Alonso et al., 2004; Plaza et al., 2009), medium (Soong and Barlow, 2004) or high (Leong and Shui, 2002) activity in avocado. Leong and Shui (2002), when measuring a range of fruit for their general antioxidant capacity based on their ability to scavenge 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), found that avocado fruit contained considerable amounts of antioxidants, while the nature of the compounds contributing to that high antioxidant capacity were not determined. Soong and Barlow...


(2004) and Bertling et al. (2007) found that the antioxidant activity of avocado was considerably higher in the seed than in the edible portion of the fruit.

### 3.4.2 Cancer studies

Several recent reports have focused on the chemopreventive activity of avocado fruit and results have shown that chloroform or acetone extracts prepared from avocado pulp have the ability to inhibit cancerous cell growth selectively (Lu et al., 2005; Ding et al., 2007, 2009). Cellular and molecular mechanisms of the phytochemicals present in the avocado extracts and involved in cancer prevention are not yet well understood. However, it has been shown that avocado-derived compounds target multiple signalling pathways and selectively induce cell cycle arrest, growth inhibition and apoptosis in precancerous and cancer cell lines (Lu et al., 2005).

A chloroform extract prepared from mesocarp of Californian cv. Hass avocado and containing phytonutrients was shown to inhibit premalignant and malignant human oral epithelial cell growth selectively and induced apoptosis (Ding et al., 2007). One of the anticancer mechanisms of this extract (Code D003) was believed to involve the targeting of the cell cycle regulatory proteins, cyclin and cyclin-dependent kinase (cdk), which are normally required for the progression and proliferation of cells (Ding et al., 2007). Apoptosis appeared to be another important target for avocado phytochemicals in eliminating cancer cells selectively from normal tissues (Oberlies et al., 1998; Lu et al., 2005; Ding et al., 2007). Apoptosis was attributed to a perturbation of the normal balance of ROS in the cancer-derived cell lines induced by D003, while normal cell lines were not affected. A more recent study by the same group confirmed that the D003 extract initiated apoptosis by acting on ROS levels, activating both the intrinsic and extrinsic pathways (Ding et al., 2009).

Previously, Lu et al. (2005) had shown that an acetone extract of avocado containing lipid-soluble carotenoids (lutein and, in minor quantities, the related carotenoids, zeaxanthin, α-carotene and β-carotene) and tocopherols (vitamin E) inhibited the growth of both androgen-dependent (LNCaP) and androgen-independent (PC-3) prostate cancer cells in vitro. Incubation of PC-3 cells with the avocado extract resulted in G2/M cell cycle arrest, with a concomitant increase in the cdk inhibitor (CDKI) p27 protein expression. When researchers assessed the biological activity of lutein alone, the anticancer effect could not be reproduced, substantiating a synergistic rather than an individual effect of the lipid-soluble bioactive substances present in the extract. This observation supports prior evidence that different carotenoids may work synergistically to reduce cancer risk (Zhang et al., 2007). Also, avocado contains large amounts of monounsaturated fat, which is likely to facilitate absorption of bioactive carotenoids into the bloodstream where, mixed with other diet-derived phytochemicals, they may exhibit biological activity against cancer. Yeum and Russell (2002) had reported previously that fats helped increase the absorption of fat-soluble vitamins, including pro-vitamin A carotenoids. Likewise, Unlu et al. (2005) demonstrated that the addition of avocado to salsa improved lycopene, lutein and carotene absorption significantly in healthy human subjects (Unlu et al., 2005).

Other potential anticancer phytochemicals present in avocado tissues with cell cycle targeting properties have been identified and are summarized by Ding et al. (2007). For instance, persin was shown to suppress progression through the cell cycle in certain breast cancer cell lines (Butt et al., 2006), while quercetin induced a G2/M arrest in a number of cell types, including U937, lung cancer, prostatic carcinoma (PC-3) cell lines and normal tumour fibroblast cells (Vijayababu et al., 2005; Lee et al., 2006). The anticarcinogenic effects of these compounds were attributed to a modulation of the level of expression of cell cycle regulatory proteins (Vijayababu et al., 2005, 2006a,b). Other alkanols isolated from unripe fruit demonstrated moderate cytotoxicity toward selected cancer cell lines (Oberlies et al., 1998). Avocado also contains apigenin (Ding et al., 2007), a very similar flavonoid to genistein, an antioxidant isoflavone
with known anticancer properties (Messina et al., 1994). Dietary intake of phytosterols, another class of compounds found in avocado fruit, has been shown to have protective effects in the development of certain cancers (Awad and Fink, 2000). The fruit is not the only source of chemicals with biological anticancer activity: Ye et al. (1996) showed that persealide, extracted from the bark of the avocado tree, was moderately cytotoxic towards selected cancer lines, including lung, breast and colon cancer.

Mannoheptulose, which is found in substantial amounts in the mesocarp of the fruit, is thought to be a glucokinase specific inhibitor, and, in a research study by Board et al. (1995), mannoheptulose caused inhibition of glucose intake by tumour cells (25–75% at 12 mM mannoheptulose) and inhibited the growth rate of cultured tumour cell lines (150, 21.4 mM) in vitro. However, there exists no study on the effects of mannoheptulose on tumours in vivo. A Japanese study, when investigating Indonesian medicinal plants, found that a complex of perseitol and K⁺ ions isolated from the leaves of Scurrula fusca (Loranthaceae), traditionally used for the treatment of cancer in Sulawesi Island, had potential cancer-prevention effects (Ishizu et al., 2002).

### 3.4.3 Osteoarthritis

Avocado combined with soybean (avocado/soybean unsaponifiables, ASU) demonstrated potential benefits in the treatment of the symptoms of osteoarthritis, in several human trials (Henrotin, 2008). The combination is thought to exert anti-inflammatory and stimulatory effects in chondrocytes. This is supported by in vitro studies that have demonstrated that ASU can increase the basal synthesis of aggrecan and reverse the reduction of aggrecan by human chondrocytes in alginate beads. It also decreased the spontaneous production of stromelysin-1, interleukin-6 and -8, prostaglandin E₂, macrophage inflammatory protein and finally it stopped fully the inhibitory effects of osteoblasts on cartilage production (Henrotin, 2008).

### 3.4.4 Lipid-lowering effects

During recent years, research has examined the effects of the consumption of different kinds of lipids and their relationship to obesity, cardiovascular disease and several types of breast and colon cancer (Steven and Bruce, 1998; Rose and Connolly, 1999). Elevated blood concentrations of total cholesterol and low-density lipoprotein cholesterol (LDL-C) increase the risk of cardiovascular disease (CVD), while higher concentrations of high-density lipoprotein cholesterol (HDL-C) have the opposite effect.

Avocado oil seemed appropriate to replace saturated fats, in order to lower total cholesterol and LDL plasma levels, and it could be a good alimentary aid, similar to olive oil, to ameliorate atherosclerosis (Alvizouri-Muñoz et al., 1992). Avocado fruit has inherently high concentrations of MUFA (mainly oleic acid) and research has shown that diets enriched with avocado pulp have a cholesterol-lowering effect (Lerman-Garber et al., 1994; Carranza et al., 1995; Ledesma et al., 1996; Carranza-Madrigal et al., 1997). There is epidemiological evidence that a diet enriched in monounsaturated fatty oils may decrease the risk of coronary heart disease (CHD), mainly via the positive effects of the oils on serum lipids. Indeed, MUFA have the ability to reduce LDL and cholesterol levels without increasing the triglyceride (TG) levels (Amunziata et al., 1999). It has been found more recently that MUFA may be more protective in the early stages of atherogenesis, when inflammatory processes and LDL oxidation take place (Kritchevsky et al., 2003). Polysaturated fatty acids, on the other hand, may be even more effective, by not providing aortic cholesterol with its preferred substrate for esterification.

In a randomized parallel controlled study conducted in Mexico, a diet enriched with MUFA derived from avocado decreased the concentrations of total cholesterol and LDL-C significantly, without affecting the HDL-C level in healthy and hypercholesterolaemic subjects (Ledesma et al., 1996). There was also a decrease in TG levels in moderately hypercholesterolaemic patients. Avocado has also been shown to improve lipid levels in a study where patients with phenotype II or...
phenotype IV dyslipidaemias have received either a diet rich in MUFA, using avocado as the principal source, or a low-saturated fat diet without avocado (Carranza-Madrigal et al., 1997). In the diet enriched with avocado, total cholesterol and LDL-C concentration decreased significantly in the patients with phenotype II dyslipidaemia, and HDL-C increased significantly in all of the patients with (phenotype II or phenotype IV) dyslipidaemias. However, Pieterse et al. (2003) found contradicting results, whereby no significant changes in plasma lipid levels were observed, when investigating the effects of avocado in an energy-restricting diet on weight loss and serum lipids in overweight and obese subjects. The difference in patients used for the trials (namely, patients with normal to high serum lipid levels versus dyslipidaemic patients) could have accounted for the discrepancies in results.

Kinetic studies have demonstrated that the lipid-lowering (or hypocholesterolaemic) effect of MUFA probably occurs via an alteration of the small, very low-density lipoprotein (VLDL) particle production rate, on which plasma LDL concentration is dependent. It has been suggested that the LDL-lowering action of dietary MUFA is mediated either by an upregulation of LDL clearance or by decreased conversion of intermediate-density lipoprotein into LDL (Sanderson et al., 2002). High MUFA diets have also been shown to decrease plasma TG levels, but the underlying mechanism for the hypotriacylglycerolaemic (triacylglycerol-lowering) effect of MUFA remains unclear (Kris-Etherton et al., 1999) and requires more research. Not only monounsaturated fatty acids but also phytosterols may contribute to the lipid-lowering effect of avocado. Moghadasian and Frohlich (1999), when reviewing 16 published studies, reported averages of a 13% reduction in LDL concentration and a 10% reduction in total cholesterol concentration in response to various dietary phytosterol mixtures (1–6 g/day).

### 3.4.5 Diabetes

Diabetes mellitus shows hyperglycaemia as a symptom induced by decreased cellular glucose metabolism and uptake, and it is divided into type 1 and 2, where individuals with type 1 require external insulin and type 2 individuals first are prescribed a diet and lifestyle change and then additional drug therapy (Gallagher et al., 2003). Non-insulin-dependent diabetes mellitus (NIDDM) patients benefit from a regulated nutrient scheme. Studies with animal models have shown the efficacy of certain plant extracts in glycaemic control (Gallagher et al., 2003).

It has been shown that the replacement of certain carbohydrates with MUFA (from avocado) in the diet of NIDDM patients improves lipid profile and maintains good glycaemic control (Lerman-Garber et al., 1994). In NIDDM patients with poor glycaemic control, a high monounsaturated fatty acid (HMUFA) diet showed no benefit over a diet high in complex carbohydrates (HCHO); thus, in these patients dietary treatment alone was not sufficient to ameliorate glucose and lipid metabolism (Lerman-Garber et al., 1995). However, in the same study, patients with the poorest metabolic control had a worse glycaemic profile with the HCHO diet, but not with the HMUFA diet.

According to official sources, 70% of the world population uses traditional medicine to improve their life with diabetes (Gondwe et al., 2008). The effectiveness of many plants to ameliorate diabetes symptoms has been tested scientifically with animal models (Swanston-Flatt et al., 1989); for example, crude methanolic extracts of African mistletoe (Loranthus micranthus), a hemiparasitic plant growing on avocado trees, showed a dose-dependent hypoglycaemic effect on alloxan-induced diabetic rats, which was comparable to that seen with the standard drug at 24 h after administration (Osadebe et al., 2004). Extracts from the avocado plant itself have been of interest to researchers: aqueous extract of dried avocado material has been shown to inhibit glucose diffusion across dialysis membrane in vitro (Gallagher et al., 2003). Aqueous extracts of dried avocado leaves given to alloxan-induced diabetic rats produced a significant dose-dependent reduction in blood glucose levels (Antia et al., 2005). It has been reported that the active ingredients in avocado leaves are...
sterols and flavonoids (Andrade-Cetto and Heinrich, 2005). Ethanolic leaf extracts of avocado had a blood glucose lowering effect in non-diabetic and streptozotocin diabetic rats (Gondwe et al., 2008). Possibly, such extracts alter the glucose metabolism in the liver, thus reducing blood glucose concentration by increasing glycogenesis; they have been recommended as a complementary remedy in diabetes (Gondwe et al., 2008). It is unknown whether the fruit would have the same effect.

3.5 Preharvest and Postharvest Continuum

3.5.1 Preharvest

There has been a significant amount of research on preharvest factors affecting postharvest quality of avocado. However, most research understandably has focused on quality parameters such as size, eating quality, ripening and occurrence of disorders rather than on phytochemical content. Some orchard management relating to harvest date has been reported to induce variations in some phytochemicals. Since the avocado, unlike many other fruit, can remain on the tree without ripening when fully mature, it is not unusual that, for economic reasons, part of the production is left hanging on the tree during the picking season, while some fruit are harvested early in the season. These differential harvest dates in the year have been reported to induce variations in the fruit phytochemical content, such as increased carotenoid content (Lu et al., 2009) and a decrease in C7 sugar concentrations (Liu et al., 1999b; Meyer and Terry, 2010) in later harvested fruit. The increase in mesocarp carotenoid content has been shown to be correlated intimately and positively with the temporal increase in lipid content.

Additionally, growing location may affect the biochemical profile of fruit, since both fatty acid and sugar profiles in avocado cv. Hass vary significantly according to production site (Landahl et al., 2009).

3.5.2 Postharvest continuum

With increasing distances between growing location and consumers, avocado can be stored for long periods. A range of technologies, such as refrigerated and controlled atmosphere (CA), as well as the ethylene inhibitor, 1-methylcyclopropene (1-MCP) (Watkins, 2006, 2008), is used commercially to maintain fruit quality and minimize losses due to premature ripening. While there has been a significant amount of research on the physical attributes of avocado fruit, less attention has been given to investigate changes in the health-related components in avocado fruit after harvest. Moreover, investigation of the effects of different storage techniques on avocado phytochemicals remains limited.

Phenolics have been investigated mostly for their role as substrates for browning enzymes (Golan et al., 1977; Kahn, 1983). Total phenolics content in avocado increases with maturity, as does mesocarp discoloration following cold storage (Cutting et al., 1992). The same authors found no difference in phenolics concentration between cold and non-cold stored fruit. Concentration of a particular anthocyanin, cyanidin 3-O-glucoside, can be affected by ripening temperature (Cox et al., 2004).

Changes in fatty acid profile during fruit growth and development have long been known. However, knowledge of such changes in the fatty acid composition of the lipid fraction after harvest is more recent. Some changes occur in the fatty acid composition during the fruit ripening process, with an increase in unsaturated and a decrease in saturated fatty acids (Ozdemir and Topuz, 2006; Meyer and Terry, 2008, 2010). However, these changes remain relatively small and a more important factor that would affect the amount of monounsaturates delivered per fruit would be the lipid content, intimately related to fruit maturity (harvest date). Research with cvs. Fuerte and Hass found that the fatty acid profile remained relatively constant during cold storage (Eaks, 1990; Luza et al., 1990; De la Plaza et al., 2003; Meyer and Terry, 2010).

Other compounds such as heptose sugars show more drastic changes after harvest, with a consistent decrease in mannoheptulose...
and perseitol during postharvest cold storage and ripening (Liu et al., 1999b, 2002; Meyer and Terry, 2010). These results have suggested that C7 contribute to the carbon reserve necessary during the respiratory process, which requires energy. Mannoheptulose pools within the fruit also tend to decline with increasing maturation on the tree.

The use of CA is effective at extending shelf life and reducing CI in avocado (Hatton and Reeder, 1972; Truter and Eksteen, 1987; Meir et al., 1995; Burdon et al., 2008). However, there is no information available on the effects of CA on the health-related compounds present in avocado.

The effects of 1-MCP on avocado physical attributes have been reviewed extensively (see Watkins, 2006, 2008), but studies on the effects of the ethylene antagonist on the bioactive compounds of avocado fruit remain limited. Recently, the effects of 1-MCP on the fatty acids and sugars content of avocado cv. Hass during cold storage and ripening have been reported (Meyer and Terry, 2010). The authors found that the fatty acid profile differed between 1-MCP-treated and non-treated fruit, although these differences remained small. 1-MCP treatment also had an effect on mannoheptulose and perseitol content, with more of the heptose sugars found in the firmer 1-MCP-treated fruit than in the untreated fruit.

Ethylene scavengers can be used to reduce ethylene in the environment and effectively delay climacteric-induced ripening. While their application is not common for avocado fruit, some significant experimental responses to ethylene removal have been recorded using a palladium (Pd)-promoted ethylene scrubber (Terry et al., 2007; Meyer and Terry, 2010). These studies observed better firmness and greenness maintenance in fruit held in the presence of the scavenger. When ripening (i.e. softening and colour change) was delayed in response to the scavenger, higher C7 content in fruit mesocarp as compared with non-treated fruit was observed. On the other hand, ethylene removal in the storage atmosphere had no effect on the fatty acid composition of the oil, as found previously (De la Plaza et al., 2003).

Processing is becoming increasingly valued in the avocado industry due to the growing popularity of avocado, suitable processing technology and increasing recognition of the health benefits of avocado (Elez-Martinez, 2005; Jacobo-Velázquez and Hernández-Brenes, 2010). Avocado can be processed into guacamole, minimally processed (MP) avocado halves and slices and avocado oil (although the latter is still used predominantly in cosmetics). The impact of minimal processing on health-promoting attributes was investigated on avocado cut in slices or in halves and packaged in plastic bags under nitrogen, air or vacuum (Plaza et al., 2009). Refrigerated storage (8°C) of slices or halves of avocado fruit induced a decrease in fatty acid content. However, halves kept under vacuum had reduced loss of fatty acids. Phytosterols presented no significant changes during refrigerated storage. Antioxidant activity, as measured using DPPH, increased toward the end of refrigerated storage for tissues held under vacuum. Minimal processing under vacuum could hence contribute to better preservation of the health-related attributes of avocado fruit.

3.6 Conclusion

The avocado is a rich source of health-related phytochemicals, although research reporting on bioactive compounds and their health properties is only relatively recent and remains limited in comparison with that on other fruit, berries for instance. The evidence from epidemiological studies suggests that consumption of avocado may help decrease risk of developing chronic diseases such as cardiovascular disease and cancer. However, it is likely that the health benefits of avocado result from the synergistic rather than the individual activity of the complex mixture of phytochemicals and bioactive compounds with different functions. Additionally, the lipid present in the mesocarp plays an important role in the assimilation of various lipid-soluble vitamins and components present in the fruit. Therefore, avocado is an excellent candidate to improve nutrient assimilation of other foodstuffs. More research is necessary to understand the mechanisms underlying...
the efficacy of avocado toward reduction of various chronic diseases.

Information on the effects of preharvest and postharvest continuum on the biochemical profile remains scant for avocado fruit. Phenolics, carotenoids, vitamin E, phytosterols and heptose sugars have not been quantified sufficiently for different cultivars, maturity (that is, early versus late season) and location sites. The effects that different storage conditions/postharvest techniques have on bioactive compounds have not been defined adequately for avocado fruit. A better understanding of the biochemical response of avocado to different storage conditions would certainly lead to improved pre- and postharvest management and practices to maintain nutritional quality and ultimately enhance the health benefits delivered to consumers.

References


Walker, K.Z. and O'Dea, K. (2001) Is a low fat diet the optimal way to cut energy intake over the long term in overweight people? *Nutrition, Metabolism and Cardiovascular Diseases* 11, 244–248.


4 Blueberry and Cranberry

Charles F. Forney and Wilhelmina Kalt

4.1 Introduction

Many fruit from the genus Vaccinium are rich in phytonutrients, including those with potent antioxidant properties. The genus contains over 500 species including blueberries, cranberries, lingonberries, bilberries and huckleberries (Vander Kloet, 2004). Plants in this genus typically are small to moderate-sized perennial shrubs that produce berries which are often richly pigmented. Fruit from several Vaccinium species have been gathered from the wild by indigenous people, who used them for food and medicine (Kalt and Dufour, 1997). In North America, teas and syrups made from blueberries were used to treat coughs, diarrhoea and various female illnesses, while in Europe bilberry fruit and leaves were used in various folk medicines to treat inflammation, infection, scurvy and urinary complaints.

Commercial species of blueberries and cranberries are native to North America. In the past two centuries their commercial cultivation has developed into a major industry. Initially, fruit were gathered from wild plants. However, in the last century, cultivars have been developed and improved through extensive breeding programmes supported primarily by state, provincial and federal governments through universities and government research centres (Carew et al., 2006). Breeding programmes have resulted in increased productivity, extended regions and seasons for production, and improved shipping, storing and eating quality of the fruit.

Blueberry species produced commercially include highbush (V. corymbosum L.), lowbush (V. angustifolium Aiton) and rabbit-eye (V. virgatum Aiton, also known as V. ashei Reade) blueberries. In addition, wild European blueberries, also known as bilberries (V. myrtillus L.), are gathered from the wild, but will not be discussed in this chapter. Highbush blueberries, which account for about 60% of total blueberry production in North America, are grown primarily in British Columbia, Michigan, New Jersey, Oregon, Washington, North Carolina and, most recently, California (Carew et al., 2006). Southern highbush blueberries are hybrids of the northern highbush blueberry and native southern species, which impart lower chilling requirements and greater tolerance to heat. Their development has enabled expansion of production in south-eastern USA. Lowbush blueberries are produced primarily in Maine, Québec and the Atlantic provinces of Canada, where they are abundant in the wild. Lowbush or 'wild' blueberry production is based on the management of wild populations. As a result, there is a diversity of genetics among the blueberry clones comprising commercial fields. In addition, the velvet leaf blueberry (V. myrtilloides L.) can comprise substantial
proportions of the wild blueberry plants in some production regions (Wood, 2004). The rabbiteye blueberry matures later in the season than the highbush blueberry. It is cultivated primarily in Florida and south-eastern USA, although it is beginning to be grown in other regions for season extension. While North America dominates world blueberry production, accounting for about 70% of world acreage, plantings in other countries have expanded over 4.7-fold between 1995 and 2005 (USHBC, 2006). South America accounts for most of this growth, followed by Europe, Australia, New Zealand and Asia.

Demand for both fresh and processed blueberry fruit has increased substantially over the past 20 years, driven in recent years by research and promotion related to the healthful properties of this fruit (Carew et al., 2006). Between 1995 and 2005, per capita consumption of blueberries in the USA increased about 1.5-fold (USHBC, 2006). In addition, total exports of blueberry fruit from the USA and Canada increased about 2.5-fold between 1995 and 2002 (USDA, 2008b). Since 1980, blueberry production in the USA and Canada has increased by about 2.7- and 5.8-fold, respectively, to supply this increasing demand (Carew et al., 2006). In addition, new plantings are being established in other parts of the world, including Chile, Argentina and New Zealand. To date, increased demand has kept pace with the growing production and fruit prices have remained fairly stable.

The American cranberry (V. macrocarpon Ait.) is native to damp acidic soils in north-east and north central USA and Canada. It is a low growing, woody perennial that forms a dense spreading mat. Fruit are borne on short uprights that emerge from horizontal stems or runners. Fruit ripen in the autumn, from late September through early November, depending on the cultivar and location. Ripe fruit are astringent and tart, with deep red skin and white flesh. The fruit float, which facilitates 'wet' harvesting. Because of the tart nature of the fruit, about 95% of harvested fruit are processed into juice cocktails, sauce and other products, while only 5% are sold fresh and then processed just prior to being consumed.

Cranberries were first domesticated in the early 1800s in Massachusetts, where wild plants were cultivated (Trehane, 2004). Since then, the cranberry industry has grown steadily in North America. The development of new products including juice cocktails and blends during the 1980s and 1990s and the development of international markets drove a steady increase in cranberry production, resulting in a 2.3-fold increase between 1980 and 1999 (Pollack and Perez, 2008). However, an oversupply of cranberries in 1997 caused a dramatic drop in cranberry prices, causing a destabilization of the cranberry industry (CCCGA, 2009). Since then, cranberry prices have stabilized and demand continues to increase, driven, at least in part, by the publicizing of health-promoting properties of the cranberry fruit. Currently, cranberries are produced on about 15,500 ha, primarily in five states: Wisconsin, Massachusetts, New Jersey, Oregon and Washington. Another 3000 ha are cultivated in the Canadian provinces of British Columbia and Quebec (CCCGA, 2009). Over 345,000 t of cranberries were produced in the USA in 2008 (USDA, 2008a) and about 43,000 t in Canada.

The medicinal properties of particular Vaccinium berries were documented as long ago as the Middle Ages (Kalt and Dufour, 1997). Blueberries and cranberries were also included among the medicinal plants used by native North Americans (Chandler et al., 1979) and, in more recent folk medicine, cranberries have been recognized for their ability to promote urinary tract health, while Vaccinium with a high anthocyanin concentration (i.e. blueberry species) are purported to benefit vision. Most recently, there has been tremendous interest in the health-promoting properties of plant foods, based on epidemiological evidence of an inverse relationship between their consumption and the incidence of degenerative disease. This most recent interest has focused on phytochemicals that possess sufficient antioxidant capacity to protect biomolecules against oxidation. Oxidative damage to biomolecules such as low-density lipoprotein, DNA and protein, is known to underlie the aetiology of degenerative diseases such as cardiovascular disease, certain cancers and the ageing process itself (Ames et al., 1993). Vaccinium berries have stood prominently among a cadre of healthful foods.
since, along with their history of medicinal use, they possess a particularly high concentration of various phenolic components that are potent antioxidants. As the research unfolds, it is becoming apparent that both the concentration and structural features of Vaccinium phenolics figure prominently in their health functionality.

The current flurry of research activity to characterize and understand the effects on human health of the consumption of fruit and vegetables has created much interest in both blueberry and cranberry by researchers and consumers alike. In the past few years, many in vitro studies have demonstrated that compounds found in blueberry and cranberry fruit can scavenge free radicals and have beneficial effects in various in vitro disease models. However, evidence of the health benefits from in vivo studies using animals or humans is more limited. The objective of this chapter is to identify the potential healthful components of Vaccinium fruit, to explore the evidence that these components can impact animal or human health, and to assess how pre- and postharvest factors can affect these components and their healthful properties.

4.2 Identity and Role of Bioactives

Blueberry and cranberry fruit contain a diverse range of phytochemicals that have been implicated in having beneficial health effects. These include vitamins A, C and E, folic acid, β-carotene, α-carotene, lutein, phytosterols such as β-sitosterol and stigmasterol, triterpene esters and phenolic molecules. Among these phytochemicals, phenolic molecules have received the greatest attention due to their abundance and diversity in Vaccinium fruit (Vinson et al., 2001; Kalé et al., 2008b). The major structural classes of phenolics found in Vaccinium fruit are flavonoids, which include anthocyanins, flavonols, flavanols and proanthocyanidins (condensed tannins), stilbenoids, phenolic acids, such as hydroxybenzoic and hydroxycinnamic acids, and lignans. In this chapter we will provide a brief overview of these phytochemicals, focusing primarily on the flavonoids, which have been associated with many of the unique health benefits attributed to Vaccinium fruit.

4.2.1 Anthocyanins

Of the many flavonoids found in blueberry and cranberry fruit, anthocyanins are one of the most abundant. Anthocyanins occur in nature as glycosides of anthocyanidins. There are six major anthocyanidins that are found most commonly in nature (Fig. 4.1). The most abundant sugar moieties bound to these anthocyanidins include glucose, galactose and arabinose. In addition, acyl groups may be bound to the sugar moieties, which may improve their stability. Anthocyanins are responsible for the dark blue and red colour of ripe blueberry and cranberry fruit, respectively. They are synthesized rapidly during fruit ripening and, with some notable exceptions (e.g. V. myrtillus L.), typically are found only in the peel tissue (Riihinen et al., 2008).

Anthocyanin concentration in blueberries can range from 0.7 to 5.2 mg/g FW and comprises about 30–47% of the total phenolics (Moyer et al., 2002). Blueberry anthocyanins include five of the six major anthocyanidins, namely, delphinidin, malvidin, petunidin, cyanidin and peonidin. Blueberries do not contain pelargonidin. When anthocyanins were analysed in various lowbush and highbush clones and cultivars, 14 different anthocyanins and 11 different anthocyanins that were acylated with acetic acid were identified (Kalé et al., 1999b; Prior et al., 2001). In a more recent study, Wu and Prior (2005), using high performance liquid chromatography electrospray ionization tandem mass spectroscopy (HPLC-ESI-MS/MS), tentatively identified five additional anthocyanins present in trace amounts in lowbush blueberries.

Cranberry anthocyanin concentrations range from 0.2 to 3.6 mg/g FW (Prior et al., 2001; Wang and Stretch, 2001). The major anthocyanins are galactosides and arabinosides of cyanidin and peonidin (Neto, 2007). However, Wu and Prior (2005) recently reported that trace amounts of anthocyanins based on pelargonidin, delphinidin, petunidin and malvidin had been found in cranberry fruit.

4.2.2 Proanthocyanidins

Blueberry and cranberry fruit are both rich in proanthocyanidins (PACs), which are also
referred to as condensed tannins, and can occur as procyanidins or prodelphinidins (Fig. 4.2). These molecules are made up of oligomers and polymers of variously linked flavan-3-ols and flavan-3,4-diols and are found primarily in the peel and seeds of the fruit. Commonly found flavan-3-ols are catechin, epicatechin and galloylated catechins. These monomers typically are linked by single C–C bonds between adjacent flavanol monomers at the 4-6 or 4-8 positions (B-type). *Vaccinium* fruit are notable for the presence of PACs whose monomers are linked by two bonds at the 4-8 and 2-O-7 positions (A-type), which is thought to confer structural rigidity and unique properties (Prior et al., 2001; Gu et al., 2003; Schmidt et al., 2004). This structural feature is not widespread in the plant kingdom. Cranberry PACs are composed primarily of epicatechin units that include A-type linkages, while blueberry PACs are composed primarily of catechin and epicatechin units and have few A-type linkages (Schmidt et al., 2004).

PACs are notoriously difficult to analyse because of their structural diversity and propensity to rearrange, particularly after extraction and processing. A major structural feature that can be analysed is their degree of polymerization. HPLC analysis is conducted using normal phase and fluorescent detection, but cannot characterize heterogeneous and A-linked PACs adequately. MALDI-TOF, a sophisticated and costly technique which requires a relatively purified sample, is currently the most informative approach in the analysis of cranberry PACs (Howell, 2007).

The PAC concentration in blueberries averages 0.65 for lowbush and 0.44 mg/g FW for highbush fruit (Neto, 2007). Cranberry fruit have the highest concentration of PACs among fruit listed in the USDA PAC database.
Fig. 4.2. The structure of an A-type proanthocyanin commonly found in cranberry fruit. Note the A-type linkage in bold.

Fig. 4.3. Hydroxycinnamic acids and their esters are abundant in blueberry fruit peel and flesh, of which chlorogenic acid is predominant (Fig. 4.3). Fruit in the highbush blueberry cv. Sierra contained 0.65 mg/g FW chlorogenic acid, which comprised about 29% of the total phenolic compounds in the fruit (Zheng and Wang, 2003). Taruscio et al. (2004) found the concentration of phenolic acids of several highbush blueberry cultivars to average 1.3, 0.18, 0.044 and 0.005 mg/g FW for chlorogenic, caffeic, ferulic and p-coumaric acids, respectively. In cranberry fruit, Zuo et al. (2002) reported the most abundant phenolic acids to be coumaric, sinapic, caffeic, o-hydroxycinnamic, ferulic and 2,4-dihydroxybenzoic, having concentrations of 0.25, 0.21, 0.16, 0.089, 0.088 and 0.043 mg/g FW, respectively.

4.2.3 Other phenolics

Flavonols are plentiful in both blueberry and cranberry fruit and are found primarily in the skin (Riihinen et al., 2008). Total flavonol concentration of blueberry cvs. Northblue and Northcountry was reported to be 1.2 and 0.7 mg/g DW, respectively (Häkkinen et al., 1999), and the concentration in cranberry fruit ranged from 0.2 to 0.4 mg/g FW (Vvedenskaya et al., 2004; Neto, 2007). The primary flavonols found in blueberries and cranberries are quercetin and myricetin, which exist in several gycosidic forms. Quercetin glycosides comprise about 75–85% of the total flavonols in blueberry and cranberry fruit (Häkkinen et al., 1999; Taruscio et al., 2004; Neto, 2007).

4.2.4 Other bioactive phytochemicals

Blueberry and cranberry fruit contain triterpenes. Blueberry fruit were reported to contain ursolic acid, pomolic acid and β-amyrin (Neto, 2007). Ursolic acid was found in cranberry fruit at a concentration of 0.6–1.1 mg/g
HO
HO
OH

Fig. 4.3. The structure of chlorogenic acid, a prominent hydroxycinnamic acid in blueberry fruit.

Both blueberries and cranberries contain small amounts of stilbenes. Resveratrol concentrations as high as 1.7 µg/g DW were reported for blueberry fruit and 0.9 µg/g DW for cranberry fruit (Rimando et al., 2004). Pterostilbene and piceatannol, analogues of resveratrol, were also found in rabbiteye and highbush blueberry fruit, respectively, at a concentration ranging from 0.1 to 0.42 µg/g DW.

4.2.5 Antioxidants

Blueberries and cranberries rank highly among fruit for their antioxidant capacity, which has been attributed to their high phenolic content (Prior et al., 1998; Vinson et al., 2001; Moyer et al., 2002). Total phenol antioxidant index (PAOXI) values for blueberry and cranberry fruit were 40.5 × 10³ and 31.2 × 10³, which ranked third and sixth, respectively, among 20 fruit assayed (Vinson et al., 2001). The antioxidant capacity of blueberry fruit, measured by the oxygen radical absorbance capacity (ORAC) assay, ranged between 19 and 130 µmol Trolox (a water-soluble vitamin E analogue) equivalents per g FW among 20 blueberry genotypes representing lowbush, highbush and rabbiteye fruit (Moyer et al., 2002). When antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) assay, for these same 20 blueberry genotypes, values ranged from 19 to 161 µmol/g FW and results were similar to those of the ORAC assay (Moyer et al., 2002). It was estimated that anthocyanin content accounted for about 50% of the antioxidant capacity of blueberry and cranberry fruit (Zheng and Wang, 2003). Chlorogenic acid andpeonidin 3-galactoside were reported to be the most important antioxidants in blueberry and cranberry fruit, respectively (Zheng and Wang, 2003). Prior et al. (2001) reported that PACs accounted for 32 and 54% of the total ORAC measured in blueberry and cranberry fruit, respectively. Ascorbate contributed <5% of the total ORAC antioxidant capacity in blueberry fruit (Prior et al., 1998; Kalt et al., 1999a).

4.3 Health Benefits of Blueberry and Cranberries

In order to interpret the potential significance of results, it is important to discuss the experimental approaches taken in food and health research. Research conducted using *in vitro* assays is designed to simulate parameters of a specific physiological state (e.g. disease) using appropriate biochemicals and often specific cell types. In *in vitro* testing, the user defines the concentration of, for example, *Vaccinium* extract to be added to an assay, which may or may not be cell based. When tested *in vitro*, components of interest bypass the normal processes of digestive absorption and other factors that determine their bioavailability. While *in vitro* results demonstrate biochemical interactions among test components and may suggest a possible mechanistic basis for possible health effect(s), they provide limited information on the effects that may be expected in a whole living organism. Alternatively, effects shown *in vivo* demonstrate that
component(s) in the dietary intervention are adequately bioavailable and in a form capable of producing such effects. Whether in vitro or in vivo, studies employ ‘models’ which include specific cell types (in vitro) or animals (in vivo, often rodents) that may be genetically defined, or treated in such a way to induce a particular condition, often in an attempt to simulate processes related to disease. Along with the important distinctions between in vitro and in vivo evidence, it is also important to recognize that significant differences exist between the physiology of rodents and humans, as well as the myriad processes that may contribute to disease outcomes. Rigorously designed human clinical trials constitute the current ‘gold standard’ for evidence of relationships between plant components and human health outcomes. For the reasons outlined above, the following discussion of the health functionality of Vaccinium berries focuses primarily on in vivo evidence, while the vast abundance of in vitro evidence suggestive of the beneficial health effects of Vaccinium berries will not be discussed in depth.

4.3.1 Blueberries may protect the brain

Blueberry consumption may protect the brain during normal ageing and when neurodegenerative disease is present. The interest in blueberries as neuroprotective agents arose from epidemiological research supporting a role for fruit and vegetable consumption in reducing the risk of various degenerative diseases (Ames et al., 1993), including neurodegenerative disease (see references in Lau et al., 2007). Concurrently, extensive in vitro research suggested that specific fruit and vegetable components might confer health protection via their action as antioxidant agents in biological systems. Since the late 1990s, research on the neuroprotective effects of blueberries has expanded dramatically, with evidence for their benefits extending beyond their activity as antioxidants. Importantly, research supports a role of blueberries as anti-inflammatory agents, due to their ability to affect cell signal transduction pathways to reduce inflammatory processes that can influence various site-specific aspects of brain ageing and neuropathologies (for review see Lau et al., 2007). Importantly, the beneficial effects of blueberries in the brain have been demonstrated in various in vivo rodent models (for review see Lau et al., 2007). These in vivo results have been complemented with further ex vivo and in vitro characterization. Typically, these studies involve the imposition of a physiological stress (e.g. genetic predisposition, ageing, inflammatory insult, oxidative stress), after which cognitive function (e.g. memory, motor abilities) is tested and subsequently neuronal physiology and various biomarkers are examined. Together, results from in vivo, ex vivo and in vitro studies are correlated with specific dietary interventions (e.g. blueberry feeding).

Neuroprotection by various plant and food components has been reported (Ramassamy, 2006). Among the berries, blueberries appear generally to be more potent and distinctive in their effects. In an early study by Joseph et al. (1999), rats that demonstrated age-related cognitive impairment were fed diets enriched in spinach, strawberry, or blueberry extract and this resulted in memory improvement in all treatment groups compared with controls, based on the rats’ performance in a water maze test. However, only the blueberry-fed rats demonstrated significant improvement in their motor abilities. In another study, rats were fed diets enriched in either strawberries or blueberries before receiving whole-body exposure to high-energy $^{56}$Fe irradiation that disrupted dopamine-sensitive neuronal systems, which normally decline during ageing (Rabin et al., 2005). Both blueberry and strawberry feeding preserved the capacity for release of the neurotransmitter, dopamine, compared with the rats fed non-supplemented diets. Also, both strawberry- and blueberry-enriched diets protected rats against cognitive deficits (water maze test performance). However, differences in water maze performance suggested that strawberry supplementation had a greater effect on the hippocampus, which controlled spatial memory, while blueberry supplementation had a greater effect on the striatum, which influenced relearning. The results suggested that not only might the total concentration of dietary phenolics be
important in neuroprotection, but also the specific types of phenolics present. Blueberries are notable for their high anthocyanin concentration, while they contain little, if any, ellagitannins. Strawberries contain ellagitannins, but a significantly lower concentration of anthocyanins. These noted differences may aid in the development of products containing optimized blends of phytochemicals to elicit specific health benefits.

The beneficial effects of blueberry supplementation in various models of brain aging and disease have been reviewed recently (Joseph et al., 2007; Lau et al., 2007). These reviews describe the fundamental role played by inflammation and oxidative stress in the degenerative processes underlying brain aging and neuropathologies like Alzheimer’s disease and Parkinson’s disease. Inflammation is a highly regulated process involving numerous intermediates (especially cytokines) that work either to amplify or to dampen the cellular processes involved in inflammation. The immune system is finely tuned to launch rapid responses of an appropriate magnitude, which is critical to the beneficial role of inflammation in acute conditions such as infection and trauma. However, it is widely recognized that chronic low-level inflammation, and the oxidative stress arising from inflammation, are both the hallmark of, and a damaging element in, degenerative conditions such as cardiovascular disease, diabetes, neurodegenerative disease and the aging process itself. Phytochemicals that can reduce the activity of proinflammatory signal transduction pathways constitute a potentially powerful means to mitigate the degenerative processes of ageing and disease. Numerous lines of evidence suggest that blueberries possess this characteristic.

In the brain, glial cells mediate immune responses and their activation is a definitive marker of neuroinflammation. Activated glial cells produce proinflammatory cytokines (e.g. tumour necrosis factor, interleukins 1 and 6), various growth factors and other proteins that lead to further glial cell activation. Some rodent models designed to examine inflammation involve administration into the brain of the proinflammatory compounds, lipopolysaccharide or kainic acid. Responses are thought to mimic the effects of inflammatory and oxidative metabolism on cognition and motor function that are evident in disease and ageing (Lau et al., 2007).

Transgenic mice that are predisposed to the development of symptoms of Alzheimer’s disease, including amyloid plaque development in several brain regions, and cognitive deficits at middle age, were used to examine the effect of blueberry supplementation on parameters related to the development of the disease (Joseph et al., 2003). After 8 months, blueberry-supplemented transgenic mice performed as well as non-transgenic mice in a Y-maze test, and significantly better than the control transgenic mice. Compared with control transgenic mice, blueberry-fed transgenic mice had a higher activity of specific kinases that played a role in cognitive function, especially in conversion of short- to long-term memory (Joseph et al., 2003).

Old rats that were equivalent to 70-year-old humans received blueberries in their diet and were reported to perform better in an object recognition test than their age-matched counterparts that did not receive berry extract. The blueberry-supplemented rats also had a lower level of NF-κB, a biomarker whose expression was correlated with inflammation and oxidative stress. The effects of blueberries on NF-κB expression varied among brain regions, suggesting additional specificity in the scope of neuroprotective responses to blueberry feeding (Goyarzu et al., 2004).

Different lines of evidence suggest that blueberry components may stimulate the regenerative capacity of the brain. The hippocampus, a region of the brain that functions in learning and spatial recognition, undergoes new cell formation, although the rate of this neurogenesis declines with ageing. Rats that received blueberries for 8 weeks, followed by bromodeoxyuridine, an analogue of uridine that could be detected histochemically, had a significantly higher density of new cells compared with rats that did not receive blueberries (Casadesus et al., 2004). These rats also had higher levels of an insulin-like growth factor that was a key modulator of hippocampal neurogenesis, and higher activity of a kinase that was critical in neuronal signal transduction.
Transplantation of embryonic dopamine neurons can mitigate the progression of Parkinson’s disease by restoring dopamine production. However, this approach suffers from poor survival of transplants, in spite of anti-inflammatory and antioxidant therapy. A rat model that simulated this type of human cell transplantation therapy was employed in a study designed to improve the environment of the host tissue prior to transplantation (McGuire et al., 2006). Using this model, diet supplementation with rabbiteye blueberry cv. Tifblue increased the survival of dopamine neurons significantly and reduced behavioural responses associated with poor survival. In another study involving transplantation of neuronal tissue, portions of fetal hippocampus tissue were transplanted into the eye of middle-aged (4-month-old) rats (Willis et al., 2005). The survival, growth and cellular organization of the chimeric grafts were improved significantly in the rats that received blueberries in the diet for 1 week prior to and 6 weeks after the transplantation procedure.

Another study that suggested that blueberry consumption might improve the cellular viability and regenerative capacity of brain tissues employed a rat model of ischaemic stroke (Sweeney et al., 2002). During ischaemic stroke, affected regions of the brain are subjected to hypoxia and then reperfusion when the blockage is relieved. During reperfusion, extremely high levels of reactive oxygen species are released, contributing further to cellular damage arising from hypoxia. Rats that received a lowbush blueberry-supplemented diet for 6 weeks prior to a surgically induced ischaemic stroke were compared with non-supplemented rats 1 week after surgical treatment. When specific regions of the hippocampus were compared histologically for the presence of damaged cells, tissues from blueberry-supplemented rats had significantly less damage in two of the three regions of the hippocampus.

The neuroscience studies summarized above suggest that blueberry consumption may protect the brain in a variety of ways. These observations prompt the question of what is distinctive about the phytochemical profile of blueberries that may account for these benefits. Clearly, blueberries contain a high concentration of anthocyanins compared with other fruit, and even many berries, such as strawberries and grapes. Whether anthocyanins are contributing significantly to these benefits remains to be elucidated, however. In this regard, it is interesting to note a study involving blueberries and longevity (Wilson et al., 2006). The lifespan of a soil nematode (Caenorhabditis elegans) is approximately 1 month and is therefore a convenient in vivo model for longevity. Including blueberries in the diet of these nematodes extended their lifespan by more than 25%. However, when individual blueberry components were fed, it was found that PACs, and not anthocyanins, were responsible for these effects. The authors also showed that PACs contributed not only to lifespan extension, but also to thermotolerance in these organisms.

4.3.2 Blueberries may benefit vision

Bilberry (V. myrtillus L.) anthocyanins have a long history in folk medicine for their purported benefits in night vision. European research conducted mainly between the 1960s and 1980s examined various aspects of anthocyanins in vision physiology both in vitro and in vivo. One suggestion, which has been supported recently (Matsumoto et al., 2003), is that anthocyanins increase the rate of rhodopsin regeneration. Interestingly, cyanidin, but not delphinidin, glycosides were found to be effective. Rhodopsin is the photoreceptor primarily responsible for vision in low light and darkness. Most recently, molecular modelling studies have been employed to understand the mechanistic basis for the rhodopsin interaction and if and how anthocyanins may be involved (Tirupula et al., 2009).

Clinical benefits to vision arising from the consumption of Vaccinium berries with a high anthocyanin concentration (i.e. blueberry species) were reviewed in 2004 by Canter and Ernst (2004). Their meta-analysis of placebo-controlled trials of V. myrtillus (i.e. bilberry) effects on night vision concluded that rigorous trials did not support a beneficial role for bilberry in night vision. Of 12 placebo-controlled trials, four trials that used a randomized...
controlled (RC) design had a negative outcome. A fifth RC trial and seven non-RC trials reported positive effects of bilberry on outcome measures relevant to night vision. Negative outcomes in these trials were associated with more rigorous methodologies and with lower anthocyanin dosage levels. Factors such as the age of subjects and the duration of intervention should also be considered in such trials, since human night vision begins to decline during middle age. Also, the time dependency of the possible effect(s) of anthocyanins on vision parameters is not known.

4.3.3 Cranberries support urinary tract health

The effect of cranberries in maintaining urinary tract health is arguably the most widely recognized and thoroughly studied human health benefit of any fruit. While the benefit of cranberries in urinary tract health has been recognized for over 100 years, conclusive clinical evidence, and the characterization of the effects and their mechanistic basis, is only now becoming clear.

It was thought originally that the high acid concentration of cranberries was sufficient to acidify urine and confer a bacteriostatic effect; however, such a low pH is achieved rarely with cranberry consumption. Instead, research results from the past 20 years favour an antibacterial adhesion mechanism as the basis for their effect. Numerous in vitro and ex vivo studies support the notion that specific cranberry components possess an antiadhesion property that can reduce or prevent the adhesion of uropathogenic bacteria to the wall of the bladder and urinary tract, which is otherwise essential to the initiation and progression of a urinary tract infection (UTI) (Howell, 2002).

Escherichia coli is the predominant bacterium involved in UTI. Interaction of the proteinaceous fimbriae on the surface of specific E. coli with receptor types on the mucosal surface of uroepithelium is the mechanism by which these bacteria adhere, multiply and colonize the urinary tract (Howell, 2002). Most uropathogenic E. coli express type-1 fimbriae. However, E. coli possessing the P-type fimbriae, which are correlated with cystitis (bacteria in the bladder) and pyelonephritis (infection of the kidneys), appear to be affected specifically by cranberry components. Based on recent in vitro studies, cranberry components appear to induce conformational changes in P-type fimbriae, including a reduction in their length and density (Liu et al., 2006). Numerous lines of evidence suggest that cranberry phenolics, specifically their PACs possessing A-linkages, are most important in affecting the action of P-fimbriated E. coli in UTIs.

The impact of UTI in society is significant. It is estimated that in the USA 11 million women are affected annually and that 25% of these women suffer from recurring UTI (Howell, 2007). While the typical treatment is to eliminate uropathogenic bacteria using antibiotic therapy, there is growing concern that the widespread use of antibiotics is contributing to the development of antibiotic-resistant bacteria. Cranberries may provide an alternative means to reduce the risk of recurring infections, as well as to treat infections by a non-bacteriostatic means. Therefore, the clinical benefit of cranberry consumption for various types of UTI is actively being investigated. Jepson and Craig (2007) have systematically reviewed clinical trials focused on this topic. Using rigorous criteria, which included only randomized controlled trials (RCT), Jepson and Craig (2008) used meta-analysis to examine selected types of RCT, participants, interventions and outcome measures. On the basis of these criteria, nine clinical trials were included in the meta-analysis and included the outcomes of just over 1000 participants, with interventions using cranberry juice, cocktail and capsules, tablets and a lingonberry–cranberry juice blend. The meta-analysis examined three groups that included women with recurring UTIs, an elderly population and a population requiring intermittent or continuous catheterization (e.g. spinal cord injury patients). This rigorous meta-analysis concluded that cranberry consumption could be beneficial in certain subpopulations of women (uropathogen-specific) with recurring UTIs. This outcome is supported by the notion that the cranberry A-linked PACs confer
Blueberry and Cranberry

61

protection only against the adhesion and further colonization of P-fimbriated *E. coli*.

The antibacterial adhesion mechanisms of cranberry components including A-linked PAC in urinary tract health, or cranberry nondialysable material in oral health (see below), constitute a novel and potentially powerful means to reduce risk and promote wellness through the consumption of an innocuous food product instead of a pharmaceutical product. A secondary and possibly even more significant beneficial outcome of the antiadhesion effects of cranberries is that their use may reduce the requirement for antibiotic therapy and mitigate the selection for survival of antibiotic-resistant bacteria.

### 4.3.4 Cranberries may benefit oral health

Dialysis (12,000–15,000 MW cut-off) of cranberry extracts against water gives rise to a fraction called simply non-dialysable material (NDM), which has various bioactivities that may promote oral health (Weiss et al., 1998). The development of dental caries and periodontal disease arises from the effects of dental plaque, which is a dynamic and complex matrix of biotic and abiotic components attached strongly to the teeth. Bacteria are also associated with epithelial cells of the mouth and the saliva. Most significant perhaps is the abundant diversity of proliferating microorganisms that adhere to each other to form biofilms via interspecific cell-to-cell interactions. Biofilms are being reformed constantly after their physical disruption; however, without ongoing intervention through dental hygiene, biofilms become mature stable entities that are resistant to removal and create microconditions for the development of dental caries and periodontal disease.

Various lines of *in vitro* evidence support the notion that cranberry NDM may benefit oral health. An early *in vitro* approach was to examine the extent of bacterial coaggregation, a critical event in the formation of biofilms, among numerous pairs of oral bacteria following preincubation with NDM (Weiss et al., 1998). Cranberry NDM prevented the coaggregation of bacterial pairs at concentrations as low as 0.04 mg/ml. When a non-dialysable fraction was prepared from various other fruit juices, none was found to prevent coaggregation of pairs of bacteria *in vitro*, with the exception of blueberry, which possessed only a weak activity (Bodet et al., 2008). Once coaggregated, bacterial pairs could be dissociated by an approximately five times greater concentration of NDM. Since *in vivo* NDM will encounter already formed bacterial biofilms, it is notable that NDM could also reverse the aggregation of some bacteria in biofilms. When NDM was tested at the concentration level that occurred in cranberry juice (2.5 mg/ml), a reversal of coaggregation occurred in more that 50% of the bacterial combinations examined, while 90% of the remaining bacterial pairs were reversed completely at a concentration four times greater.

When the effects of saliva and NDM were examined individually *in vitro*, neither saliva nor NDM led to coaggregation of selected bacterial species. However, in combination, saliva and NDM gave rise to significant bacterial aggregation. It was concluded from this result that NDM would improve the ability to remove bacteria from the mouth via the saliva (Weiss et al., 2002).

Hydroxyapatite has been used experimentally to simulate dental enamel, and saliva-coated hydroxyapatite beads were employed as an *in vitro* model to examine factors influencing oral biofilm formation. Yamanaka et al. (2004) tested bacterial adherence to these beads and found that cranberry juice inhibited the adherence of several important oral bacteria by up to 95%. It is important to note that, at the concentration employed, NDM did not appear to be affecting bacterial viability, but simply their coaggregation capacity (Duarte et al., 2006). Therefore, the floral profile may not shift to more resistant populations, which is considered a beneficial outcome.

An early stage in the development of dental biofilms is the formation of an underlying pellicle. The pellicle is attached firmly to the dental enamel, and is composed of cell-free and saliva components, including polysaccharides. Polysaccharin- and polyfructan-mediated binding of oral bacteria is considered an important mechanism in bacterial biofilm formation (Bodet et al., 2008). These polysaccharides use sucrose as a substrate
and are formed through the activity of glucosyl and fructosyl transferase. Cranberry NDM has been shown to inhibit the activity of these enzymes, but is less effective when they are immobilized on hydroxyapatite, since they then become less accessible to NDM. Cranberry juice reduced bacterial adhesion to glucan binding sites on the pellicle by 40–85% and reduced the final biofilm mass (Bodet et al., 2008). Since the formation of the polysaccharide layer by glucosyl and fructosyl transferase will be influenced by the availability of sugar substrates, NDM, and not cranberry cocktails that are rich in simple sugars, will be the more logical material from which to develop cranberry products to support oral health. Dental caries arise due to acid-mediated demineralization of tooth enamel. This occurs when cariogenic bacteria, particularly Streptococci mutans, form lactic acid, which occurs when the pH is less than about 5.5. Cranberry extract was found to increase the pH of biofilms and mitigated the formation of lactic acid, which might otherwise have demineralized dental enamel.

The composition of cranberry NDM is not well described. However, in their recent review, Bodet et al. (2008) compared cranberry NDM with cranberry PACs, which possess antiadhesin effects on uropathogenic P-fibriated E. coli. The NDM and PAC fractions differ in their solubility properties, as well as their NMR and MALDI-TOF spectra. While cranberry PACs possess an astringent taste, NDM does not. The mass of NDM and PACs in cranberry cocktail differs. Their concentration required to affect bioactivities in relation to oral pathogens and uropathogenic P-fibriated E. coli also differs. Cranberry NDM contains about 65% phenolic-like materials (based on colorimetric measurements) and is devoid of sugars or acids.

### 4.3.5 Evidence for cardioprotection by Vaccinium fruit

Neto (2007) has reviewed evidence recently for the ability of Vaccinium fruit to reduce the risk and mitigate the symptoms of cardiovascular disease. Also included in the review is an overview of the phytochemical composition of major commercial Vaccinium species, including blueberries and cranberries. A major risk factor in cardiovascular disease is the presence of atherosclerotic plaque in coronary arteries; plaque can restrict blood flow, contribute to hypertension and damage the heart. Development of atherosclerotic plaque involves the uptake of oxidatively modified low-density lipoprotein (LDL) into the lining of the vascular endothelium. Plant phenolics possess potent antioxidant properties. Therefore, provision of dietary phenolics, such as those in Vaccinium berries, has been considered an important mechanism to protect plasma LDL from oxidation. However, it should be considered that there are abundant endogenous antioxidants already present in the plasma. This factor, along with research reporting low plasma bioavailability of phenolics, has tempered current opinion on the potential importance of dietary phenolic antioxidants in the protection of LDL against oxidative modification. Other factors considered to be of importance to cardioprotection by Vaccinia are their antiplatelet effects, which reduce the propensity of blood to clot and create vascular occlusion. While antiplatelet effects by phenolics have been demonstrated in vitro (Keevil et al., 2000), they have not been shown for blueberries in vivo (Kalt et al., 2008a), owing perhaps to the low plasma bioavailability of the phenolics. It should be noted, however, that in a clinical study involving the consumption of progressively higher amounts of low-calorie cranberry juice, Ruel et al. (2008) reported a lower concentration of oxidized plasma LDL, and favourable effects on intercellular and vascular cellular adhesion molecules, which are involved in atherosclerotic plaque deposition.

The phenolics, stilbene and pterostilibene, which are found in blueberries at low concentrations (Rimando et al., 2004, 2005), have been reported to reduce LDL concentration significantly and improve the LDL/HDL ratio in vivo in hypercholesterolaemic hamsters (Rimando et al., 2004). The mechanism underlying this benefit was the induction of peroxisome proliferator activator receptor (PPARα). It is interesting to note that the drug, ciprofibrate, which works via PPARα, was deemed less effective in this respect than pterostilibene (Rimando et al., 2005).
Blueberry components can bind bile acids, resulting in reduced serum cholesterol. Indeed, freeze-dried blueberry was shown in vitro to bind almost half the amount of bile acids as the cholesterol-lowering drug, cholestyramine, which works via this mechanism (Kahlon and Smith, 2007). Binding of bile acids favours cholesterol excretion to affect plasma lipid balance beneficially and confer a cardioprotective benefit.

Blueberry feeding was shown recently to reduce total cholesterol and affect the LDL/HDL ratio of pigs favourably (Kalt et al., 2008a). Pigs are considered a good model for cardiovascular studies since several markers of disease, which vary among species (e.g. LDL metabolism, plaque deposition), are similar between pigs and humans. Also, pigs are omnivores and have similar body weight to humans. Plasma cholesterol reduction was dose-dependent among diets containing 0, 1 and 2% blueberries, and levelled off between 2 and 4%. The effects of blueberries on cholesterol lowering was greater when the pig’s basal diet was rich in other plant foods (70% soy, oats and barley) than when less (20%) plant foods made up the basal diet. Together, the results suggested that blueberries might affect plasma lipids beneficially when consumed in reasonable doses and as part of a diet rich in plant-based foods.

Ischaemic stroke, another adverse outcome of cardiovascular disease, arises from transient occlusion of blood vessel(s) in the brain. As reported in the discussion of the neuroprotective effects of Vaccinium fruit, long-term blueberry feeding mitigated damage to specific regions of the hippocampus in a rat model of ischaemic stroke (Sweeney et al., 2002). Results supporting a beneficial role for blueberries in reducing ischaemic stroke damage were also later reported by Wang et al. (2005).

4.3.6 Evidence for cancer chemoprevention

Neto (2007) has recently reviewed the literature on Vaccinium fruit in cancer chemoprevention. In this review, the anticancer bioactivity of Vaccinium (mainly blueberries and cranberries) phytochemicals (mainly phenolics) is illustrated in a wide range of in vitro cancer cell models. Such in vitro models can provide valuable mechanistic information regarding the initiation and progression of cancerous cellular metabolism. To date, anticancer mechanisms suggested for Vaccinium phytochemicals include: (i) induction of enzymes involved in the detoxification of carcinogens (e.g. quinone reductase); (ii) promotion of programmed cell death (i.e. apoptosis); and (iii) inhibition of enzymes involved in metastasis (i.e. matrix metalloproteinases). The effect of Vaccinium phytochemicals on apoptosis is the most widely cited.

While in vitro studies, whether related to cancer or other diseases, can provide valuable information regarding possible mechanisms of action, significant questions remain regarding the ability of specific phytochemicals to provide protection in vivo. Indeed, in order to be considered beneficial even in vitro, effects must be specific to the cells that manifest the disease (i.e. cancer) instead of affecting both diseased and normal cells. For in vitro effects to translate into significant human health benefits, their effects must be demonstrable in vivo. Implicit in the demonstration of in vivo effects is that putative beneficial phytochemicals are sufficiently bioavailable and effective even after their metabolism by the body. Since it is often difficult to purify sufficient quantities of specific target compounds to conduct long-term in vivo studies, beneficial effects are demonstrated most often after feeding these compounds in a complex food source.

The low in vivo bioavailability of phenolics may limit their potential benefit in tissues, with one possible exception. Since the concentration of phenolics remains high in the gastrointestinal tract, these compounds (and their gut microfloral catabolites) may protect gastrointestinal tissues against dietary prooxidant and carcinogenic molecules (e.g. nitrosamines) (Halliwell, 2007).

4.4 Effect of Preharvest and Postharvest Continuum

Many factors can affect the composition of blueberry and cranberry fruit and their products, which in turn may impact their potential
health benefits. While the mechanisms by which these fruit affect human health and well-being continue to be pursued, the effects of pre- and postharvest factors on their health benefits have not been determined. However, research has been conducted to determine the effects of these factors on the chemical composition and properties of the fruit. Therefore, we will discuss factors that alter the phytochemical profile and properties that have been implicated to contribute to the biological effects of these fruit.

4.4.1 Cultivar

Genotype has a large effect on determining the chemical composition of blueberry and cranberry fruit, including the content of phenolic and other health-promoting compounds. Numerous studies have demonstrated a wide variation in the total phenolic content and the antioxidant capacity of different blueberry genotypes (Ehlenfeldt and Prior, 2001; Kalt et al., 2001; Connor et al., 2002b; Howard et al., 2003). In a survey of 80 highbush cultivars and 155 lowbush clones, chemical composition varied, especially for the lowbush clones, where total anthocyanins, total phenolics and antioxidant capacity varied 5-, 1.6- and 3.3-fold, respectively (Kalt et al., 2001). In a different survey of 87 highbush and species-introgressed highbush blueberry cultivars, similar variation in anthocyanins, phenolics and antioxidant capacity was reported (Ehlenfeldt and Prior, 2001). Extremes in anthocyanin and total phenolic concentration and ORAC antioxidant capacity varied 3-, 10- and 6.8-fold, respectively (Kalt et al., 2001). In a study with 52 genotypes, Connor et al. (2002b) found that fruit from genotypes with ancestry from V. myrtillus and V. constablæi × V. ashei had the highest antioxidant capacity, while those from V. corymbosum and V. angustifolium had similar but lower capacities.

In addition to total phenolic concentration and antioxidant capacity, blueberry genotypes can vary substantially in their phenolic composition. Mi et al. (2004) compared the flavonoid glycoside content of five blueberry genotypes comprised of a northern and a southern highbush cultivar and three advanced interspecies hybrid selections. Total anthocyanin concentration ranged from 1.4 to 8.2 mg/g FW, but the relative distribution of anthocyanidins among the genotypes was similar. The blueberry cv. Bluecrop and the selection A-98 were the only genotypes that had a significant concentration of acylated anthocyanins, and Bluecrop had about half the concentration of anthocyanin galactosides and 7- to 13-fold more glucosides than the other genotypes. The predominant flavonol glycoside also varied among genotypes, being quercetin 3-galactoside in the three advanced selections, quercetin 3-glucoside + rutinoside in the blueberry cv. Bluecrop and quercetin 3-acetylhamnoside in blueberry cv. Ozark-blue. In a comparison of three rabbiteye and three highbush blueberry cultivars, total anthocyanin concentration was greater in the rabbiteye cultivars, ranging from 10.1 to 13.7 compared with 5.8 to 9.6 g cyanidin 3-glucoside equivalents/kg DW for the highbush cultivars (Lohachoompol et al., 2008). Anthocyanin concentration among these cultivars was similar, but proportions of each compound were cultivar dependent. Concentrations of chlorogenic acid also varied among 38 blueberry cultivars, ranging from 0.24 to 1.11 mg/g FW (Giongo et al., 2006).

The heritability of anthocyanin and phenolic content as well as antioxidant capacity in blueberry fruit has been assessed to determine the feasibility of breeding new cultivars that produce fruit with enhanced healthful properties. In a preliminary study, Ehlenfeldt and Prior (2001) suggested that inheritance of the antioxidant capacity of fruit may be additive, based on the evaluation of 11 highbush blueberry cultivar pedigrees. However, data from the Rubel × Duke family suggested that antioxidant content was controlled by epistatic gene action in the blueberry cv. Rubel, which was broken up when it was used as a parent. This might explain why the high antioxidant capacity of the blueberry cv. Rubel was not transferred effectively to progeny. In a heritability study using 20 crosses of V. corymbosum, V. angustifolium and hybrids between these species, Connor et al. (2002c) found narrow-sense heritability estimates of 0.43, 0.46 and 0.56 for antioxidant capacity, total phenolics and total anthocyanins, respectively.
The authors suggested that these moderate values indicated that reasonable progress could be made to improve these traits through breeding.

Anthocyanin content of cranberry fruit also varies among cultivars. The average total anthocyanin concentration of dark red fruit from six cultivars ranged from 0.30 to 0.63 mg/g FW, with smaller fruit having higher concentrations than larger fruit (Sapers et al., 1986a). Similarly, when the total anthocyanins of 12 cultivars were measured over five seasons, concentrations ranged from 0.43 to 0.95 mg/g FW (Schmid, 1977). However, the anthocyanin content within a cultivar can be highly variable among individual fruit, with some having up to fourfold greater anthocyanin concentrations than the mean (Sapers et al., 1986b).

Flavonol composition of cranberry fruit has also been shown to differ among cultivars. The concentration of quercetin and myricetin in six cranberry cultivars ranged from 0.07 to 0.25 and 0.004 to 0.027 mg/g FW, respectively (Bilyk and Sapers, 1986). Within a cultivar, the concentration of each of these flavonols was greater in dark red fruit than in medium red fruit. Kaempferol was detected in only three of the six cultivars at concentrations ranging from 0.001 to 0.003 mg/g FW.

### 4.4.2 Other preharvest factors

While genetics play a major role in fruit phenolic composition, variation within cultivars is also influenced by the growing environment. Flavonoid biosynthesis can be enhanced by a variety of environmental conditions including light, UV-radiation, water stress, temperature, ozone and pathogen infection (Treutter, 2005). When fruit were harvested over 2 years from 16 highbush and interspecific hybrid blueberry cultivars grown in three locations (Minnesota, Michigan and Oregon), their phenolic and anthocyanin content and antioxidant capacity varied significantly due to both year and location (Connor et al., 2002d). Differences in total phenolic and total anthocyanin content among fruit of the same cultivar harvested from different locations and years ranged from 1.1- to 2.0-fold and 1.2- to 2.0-fold, respectively. Similarly, variation in antioxidant capacity was observed to range from 1.2- to 2.6-fold. Total anthocyanin and hydroxycinnamic acid concentration and antioxidant capacity of southern highbush blueberry fruit also varied significantly between growing seasons (Howard et al., 2003).

A variety of cultural factors may influence fruit composition. Blueberry fruit produced using organic cultural practices had higher phenolic content than fruit produced using conventional culture, which might have been a result of higher levels of stress arising as a result of the organic cultural methods (Wang, S.Y. et al., 2008). The total phenolics, total anthocyanins and antioxidant capacity of organic-grown blueberries cv. Bluecrop from five commercial farms averaged 1.7-, 1.6- and 1.5-fold higher, respectively, than fruit produced conventionally. Treatment of rabbiteye blueberry fruit with ethephon, an ethylene-releasing compound, stimulated anthocyanin formation 1.9- and 2.2-fold 4 and 8 days after application, respectively (Ban et al., 2007). Treatment of lowbush blueberries cv. Fundy with methyl jasmonate 3 weeks prior to harvest caused a slight increase in total phenolic and anthocyanin concentration by reducing fruit size, but treatments with ReTain®, riboflavin or abscisic acid (ABA) had no effect (Percival and MacKenzie, 2007). Light exposure of cranberry fruit stimulates anthocyanin synthesis both on and off the plant. On the plant, red light was shown to be most effective in stimulating anthocyanin production when compared with white, far-red, green or UV light (Zhou and Singh, 2002). Stimulation of the production of specific anthocyanins was also affected by light quality. Red and far-red light was more effective in stimulating production of cyanidin 3-glucoside than natural sunlight, but less effective in stimulating the production of cyanidin 3-galactoside, cyanidin 3-arabinoside, peonidin 3-galactoside and peonidin 3-glucoside (Zhou and Singh, 2004).

Ripeness affects the phenolic composition of Vaccinium fruit significantly, particularly when anthocyanin concentration increases dramatically as berry surface colour changes from green to red and blue. In highbush blueberry fruit, total phenolics, hydroxycinnamic acids and flavonols all decreased as fruit
ripened from green to blue, while anthocyanins increased during blue colour formation (Kalt et al., 2003; Castrejón et al., 2008). The antioxidant capacity of the fruit decreased as the fruit matured and ripened but, depending on cultivar, it could also increase slightly as ripe fruit continued to accumulate anthocyanins (Castrejón et al., 2008). Among different ripeness stages of lowbush blueberries, total anthocyanin concentration increased from 0 to about 11 mg/g DW in fully ripe fruit, while levels of chlorogenic acid did not differ among slightly unripe, ripe and overripe fruit (Kalt and McDonald, 1996). Late harvested rabbiteye blueberry fruit had greater anthocyanin and total phenolic content than fruit harvested during a normal commercial harvest (Prior et al., 1998). Similar changes in fruit phenolic content occurred during ripening of cranberry fruit. In cranberry fruit cvs. Ben Lear and Stevens, anthocyanins increased dramatically as fruit ripened, while flavonols and PACs tended to decline during fruit growth but then remained constant or increased during ripening (Vvedenskaya et al., 2004). Ascorbic acid declined during cranberry cv. Pilgram fruit ripening, going from 2.1 to 0.7 mg/ml in green and dark red fruit, respectively (Çelik et al., 2008).

### 4.4.3 Postharvest handling and storage

When ripe blueberry fruit are stored, changes in anthocyanins, phenolics and antioxidant capacity are minimal. Total phenolic and anthocyanin concentration and antioxidant capacity of fruit representing eight cultivars of highbush blueberries did not change significantly during storage at 5°C for up to 7 weeks, with the exception of blueberry cv. MSU-58, which had a 29% increase in antioxidant capacity (Connor et al., 2002a). Storing fully ripe fruit of the highbush blueberry cv. Pilgram fruit ripening, going from 2.1 to 0.7 mg/ml in green and dark red fruit, respectively (Çelik et al., 2008).

Controlled atmosphere storage, comprised of O₂ and CO₂ concentrations ranging from 1 to 15 and 0 to 15 kPa, respectively, of three cultivars of highbush blueberries at 0°C had no beneficial effects on preserving phenolics, anthocyanins, or antioxidant capacity of the fruit (Forney et al., 2008). After 9 weeks of storage, total phenolics, total anthocyanins and antioxidant capacity decreased 5–16%, 8–18% and 6–14%, respectively, depending on cultivar, but this decline was not affected by storage atmosphere composition.

Other postharvest treatments of blueberry fruit have had variable effects on fruit composition. Irradiation of blueberries with 2 or 4 kJ/m² UV-C irradiation increased the anthocyanin content of fruit of the blueberry cv. Bluecrop by 10% following storage for 7 days at 5°C plus 2 days at 20°C (Perkins-Veazie et al., 2008). In addition, 4 kJ/m² UV-C irradiation increased the antioxidant capacity of Bluecrop fruit. However 2 kJ/m² UV-C radiation reduced total anthocyanin and phenolic concentration and antioxidant capacity in blueberry cv. Collins fruit following storage. Treatment with any of three naturally occurring essential oils, carvacrol, anethole and perillaldehyde, increased total anthocyanin and total phenolic concentration of blueberry cv. Duke fruit about 1.1- to 1.2-fold and antioxidant capacity 1.4- to 1.6-fold (Wang, C.Y. et al., 2008).
Changes in the phenolic composition of cranberry fruit appear to be greater than those of blueberry fruit during storage. Wang and Stretch (2001) reported that total anthocyanin and total phenolic content as well as antioxidant capacity increased during 3 months of storage at temperatures ranging from 0 to 20°C in ten cultivars. Increases were greatest at 15°C, where total anthocyanins, total phenolics and antioxidant capacity increased by an average of 2.3-, 1.5- and 1.5-fold, respectively. However, storing cranberry fruit in controlled atmospheres of 0–70 kPa O₂ with 0–30 kPa CO₂ at 3°C had no effect on total phenolic or total flavonoid content and inhibited the increase in antioxidant capacity that occurred in air-stored fruit (Gunes et al., 2002). Postharvest treatment with ethylene and exposure to light increased anthocyanin content (Craker, 1971). Postharvest light exposure also increased the total phenolic content and antioxidant capacity of fruit, but ABA treatments had no effect (Forney et al., 2009).

4.4.4 Processing

Processing can reduce the phenolic content and antioxidant capacity of blueberry and cranberry fruit. Maceration, heat and various processes can cause oxidation, thermal degradation, leaching and other events that can reduce the fruit’s healthful properties (Kalt, 2005). In a survey of processed blueberry products, the greater the processing the lower the antioxidant capacity, with fresh and frozen fruit having the highest levels and products subjected to extensive heat or drying having the lowest (Kalt et al., 2000). When compared with fresh or frozen fruit, drying blueberries reduced their anthocyanin concentration 41%, which was reduced further by 49% if preceded by osmotic dehydration (Lohachoompol et al., 2004). Processing results in a loss of monomeric anthocyanins due to their enzymatic polymerization or degradation, which continues during storage (Brownmiller et al., 2008). Heating and storage conditions reduced phenolic concentration and biological activity, measured as inhibition of cancer cell proliferation, in blueberry extracts stored in glass bottles following several months of storage (Srivastava et al., 2007). In canned products, significant leakage of anthocyanins can occur from the fruit into the liquid canning medium.

Approaches to reduce the impact of processing on degradation of fruit composition have been identified. More gentle processing methods such as freeze-drying and hot air-drying/microwave vacuum-drying retained higher concentrations of phenolics and anthocyanins than hot air-drying or microwave vacuum-drying alone (Mejia-Meza et al., 2008). Blanching frozen fruit at 95°C for 2 min or adding 50 µl/l SO₂ increased the yield of anthocyanins in extracted blueberry juice (Lee et al., 2002). Low pH and exclusion of oxygen also helped to preserve anthocyanins, phenolics and antioxidant capacity and prevent brown colour formation in blueberry juice due to polymeric phenolics (Kalt et al., 2000).

Cranberry composition may also be altered during processing. Cranberry juice had primarily monomer, dimer and A-type trimer PACs and lacked the higher oligomers observed in whole fruit (Prior et al., 2001). In addition, cranberry fruit processed into juice or powder contains the flavonol aglycones, myricetin and quercetin, as well as quercetin-3-O-(6"-benzoyl)-β-galactoside, which were not found in whole fruit (Vvedenskaya et al., 2004). In a study comparing the stability of phenolics and antioxidant capacity of six fruit juices, including blueberry and cranberry, cranberry juice was found to be the most stable, having the least loss of antioxidant capacity after 29 days of storage (Piljac-Zegarac et al., 2009).

4.5 Conclusions

Evidence continues to accumulate to support a role for both blueberries and cranberries in human health. Based on the nature and strength of the evidence described above, one can conclude that blueberries are most notable for their ability to protect the brain during ageing and under the stresses imposed by neurodegenerative disease. Cranberries are most noted for their beneficial effects in urinary
tract health and, due to related properties, may also confer benefits in oral health. Both blueberries and cranberries are generally considered to protect cardiovascular health, and have been found to provide benefits in various models of cancer.

The preponderance of evidence suggests that the phenolic compounds, and particularly the flavonoids, are the principal bioactive components in these berries. *Vaccinium* berries, compared with other fruit and even other berries, have a high concentration of phenolics. Indeed, the high concentration of specific flavonoids is apparent in both these berries. The deep blue coloration of blueberries is due to their high concentration of anthocyanins, and the astringency of cranberries is due to their high concentration of PACs. Since all phenolics possess potent antioxidant properties, both cranberries and blueberries are notable for their high antioxidant capacity compared with other plant foods. Typically, the concentration of total phenolics correlates very well with antioxidant capacity. Although oxidative stress plays a significant role as an underlying element in the degenerative processes of ageing and disease, a decade of research has shown that dietary phenolics per se do not contribute significantly to the antioxidant defences of the body. The reason for their limited role as biological antioxidants is that they are poorly absorbed by the body, and therefore do not bolster the already abundant antioxidant defence machinery present. This finding is an excellent example of how *in vitro* results cannot be interpreted as strong evidence for *in vivo* benefits. In spite of this new knowledge, research continues to demonstrate that *Vaccinium* fruit do provide benefits *in vivo*; however, not likely due to their antioxidant effects. The property of flavonoids as anti-inflammatory agents is highly significant and we can expect that this functionality will continue to be explored in various models of disease and ageing.

Finally, biomedical research is also showing that the specific structure of flavonoids influences their health functionality strongly, whether it is their ability to be absorbed by the body, or their activity in a particular physiological condition. This structure/function specificity creates many exciting opportunities for the fields of horticulture, food chemistry and engineering as specific phytochemical targets are revealed through biomedical research.

Concentrations of the phytochemicals in blueberry and cranberry fruit that have been associated with health benefits can be affected by numerous factors. Genetic manipulation of fruit, particularly through the development of new cultivars, holds the greatest potential for enhancement of specific phytochemicals of interest. Other environmental factors that are important during production or postharvest handling of the fruit can influence the concentration of these phytochemicals and could be used to enhance further the gains obtained through genetics. Processing of fruit can degrade phytochemical content and therefore innovative new methods are needed to preserve fruit health functionality in processed products. As specific modes of action are identified, more directed methods to enhance and preserve their properties can be developed.

References


5 Brassicas

Peter Glen Walley and Vicky Buchanan–Wollaston

5.1 Introduction

Brassica vegetables have received a great deal of attention in the past 5–10 years as consumers have developed an increased awareness of the nutritional content of foodstuffs in their diet, coupled with government-led strategies to promote the consumption of at least five fruit or vegetable portions per day as part of a healthy diet in order to derive the maximum benefit from the anticarcinogenic properties, for example, that their diet can provide.

Brassica is a genus within the family Brassicaceae (Cruciferae) belonging to the order Brassicales (Hall et al., 2002; Al Shehbaz et al., 2006; Warwick et al., 2006). The Brassicaceae contains more than 3000 species in 370 genera. Several domesticated species in the genus Brassica comprise the crops related most closely to Arabidopsis thaliana (mouse-ear or thale cress), which can thus function as model eudicot crops for comparative genomics (King, 2006; Paterson, 2006). Relationships among the genomes of the Brassica spp. were first delineated by U (1935). In this work, U outlined relationships between the three main Brassica diploid crop species and their amphidiploid species (Fig. 5.1). The underlying taxonomy of the Brassicaceae is complex, with species- and genus level classification often open to question (Spooner et al., 2003). Indeed, it has been found that Raphanus sativus (radish), although being classified as a different genus, can be crossed, although with difficulty, with B. oleracea (Karpechenko, 1927), B. rapa (Lange et al., 1989) and, with great difficulty, B. napus (Metz, 1995) (see Fig. 5.1). Lysak et al. (2005) suggest that R. sativus is related more closely to B. oleracea than is B. nigra – an observation that has been both supported and disputed (see Flannery et al., 2006; Koch et al., 2007).

5.1.1 Overview of the crop and harvested products

The growing season for broccoli in the UK is between early June to late October. Traditionally, other Brassica such as Brussels sprouts are supplied during November to April; however, increasingly the UK supply of fresh broccoli and other Brassica is supplemented by imports, for example from Spain and Portugal, throughout the year. Environmental and genetic variation in products from internal and external suppliers has a considerable effect on the shelf-life quality of the products (Jeffery et al., 2003). In terms of quantity produced worldwide, cabbage and other Brassica exceed over 69.2 million tones (Mt), with the UK producing over 250 kt of this value (FAOSTAT, 2008).
The majority of Brassica vegetables are purchased and consumed as fresh vegetables, mostly following cooking. The time and methods of cooking have a considerable influence on nutrient availability and content (see below). There is also considerable consumption of raw or pickled cabbage in products such as coleslaw and sauerkraut. Also, the Brassica components of prepared salad bags (e.g. mizuna chard, kale, mustard and turnip greens, and Asian Brassica such as red Chinese mustard, tat soi and napa cabbage) are also eaten raw (see Chapter 10 of this volume). In addition, there is a market for frozen Brassica vegetables, in particular broccoli, Brussels sprouts and cauliflower.

5.2 Phytochemical Composition

5.2.1 Health-promoting compounds in Brassica

Epidemiological studies have shown that increased consumption of vegetables and fruit is associated with a lower risk of degenerative diseases such as cancer, cardiovascular disease, cataracts and brain and immune dysfunction (Block et al., 1992; Hu, 2003). Brassica vegetables have been identified as important components of a healthy diet because of their high levels of constituents that may have a beneficial health-promoting role (Van Poppel et al., 1999; Lampe and...
P.G. Walley and V. Buchanan-Wollaston

Peterson, 2002; Finley, 2003b; Jeffery and Araya, 2009). These vegetables are also known to be beneficial in the prevention of other major illnesses such as Alzheimer’s disease, cataracts and some of the functional declines associated with ageing (Verhoeven et al., 1997). The main health-providing properties identified in Brassica are dietary flavonoids, essential vitamins and minerals and glucosinolates (and their breakdown products) (Heber, 2004a,b; Moreno et al., 2006).

Brassica vegetables are an excellent source of a variety of vitamins, minerals and dietary fibre (Table 5.1). Vitamin and provitamin antioxidants such as ascorbic acid (vitamin C), tocopherols (vitamin E) and carotenoids are compounds present at high levels in the vegetable Brassica and are likely to contribute to the beneficial effects of these vegetables in the diet (Kurilich et al., 1999; Jeffery and Araya, 2009). In addition, Brassica vegetables provide significant levels of vitamin A, B12 (riboflavin), B6 (pyridoxine), K and folic acid (McKillop et al., 2002). Folic acid is particularly important during pregnancy. Folate supplementation prior to conception can reduce the incidence of neural tube defects significantly (Bailey and Gregory, 1999). Folate deficiencies have also been implicated in the aetiology of megaloblastic anaemia, Spina bifida, neuropsychiatric disorders and various forms of cancer.

Important minerals supplied by Brassica vegetables include calcium, potassium, iron, zinc, magnesium and selenium (Farnham et al., 2000; De Pascale et al., 2005; Moreno et al., 2006; Bradley et al., 2008). The calcium content of certain Brassica vegetables, including broccoli, has good bioavailability, making it a good source of calcium for lactose-intolerant people (Heaney et al., 1993). Supplementation studies with high-selenium broccoli have demonstrated the efficacy of selenium for prevention of colon cancer (Finley et al., 2000). The metabolism of selenium depends on its chemical form, and that which occurs in broccoli appears to be particularly effective at protecting laboratory animals against cancer (Finley, 2003, 2003b).

Flavonoids and hydroxycinnamic acids are phenolic compounds, found in many vegetables, which have been implicated as having a role in reducing the risk of heart disease (Hertog et al., 1993; Knekt et al., 1996) and which may act as both antioxidants and anti-mutagenic agents. Brassica vegetables such as broccoli are a significant source of such compounds (Vinson et al., 1998; De Rijke et al., 2006) (Table 5.2). Flavonoids such as quercetin and kaempferol occur in relatively high concentration in some Brassica compared with other fruit and vegetables. Also, many Brassica contain other phenolics, such as flavonol glycosides and hydroxycinnamic acid esters. In a comparison between ten common vegetables, broccoli was found to have the highest levels of phenolic-derived antioxidant activity (Chu et al., 2002). The various Brassica species contain differing profiles of phenolic compounds (Heimler et al., 2006), which may be relevant to their health-conferring quality.

5.2.2 Produce type specific bioactives

The anticarcinogenic properties of Brassica vegetables have been attributed mainly to their relatively high content of glucosinolates and other sulfur-containing compounds (Verhoeven et al., 1997; Traka and Mithen, 2009). The glucosinolate core structure is made up of a β-D-thioglucose group linked to a sulfonated aldoxime moiety and a variable side-chain-derived amino acid. Glucosinolates can be grouped into three different classes: aliphatic, from aliphatic amino acids (methionine, alanine, valine, leucine, isoleucine); aromatic (tyrosine, phenylalanine); and indole (tryptophan). Structural diversity is achieved by amino acid side-chain modifications (elongations), followed by a host of specific secondary modifications to the glucosinolate side-chain (thiol oxidation, desaturation, esterification, hydroxylation) and/or the glucose moiety (Rosa, 1999; Mithen, 2001; Bones and Rossiter, 2006).

The most important glucosinolates in vegetable Brassica are the methionine-derived glucosinolates (Table 5.2; see Halkier and Gershenson, 2006). Glucosinolates are hydrolysed to unstable aglucones, which rearrange into biologically active compounds, typically isothiocyanates and indoles, catalysed by myrosinase, an enzyme
Table 5.1. Nutrients in *Brassica* vegetables (per 100 g) (from USDA nutrition data, http://www.nutritiondata.com).

<table>
<thead>
<tr>
<th>Produce and form tested</th>
<th>Kale</th>
<th>Kale</th>
<th>Kale</th>
<th>Kale</th>
<th>Broccoli</th>
<th>Broccoli</th>
<th>Broccoli</th>
<th>Broccoli</th>
<th>Brussels sprouts</th>
<th>Brussels sprouts</th>
<th>Brussels sprouts</th>
<th>Brussels sprouts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>raw</td>
<td>boiled</td>
<td>raw</td>
<td>boiled</td>
<td>raw</td>
<td>boiled</td>
<td>raw</td>
<td>boiled</td>
<td>raw</td>
<td>boiled</td>
<td>raw</td>
<td>boiled</td>
</tr>
<tr>
<td>Calories</td>
<td>50</td>
<td>28</td>
<td>28</td>
<td>30</td>
<td>34</td>
<td>29</td>
<td>35</td>
<td>28</td>
<td>43</td>
<td>41</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2.6</td>
<td>3</td>
<td>3.3</td>
<td>3</td>
<td>3.8</td>
<td>3.8</td>
<td>2.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>3.3</td>
<td>2.7</td>
<td>1.9</td>
<td>2.8</td>
<td>2.8</td>
<td>3.1</td>
<td>2.4</td>
<td>3.1</td>
<td>3.4</td>
<td>3.8</td>
<td>2.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>15,376</td>
<td>6253</td>
<td>13,623</td>
<td>14,705</td>
<td>623</td>
<td>1138</td>
<td>1548</td>
<td>1118</td>
<td>754</td>
<td>617</td>
<td>775</td>
<td>926</td>
</tr>
<tr>
<td>Retinol activity (μg)</td>
<td>769</td>
<td>313</td>
<td>681</td>
<td>735</td>
<td>31</td>
<td>57</td>
<td>77</td>
<td>56</td>
<td>38</td>
<td>31</td>
<td>39</td>
<td>46</td>
</tr>
<tr>
<td>β-Carotene (μg)</td>
<td>9226</td>
<td>–</td>
<td>8174</td>
<td>8824</td>
<td>361</td>
<td>675</td>
<td>929</td>
<td>663</td>
<td>450</td>
<td>370</td>
<td>465</td>
<td>555</td>
</tr>
<tr>
<td>Lutein + zeaxanthin (μg)</td>
<td>39,551</td>
<td>–</td>
<td>18,248</td>
<td>19,698</td>
<td>1403</td>
<td>1525</td>
<td>1000</td>
<td>1498</td>
<td>1590</td>
<td>–</td>
<td>1290</td>
<td>1541</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>120</td>
<td>39.3</td>
<td>41</td>
<td>25.2</td>
<td>89.2</td>
<td>68.3</td>
<td>40.1</td>
<td>85</td>
<td>74.1</td>
<td>62</td>
<td>45.7</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.3</td>
<td>0.9</td>
<td>–</td>
<td>0.4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>(α-Tocopherol) (mg)</td>
<td>–</td>
<td>–</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>1.3</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Vitamin K (mg)</td>
<td>817</td>
<td>817</td>
<td>882</td>
<td>102</td>
<td>101</td>
<td>141</td>
<td>99.5</td>
<td>177</td>
<td>–</td>
<td>140</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>1</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>29</td>
<td>17</td>
<td>13</td>
<td>14</td>
<td>63</td>
<td>94</td>
<td>108</td>
<td>30</td>
<td>61</td>
<td>123</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>135</td>
<td>136</td>
<td>72</td>
<td>138</td>
<td>47</td>
<td>41</td>
<td>40</td>
<td>51</td>
<td>42</td>
<td>26</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>1.4</td>
<td>0.9</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>34</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>21</td>
<td>16</td>
<td>21</td>
<td>20</td>
<td>23</td>
<td>20</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>56</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>66</td>
<td>59</td>
<td>67</td>
<td>55</td>
<td>69</td>
<td>62</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>447</td>
<td>333</td>
<td>228</td>
<td>328</td>
<td>316</td>
<td>250</td>
<td>293</td>
<td>180</td>
<td>389</td>
<td>370</td>
<td>317</td>
<td></td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>43</td>
<td>15</td>
<td>23</td>
<td>15</td>
<td>33</td>
<td>17</td>
<td>41</td>
<td>24</td>
<td>25</td>
<td>10</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>0.3</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>2.5</td>
<td>1.9</td>
<td>1.6</td>
<td>1.6</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Water (g)</td>
<td>84.5</td>
<td>91.1</td>
<td>91.2</td>
<td>90.5</td>
<td>89.3</td>
<td>90.6</td>
<td>89.3</td>
<td>90.7</td>
<td>86</td>
<td>87.1</td>
<td>88.9</td>
<td>86.7</td>
</tr>
</tbody>
</table>
Table 5.2. The major glucosinolates occurring in the Brassicaceae (nomenclature adopted from Bjerg and Sorensen, 1987).

<table>
<thead>
<tr>
<th>Chemical grouping</th>
<th>Chemical name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic glucosinolates</td>
<td>2-Propenyl or allyl glucosinolate</td>
<td>Sinigrin</td>
</tr>
<tr>
<td></td>
<td>But-3-enyl glucosinolate</td>
<td>Gluconapin</td>
</tr>
<tr>
<td></td>
<td>Pent-4-enyl glucosinolate</td>
<td>Glucobrassicinapin</td>
</tr>
<tr>
<td></td>
<td>2-Hydroxybut-3-enyl glucosinolate</td>
<td>Progoitrin</td>
</tr>
<tr>
<td></td>
<td>2-Hydroxypent-4-enyl glucosinolate</td>
<td>Gluconapoleierin</td>
</tr>
<tr>
<td></td>
<td>3-Methylsulfinylpropyl glucosinolate</td>
<td>Glucoiberin</td>
</tr>
<tr>
<td></td>
<td>4-Methylsulfinylbutyl glucosinolate</td>
<td>Glucoraphanin</td>
</tr>
<tr>
<td>Aromatic glucosinolates</td>
<td>2-Phenethyl glucosinolate</td>
<td>Glucorasturtin</td>
</tr>
<tr>
<td>Indole glucosinolates</td>
<td>Indole-3-ylmethyl glucosinolate</td>
<td>Glucobrassicin</td>
</tr>
<tr>
<td></td>
<td>1-Methoxyindol-3-ylmethyl glucosinolate</td>
<td>Neoglucobrassicin</td>
</tr>
<tr>
<td></td>
<td>4-Hydroxyindol-3-ylmethyl glucosinolate</td>
<td>4-Hydroxyglucobrassicin</td>
</tr>
<tr>
<td></td>
<td>4-Methoxyindol-3-ylmethyl glucosinolate</td>
<td>4-Methoxyglucobrassicin</td>
</tr>
</tbody>
</table>

that is released from damaged plant cells (Mithen, 2001; Agerbirk et al., 2008). Postharvest physical disruption of the plants (e.g. chewing, cooking, freezing/thawing and high temperature) leads to loss of cellular compartmentalization and subsequent mixing of glucosinolates and myrosinase (usually confined to specialized myrosin cells) to form the biologically active compounds (Rosa et al., 1997). Hydrolysis products are governed by glucosinolate side-chain structure, the plant species, hydrolysis conditions, such as pH, and the presence of epithiospecifier protein (ESP) and thiocyanate-forming protein (TFP) (Bernardi et al., 2000; Foo et al., 2000; Burow and Wittstock, 2009).

The anticarcinogenic activity in broccoli is most likely due to activity of the isothiocyanates, iberin and sulforaphane, which are degradation products of, respectively, 3-methylsulfinylpropyl (glucoiberin) and 4-methylsulfinylbutyl (glucoraphanin) glucosinolates that accumulate in the florets of broccoli. Other members of B. oleracea contain differing concentrations of the cleavage products, 2-propenyl (sinigrin), 3-butenyl (gluconapin) and 2-hydroxy-3-butenyl (progoitrin) (Mithen et al., 2003; Halkier and Gershenzon, 2006).

Sulforaphane has been shown to be a powerful inhibitor of phase 1 and inducer of phase 2 enzymes in human and animal cell lines (Maheo et al., 1997; Juge et al., 2007). Important phase 1 enzymes are the cytochrome P450s, and phase 2 enzymes include glutathione-S-transferases (GSTs) and UDP-glucuronyl transferases. These enzymes act to metabolize and excrete potential carcinogens and this activity could explain the potential anticarcinogenic effects of these molecules. Other potential activities of such products include initiating apoptosis and inducing cell cycle arrest (Bonnensen et al., 1999; Sarikamis et al., 2006). Another sulfur-containing compound, S-methyl cysteine sulfoxide, and its breakdown product, methyl methane thiosulfinate, have been found to inhibit chemically induced genotoxicity in mice (Stoewsand, 1995).

The anticarcinogenic activities of Brassica vegetables are more effective with certain human genotypes. Individuals who have homozygous null mutations in the GST genes, GSTM1 and GSTT1, appear to gain less cancer protection from broccoli than those who can express the functional gene (Hayes and Strange, 2000; Joseph et al., 2004). Between 39 and 63% of the population have the homozygous null GSTM1 gene. While these people may gain less cancer protection from consuming broccoli, it is likely that they gain more cancer protection from eating...
other types of crucifers, such as cabbages and Chinese cabbage. The high content of different chemical compounds that may modulate carcinogenesis identify *Brassica* vegetables as very attractive subjects for chemopreventative studies. Most studies have been carried out with single, pure substances. By analysing the health-promoting effects of complex mixtures of compounds, as found in broccoli for example, the combined effects of various anticarcinogens may be elucidated (for example, Finley, 2003a, 2005; Jeffery and Araya, 2009). Combined effects are likely to be different from those observed using single substances and, in the case of cancer prevention, the combined effect is especially relevant since carcinogenesis is a multistage disease divided into several qualitative steps.

### 5.2.3 Flavour

The characteristic sulfurous and bitter taste of *Brassica* vegetables is assumed to be due mainly to the glucosinolate composition (Fenwick et al., 1983; Hansen et al., 1997; van Doorn et al., 1998). The glucosinolates sinigrin and progoitrin have been correlated to bitterness in Brussels sprouts (van Doorn et al., 1998), while a combination of neoglucobrassicin and sinigrin causes the bitter taste in cooked cauliflower (Engel et al., 2002). The lengths and structures of the aliphatic side-chains of the glucosinolate moieties determine their biological activity including flavour. Following tissue disruption, myrosinase acts to release isothiocyanates, the major volatile flavour components of cruciferous crops. The flavour of the crops is determined partially by the total amount of glucosinolates, the side-chain structures and the activity of the myrosinase. The presence of certain isothiocyanates results in varying degrees of bitterness, which make particular cultivars unacceptable to the consumer (Schonhof et al., 2004). Taste tests indicated that consumers preferred cultivars with low levels of bitter-tasting glucosinolates and higher sucrose content, and it was suggested that the bitterness of the beneficial glucosinolates could be masked by increasing sucrose contents (Schonhof et al., 2004). However, Baik et al. (2003) compared flavour profiles among 19 different broccoli cultivars and did not find a link between glucosinolate content and flavour. Interestingly, the major isothiocyanate cleavage compounds in broccoli, sulforaphane and iberin, are non-volatile; therefore, they do not contribute to the flavour. To add to this complexity, another investigation of 113 varieties of turnip greens concluded that other phytochemicals as well as glucosinolates and their breakdown products were probably involved in flavour attributes (Padilla et al., 2007).

### 5.2.4 Antinutrients

As well as having a potential beneficial health-promoting function, glucosinolates can be considered as having antinutrient activity in certain situations. This is clearly the case in oilseed rape varieties where the breakdown products may exhibit goitrogenic or antithyroid activity. The isothiocyanates give rise to the most actively goitrogenic compounds by being cyclized to form oxazolidone-2-thiones (Chubb, 1982). The most goitrogenic compound is 5-vinyl-oxazolidine-2-thione, commonly known as goitrin. The glucosinolate that gives rise to goitrin is 2-hydroxy-3-butenyl glucosinolate, or progoitrin (Chubb, 1982). This is the predominant glucosinolate in oilseed rape, representing between 50 and 70% of the total glucosinolate concentration (Zhao et al., 1994). However, the glucosinolate composition of vegetable *Brassicas*, apart from contributing to an unacceptable taste, does not appear to have an antinutrient effect. Levels of progoitrin are low in broccoli, for example (Table 5.3), and there is no evidence for any goitrogenic effect on humans from *Brassica* consumption (Mithen, 2001). The toxicity of isothiocyanates against insect pests such as the black vine weevil (Otiorhynchus sulcatus F.) has been demonstrated (Borek et al., 1988). These data suggest that *Brassica* spp. can be used in integrated pest management systems; see Hopkins et al. (2009).
Table 5.3. Glucosinolate content and standard deviation (mg/100 FM) of cauliflower and broccoli ($n = 9$). Redrawn from Schonhof et al. (2004).

<table>
<thead>
<tr>
<th>Glucosinolate (mg/100 g FM)</th>
<th>Broccoli cultivars</th>
<th>Cauliflower cultivars</th>
<th>HSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emperor</td>
<td>Shogun</td>
<td>Marathon</td>
</tr>
<tr>
<td>Glucoiberin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glucoiberin</td>
<td>0.27 ± 0.16</td>
<td>1.97 ± 1.97</td>
<td>2.65 ± 1.57</td>
</tr>
<tr>
<td>Glucoerucin</td>
<td>ND</td>
<td>0.75 ± 0.24</td>
<td>ND</td>
</tr>
<tr>
<td>Glucoraphanin</td>
<td>11.58 ± 10.62</td>
<td>33.95 ± 11.77</td>
<td>21.10 ± 14.33</td>
</tr>
<tr>
<td>Sum alkyl glucosinolates</td>
<td>11.58 ± 10.76</td>
<td>36.67 ± 12.39</td>
<td>23.75 ± 15.80</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>ND</td>
<td>0.88 ± 0.40</td>
<td>ND</td>
</tr>
<tr>
<td>Glucobrassicin</td>
<td>0.57 ± 0.23</td>
<td>10.28 ± 3.39</td>
<td>1.10 ± 0.47</td>
</tr>
<tr>
<td>Progoitrin</td>
<td>0.43 ± 0.18</td>
<td>0.53 ± 0.49</td>
<td>0.86 ± 0.28</td>
</tr>
<tr>
<td>Sum alkenyl glucosinolates</td>
<td>1.00 ± 0.36</td>
<td>12.70 ± 3.44</td>
<td>1.97 ± 0.66</td>
</tr>
<tr>
<td>Glucobrassicin</td>
<td>6.96 ± 6.14</td>
<td>7.63 ± 2.51</td>
<td>10.29 ± 4.79</td>
</tr>
<tr>
<td>Neoglucobrassicin</td>
<td>2.60 ± 3.69</td>
<td>6.35 ± 3.74</td>
<td>2.66 ± 1.63</td>
</tr>
<tr>
<td>4-Hydroxyglucobrassicin</td>
<td>0.06 ± 0.04</td>
<td>0.36 ± 0.48</td>
<td>0.29 ± 0.27</td>
</tr>
<tr>
<td>4-Methoxyglucobrassicin</td>
<td>0.43 ± 0.35</td>
<td>0.92 ± 0.38</td>
<td>1.32 ± 0.70</td>
</tr>
<tr>
<td>Sum indole glucosinolates</td>
<td>10.05 ± 10.02</td>
<td>15.26 ± 6.15</td>
<td>14.55 ± 6.70</td>
</tr>
</tbody>
</table>

Note: HSD = Tukey's honest significant difference; ND = not detectable.
5.3 Factors Affecting Composition

The chemical composition of *Brassica* vegetables is affected by a combination of genetics and both pre- and postharvest environmental conditions and treatments. For example, studies by Jeffery *et al.* (2003) found that the glucosinolate, vitamin and flavonoid contents of broccoli were affected by genotype and environment, and by processing (Fig. 5.2).

### 5.3.1 Genotype

Phytochemical profiles vary considerably between *Brassica* species and the levels of individual compounds vary between cultivars (Kurilich *et al.*, 1999; Kushad *et al.*, 1999; Jeffery *et al.*, 2003). An analysis of 50 different broccoli cultivars indicated that glucosinolate levels varied up to 20-fold, showing a considerable genotype-dependent effect. For example, the predominant glucosinolate in broccoli, glucoraphanin, showed levels ranging from 0.8 μmol/g DW in one broccoli cultivar to 21.7 μmol/g DW in another. Concentrations of the other glucosinolates in broccoli varied similarly over a wide range. In Brussels sprouts, cabbage, cauliflower and kale, the predominant glucosinolates measured were sinigrin (8.9, 7.8, 9.3 and 10.4 μmol/g DW, respectively) and glucobrassicin (3.2, 0.9, 1.3 and 1.2 μmol/g DW, respectively). Brussels sprouts also had significant amounts of gluconapin (6.9 μmol/g DW) (Kushad *et al.*, 1999). In another study, several cultivars of broccoli and cauliflower were assayed for glucosinolate composition and similar results were obtained, as shown in Table 5.3 (Schonhof *et al.*, 2004). These authors concluded that the composition of glucosinolates was determined genetically, but the growing conditions had an impact on the concentrations.

Genetic manipulation of glucosinolate levels has been suggested as a means to provide a better source for the consumption of anticarcinogenic compounds. A hybrid broccoli cultivar containing increased levels of glucoraphanin (the precursor of sulforaphane) has been generated by crossing a commercial broccoli line with a wild species of *Brassica* (*B. villosa*) (Sarikamis *et al.*, 2006). Following cooking, this line of broccoli contained around threefold higher levels of sulforaphane than commercial broccoli; also, when consumed as a soup, it has been shown to induce the expression of carcinogen-inactivating phase 2 enzymes (Gasper *et al.*, 2007). Further manipulation of the glucosinolate pathway in *Brassica* requires improved understanding of the mechanisms of biosynthesis. This is being addressed using *Arabidopsis* as a model system (Hall *et al.*, 2001; Kliebenstein, 2009).

Antioxidant levels (carotene, tocopherol and ascorbate) measured in different *Brassica* species and in 50 different broccoli cultivars indicated that there was considerable genotype-dependent variation (Kurilich *et al.*, 1999). Genotype influence on mineral composition (particularly Ca, Mg and S) was also identified in an analysis of 11 broccoli cultivars (Rosa *et al.*, 2002), and differences in levels of flavonoids and vitamin C were reported in an analysis of 14 commercial and experimental cultivars of broccoli (Vallejo *et al.*, 2002).

### 5.3.2 Agronomy

Growth conditions and time of harvesting are likely to have an effect on the chemical composition of *Brassicas*. Glucosinolate levels were assessed in a range of genotypes grown
in three different years, and there were significant environmental effects on levels of glucoraphanin (Farnham et al., 2004). However, genotype effects were greater than environmental effects. A comparison of spring versus summer planting of 11 different broccoli lines showed that total glucosinolate levels were higher in the late (summer) crop (Rosa and Rodrigues, 2001). A study of sulforaphane levels in broccoli grown in three different seasons showed concentrations between 36.7 and 74.5 mg/100 g (Howard et al., 1997); this difference was probably due to different average temperatures in the different seasons. Cooler and/or drier conditions might have resulted in higher glucosinolate production. This hypothesis was supported by another study in which glucosinolate content of cauliflower and broccoli was influenced strongly by daily temperature (Schonhof et al., 2004). Low mean temperature in one season resulted in considerably higher levels of glucosinolates than those obtained in two subsequent warmer seasons. However, another study (Ciska et al., 2000) reported that low rainfall and high temperature resulted in higher glucosinolate levels. The conclusions from all these reports is that more controlled experiments are needed to identify the environmental factors that have a role in controlling glucosinolate content; and a better understanding of the interaction between genotype and environment is also needed, since comparisons between different cultivars in different conditions are not appropriate.

Nutritional content is affected as soon as the crop is harvested. During storage and handling of these vegetables, many sequential changes take place, both in the appearance of the vegetable and also in the content of health-promoting components, many of which are likely to be degraded rapidly (reviewed in Jones et al., 2006).

5.3.3 Recommended storage regimes for Brassica vegetables

Low temperature is extremely important in achieving adequate shelf life in broccoli. A temperature of 0°C is required to optimize broccoli storage life. Heads stored at 5°C can have a storage life of up to 14 days, yet storage at 10°C reduces shelf life by one-third. Brussels sprouts are moderately perishable and can be stored for 3–5 weeks at temperatures near 0°C, but they are often left in the field during early winter. Brussels sprouts are often hydrocooled, but can be air-cooled as well. Although they have considerable wax on their leaves, they become flaccid due to water loss if high relative humidity is not maintained. This clearly shows that the most effective way to maintain shelf life, and consequently the desired levels of bioactives in Brassica vegetables, is by cooling rapidly following harvest and maintaining the product in cool conditions at high humidity. Storage of broccoli at 20°C resulted in 82% loss of glucoraphanin content after 5 days; at 5°C this loss was only 31% (Rodrigues and Rosa, 1999). Similar results were obtained by Howard et al. (1997), who reported approximately 50% loss in sulforaphane after 21 days at 4°C. However, in another cultivar, indole glucosinolates were found to increase after 10 days' storage at 10°C, yet total glucosinolate levels did not change significantly (Hansen et al., 1995). Table 5.4 shows the change in individual glucosinolates in four types of Brassica vegetables after 7 days' storage at 4°C (Song and Thornalley, 2007).

Postharvest storage in controlled atmospheres (CAs) aimed at reducing respiration is a well-tested methodology for many fruit and vegetable products, but is rarely now applied commercially to Brassicas. It appears that extending shelf life also has a positive effect on maintaining glucosinolate levels, but this has not been demonstrated clearly (Jones et al., 2006). The shelf life of broccoli can be improved using an atmosphere of 1–2 kPa O₂ with 5–10 kPa CO₂ at a temperature range of 0–5°C. CA has been shown to reduce ethylene-induced yellowing (chlorophyll breakdown) and reduced butt-end discoloration. Similar results are observed with modified atmosphere packaging (MAP), which is a common format for some Brassicas. Broccoli quality was optimum when packaging was used to maintain 1–2 kPa O₂ and 5–10 kPa CO₂ (Jacobsson et al., 2004), and levels of carotenoids and vitamin C were also retained by this treatment (Barth and Zhuang, 1996). Glucosinolate levels appeared to be more stable under MAP
Table 5.4. Baseline individual and total glucosinolate analyte contents of fresh vegetables (adopted from Song and Thornalley, 2007).

<table>
<thead>
<tr>
<th>Produce type</th>
<th>Broccoli</th>
<th>Brussels sprout</th>
<th>Cauliflower</th>
<th>Green cabbage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucosinolate (µmol/100 g fresh weight)</th>
<th>Broccoli</th>
<th>Brussels sprout</th>
<th>Cauliflower</th>
<th>Green cabbage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucoiberin</td>
<td>17.1</td>
<td>10.6</td>
<td>1.5</td>
<td>1.14</td>
</tr>
<tr>
<td>Glucoraphanin</td>
<td>29.4</td>
<td>21.3</td>
<td>0.55</td>
<td>0.25</td>
</tr>
<tr>
<td>Glucoalyssin</td>
<td>3.86</td>
<td>3.05</td>
<td>0.33</td>
<td>0.21</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>1.4</td>
<td>1</td>
<td>8.56</td>
<td>7.08</td>
</tr>
<tr>
<td>Gluconapin</td>
<td>2.87</td>
<td>2.71</td>
<td>2.77</td>
<td>2.34</td>
</tr>
<tr>
<td>Progoitrin</td>
<td>3.33</td>
<td>3.14</td>
<td>2.41</td>
<td>1.98</td>
</tr>
<tr>
<td>Gluconasturtiin</td>
<td>4.44</td>
<td>3.71</td>
<td>1.06</td>
<td>0.76</td>
</tr>
<tr>
<td>Total</td>
<td>62.4</td>
<td>45.5</td>
<td>17.2</td>
<td>13.8</td>
</tr>
</tbody>
</table>

| Loss in storage (%)                    | 27       | 20             | 11          | 14           |

Note: LOD = limit of detection.

treatment, especially at higher temperatures (Rangkadilok et al., 2002).

5.4 Processing

5.4.1 Effects of cooking

Most traditional *Brassica* vegetables such as cabbage, broccoli, Brussels sprouts, etc., are consumed following cooking and this process can be very detrimental to the nutrient quality of the product that is actually consumed. This said, without cooking many *Brassica* are near inedible. Table 5.1 shows the effects of cooking (boiling) on vitamin levels in several *Brassica* vegetables and it is clear that some vitamins, such as vitamin C, are reduced following cooking. Other components, such as vitamin A, appear to increase following cooking, possibly because they are easier to extract.

Measurement of total and individual flavonoid components in freshly harvested broccoli cooked in four different ways showed that there were large differences with the different cooking methods (Vallejo et al., 2003). Microwaving was the most detrimental method, with 97% loss of flavonoid content. Steaming was the optimum cooking method, showing a loss of only 11%. In another paper, the same authors compared the effects of the four cooking processes on the levels of vitamin C and glucosinolates in freshly harvested broccoli (Vallejo et al., 2002). This study also showed that microwaving was very detrimental, with 40% loss of vitamin C and 74% loss of glucosinolates, and, again, steaming was found to have minimal effects on the levels of these important phytonutrients. The problem with these pieces of work is that, although they provide useful information, they do not represent reality as most consumers eat broccoli that has been stored either at retail or in the home. Another study investigated the effects of cooking (steaming and boiling) on glucosinolate profiles in broccoli (Gliszczynska-Swiglo et al., 2006). Total glucosinolate levels were reduced around 50% by conventional boiling, but were increased following steaming (Table 5.5). Gliszczynska-Swiglo et al. (2006) also showed that levels of total polyphenols and several vitamins actually appeared to increase following steaming (Table 5.6). Increased levels of carotene and lutein following both types of cooking are likely due to increased
Table 5.5. Glucosinolate content (μmol/g dry weight) in fresh, steamed and water-cooked broccoli. Redrawn from Gliszczynska-Swiglo et al. (2006) with permission from Taylor and Francis Ltd (http://www.informaworld.com).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fresh broccoli</th>
<th>Steamed broccoli</th>
<th>Water-cooked broccoli</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucoiberin</td>
<td>1.43</td>
<td>1.58</td>
<td>0.81</td>
</tr>
<tr>
<td>Progoitrin</td>
<td>0.18</td>
<td>0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>Glucoraphanin</td>
<td>9.6</td>
<td>10.19</td>
<td>5.09</td>
</tr>
<tr>
<td>Napoleiferin</td>
<td>0.31</td>
<td>0.26</td>
<td>0.14</td>
</tr>
<tr>
<td>Glucoalyssin</td>
<td>0.07</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>Gluconapin</td>
<td>Traces</td>
<td>Traces</td>
<td>Traces</td>
</tr>
<tr>
<td>Glucoibervirin</td>
<td>Traces</td>
<td>0.05</td>
<td>Traces</td>
</tr>
<tr>
<td>Glucoerucin</td>
<td>Traces</td>
<td>Traces</td>
<td>Traces</td>
</tr>
<tr>
<td><strong>Aromatic indoles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluconasturtin</td>
<td>0.1</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>4-Hydroxyglucobrassicin</td>
<td>0.63</td>
<td>0.76</td>
<td>0.43</td>
</tr>
<tr>
<td>Glucobrassicin</td>
<td>1.76</td>
<td>2.78</td>
<td>0.93</td>
</tr>
<tr>
<td>4-Methoxyglucobrassicin</td>
<td>0.36</td>
<td>0.48</td>
<td>0.3</td>
</tr>
<tr>
<td>Neoglucobrassicin</td>
<td>1.6</td>
<td>2.21</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>16.04</td>
<td>18.79</td>
<td>8.58</td>
</tr>
</tbody>
</table>

Table 5.6. Distribution of compounds analysed in fresh and domestically processed broccoli (mg/100 g). Redrawn from Gliszczynska-Swiglo et al. (2006) with permission from Taylor and Francis Ltd (http://www.informaworld.com).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fresh</th>
<th>Steamed</th>
<th>Water-cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total polyphenols</strong></td>
<td>Dry weight 886.3</td>
<td>1409.1</td>
<td>775.8</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 109.9</td>
<td>167.3</td>
<td>89.2</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td>Dry weight 25.4</td>
<td>38.7</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 3.15</td>
<td>4.59</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Phenolic acids</strong></td>
<td>Dry weight 328.1</td>
<td>417.3</td>
<td>155.9</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 40.55</td>
<td>50.05</td>
<td>17.93</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
<td>Dry weight 681.2</td>
<td>652.6</td>
<td>524.8</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 84.5</td>
<td>77.7</td>
<td>60.3</td>
</tr>
<tr>
<td><strong>β-Carotene</strong></td>
<td>Dry weight 10.5</td>
<td>19.93</td>
<td>24.61</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 1.3</td>
<td>2.37</td>
<td>2.83</td>
</tr>
<tr>
<td><strong>Lutein</strong></td>
<td>Dry weight 6.47</td>
<td>26.6</td>
<td>38.8</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 0.8</td>
<td>3.16</td>
<td>4.46</td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
<td>Dry weight 0.798</td>
<td>0.999</td>
<td>1.397</td>
</tr>
<tr>
<td></td>
<td>Fresh weight 0.099</td>
<td>0.119</td>
<td>0.161</td>
</tr>
</tbody>
</table>
availability for extraction. The action of myrosinase on the dominant broccoli glucosinolate, glucoraphanin, results in the production of the isothiocyanate derivative, sulforaphane, which has anticarcinogenic activity. However, an alternative degradation pathway involving the ESP group of enzymes results in an inactive nitrile derivative of this compound (Mithen et al., 2003; Agerbirk et al., 2008). This is not a problem when broccoli is cooked, since cooking destroys the myrosinase and the enzymes present in the gut microflora degrade the glucosinolates to active compounds. Heat treatments that inactivate the more labile ESP but not the myrosinase were found to increase the levels of active sulforaphane in the sample (Matusheski et al., 2004).

5.4.2 Effects of freezing

Warmer weather induces faster crop growth in broccoli and consequently promotes greater horticultural maturity. Because broccoli has to be harvested at a physiologically immature stage, this can cause problems as higher temperatures also lead to reduced demand from consumers. Broccoli is usually frozen into florettes when product availability outstrips demand; however, freezing can have a negative affect on health-promoting compounds. Table 5.1 shows data on the levels of nutrients in cooked frozen broccoli and Brussels sprouts compared with cooked fresh vegetables. It is clear that some nutrients such as vitamin C are lost in the freezing process. However, there is no comparison with broccoli or Brussels sprout samples that have been stored for a few days before consumption.

Freezing broccoli is usually preceded by a blanching step to inactivate enzymes that cause product deterioration. Enzymes such as lipoxygenase, peroxidase and cystine lyase contribute to the development of ‘off’ aromas in stored vegetables. The blanching step inactivates myrosinase, which means that glucosinolate levels are stable (Rodrigues and Rosa, 1999). The blanching process also causes the thermal inactivation of ESPs. ESPs act to redirect the production of isothiocyanates (such as sulforaphane from glucoraphanin) towards nitrile production (in this example, sulforaphane nitrile) (Matusheski et al., 2004; see Agerbirk et al., 2008). In the absence of blanching, glucosinolates are degraded rapidly following thawing (Rosa et al., 1997), the degree of breakdown governed primarily by the classes of glucosinolate present as a function of genotype used.

5.5 Conclusions

Increasing awareness of the beneficial phytochemicals present within the Brassicaceae has led to the promotion of these vegetable crops to ‘super-food’ status. It has become apparent that modern breeding techniques will enable the directed action required to capture the allelic variation necessary to improve public health further. In line with an increasing understanding of the interactions between genotype, preharvest environment and post-harvest conditions, an understanding of nutrient × human allele interactions is required in order to maximize the benefits associated with the consumption of this crop type. The ever-growing field of nutritional genomics seeks to integrate these diverse areas of research in a systematic approach, to deliver directed advice that may be adopted for policy guidance.

References


King, G.J. (2006) Utilization of Arabidopsis and Brassica genomic resources to underpin genetic analysis and
Kliebenstein, D.J. (2009) A quantitative genetics and ecological model system, understanding the aliphatic
glucosinolate biosynthetic network via QTLs. Phytochemical Reviews 8, 243–254.
multiple events of plastid trnF(GAA) pseudogene evolution in the Brassicaceae. Molecular Biology and
Evolution 24(1), 63–73.
of Agricultural and Food Chemistry 47, 1576–1581.
Variation of glucosinolates in vegetable crops of Brassica oleracea. Journal of Agricultural and Food
Chemistry 47, 1541–1548.
Lange, W., Toxopeus, H., Lubberts, J.H., Dolstra, O. and Harrewijn, J.L. (1989) The development of Rapar-
dish (× Brassicoraphanus, 2n = 38), a new crop in agriculture. Euphytica 40, 1–14.
McKillop, D.J., Pentieva, K., Daly, D., McPartlin, J.M., Hughes, J., Strain, J.J., Scott, J.M. and McNulty, H.
(2002) The effect of different cooking methods on folate retention in various foods that are amongst
the major contributors to folate intake in the UK diet. British Journal of Nutrition 88, 681–688.
of cytochromes P-450 and induction of glutathione S-transferases by sulforaphane in primary human and
creases sulforaphane formation in broccoli. Phytochemistry 65, 1273–1281.
Metz, P.L.J. (1995) Hybridization of radish (Raphanus sativus L.) and oilseed rape (Brassica napus L.) through
34, 91–103.
isothiocyanate-enriched broccoli and its enhanced ability to induce phase 2 detoxification enzymes in
characterisation of nutraceutical compounds of broccoli. Journal of Pharmaceutical and Biomedical Analysis
41, 1508–1522.
Paterson, A.H. (2006) Leafing through the genomes of our major crop plants, strategies for capturing
(2002) The effect of post-harvest and packaging treatments on glucoraphanin concentration in broc-
Rodrigues, A.S. and Rosa, E. (1999) Effect of post-harvest treatments in the level of glucosinolates in brocc-
vier, Amsterdam, pp. 315–357.


6 Citrus
[Orange, Lemon, Mandarin, Grapefruit, Lime and Other Citrus Fruits]

Amarat H. Simonne and Mark A. Ritenour

6.1 Introduction

Citrus, a genus of the plant family Rutaceae, is one of the most important horticultural commodities in the world. First domesticated 4000 years ago in South-east Asia, some suggest that secondary diversification of Citrus occurred later in the Mediterranean and Caribbean (Mukhopadhyay, 2004). Others believe the orange, tangerine and pomelo (or pummelo) originated in China and South-east Asia, lemon and lime in northern India and grapefruit in the Caribbean (Saunt, 2000). As one of the ancient crops, Citrus has been subjected to years of natural and man-made genetic transformation, resulting in many new species and hybrids and leading to disagreements and inconsistencies among experts on the classification and taxonomy of the plants (Mukhopadhyay, 2004; Moore et al., 2005; Khan, 2007; Laszlo, 2007; Ladaniya, 2008). To complicate the classification and taxonomy further, most citrus species are polyembryonic and apomictic, which means that seeds contain one zygotic embryo and other embryos made entirely of maternal, nucellar DNA and tissue (Mukhopadhyay, 2004; Khan, 2007; Ladaniya, 2008). To date, it is impossible to give an exact number of species for citrus. While Moore et al. (2005) stated that 150 genera and 900 species were attributed to the Rutaceae family, others stated 150 genera and 1500 species (Laszlo, 2007). Nevertheless, new genetic data derived from modern methods of analyses suggest that all citrus species are derived from three basic species: C. grandis (pomelo), C. medica (citron) and C. reticulata (mandarin) (Mukhopadhyay, 2004). Because of its long history and ability to grow in various regions of the world ranging from latitudes 40°N to 40°S (Mukhopadhyay, 2004), many scientific and contemporary reports, books and records have been published on citrus throughout the world, in many languages.

This chapter will focus on the major economically important true citrus plants that are now widely grown throughout the globe, including Citrus, Fortunella (kumquat) and Poncirus (trifoliate orange), which is used mainly as rootstock for citrus production because of its cold hardiness (Saunt, 2000). The citrus genera are divided further into the sweet orange (C. sinensis), mandarin (C. reticulata), grapefruit (C. paradisi), pummelo (C. grandis), lemon (C. limon), sour lime (C. aurantifolia), citron (C. medica) and sour orange (C.aurantium).

Total world production of citrus was estimated to be 116 million tonnes (Mt) in 2007 (FAOSTAT, 2009). Based on the world production data and availability of current research, similar crops will be grouped together. For example, mandarin, tangerine
and clementine will be grouped into the same class, despite some slight differences in genetic background (Saunt, 2000; Mukhopadhyay, 2004; Ladaniya, 2008). The sweet orange groups are by far the most important citrus species for both fresh and juice consumption worldwide, with almost 64 Mt produced in 2007, followed by tangerine (27 Mt), lemon/lime (13 Mt) and grapefruit/pummelo (5 Mt) (FAOSTAT, 2009). The top orange producing countries are Brazil, the USA and China (Ladaniya, 2008).

Mukhopadhyay (2004) classified oranges from different producing countries around the world into four types, including common or round, navel, pigmented (blood-coloured) and acidless oranges. According to Ladaniya (2008), oranges for fresh market are divided into two large groups: blood (pigmented) or non-blood (non-pigmented) oranges.

Although citrus has been grown since ancient times, only in the past 100 years has its production and processing become fully commercialized. Citrus commercialization relied on advancements in its production and postharvest research, including postharvest handling, processing and nutritional research that developed hand in hand with other scientific advances in various fields during the 20th century (Nagy et al., 1977a,b). As a result, citrus is one of the world’s major fruit commodities consumed, around the world, as fresh fruit or juice and other citrus-derived products (Boriss, 2009; UNCTAD, 2009).

### 6.2 Identity and Role of Bioactive Compounds

Since ancient times, citrus fruit and products have been recognized as important components of a healthy human diet due to various constituents such as vitamin C, folic acid, potassium, flavonoids, pectin, pigments and limonoids (Manners, 2007; Ladaniya, 2008). Citrus fruit contain numerous bioactive compounds that offer potential human health benefits. In addition to vitamin C and folic acid, which have been the traditional focus of citrus nutritional components, this chapter will focus on the potential health benefits of other citrus components (bioactive compounds). The majority of studies attribute the health benefits of natural citrus to phytochemicals such as flavonoids, limonoids, furocoumarins and pectins, which are present in citrus fruit (Patil et al., 2006a,b).

#### 6.2.1 Pigments (chlorophylls, carotenoids and anthocyanins)

Major pigments found in citrus fruit include chlorophylls, carotenoids and anthocyanins. Chlorophylls (a and b) impart green colours and predominate in the peel of citrus fruit during growth and maturation. Chlorophyll levels are high when the fruit are immature, and disappear in most citrus fruit after maturation and exposure to cool night temperatures. Most research on citrus fruit chlorophyll composition was conducted from the 1940s to 1980s. It was shown that changes in peel chlorophyll content were not a good indicator of maturity because the fruit often reached maturity while remaining green, especially in tropical or subtropical growing environments (Gross, 1987; Ladaniya, 2008). However, high chlorophyll content in the peel of most citrus fruit is often considered a negative quality characteristic because it masks the presence of carotenoids, which give the fruit its characteristic attractive orange and yellow colour. For lime, however, green colour is considered an important positive quality indicator, and thus chlorophyll maintenance is highly desirable during postharvest handling and marketing of the fruit (Win et al., 2006a,b).

Understanding the nature of chlorophyll degradation was important for developing effective degreening procedures for many fresh citrus varieties (Ladaniya, 2008). Optimum conditions for promoting chlorophyll degradation differ depending on variety and growing location and are related to chlorophyllase activity (Gross, 1987). Although the potential health benefits of chlorophyll derivatives have been explored (Egner et al., 2001; Ferruzzi et al., 2001, 2002; Fahey et al., 2005), limited documentation is available on the potential intake, bioactivity and specific health benefit of chlorophyll pigments from citrus.
Carotenoids are major pigments found in the peel, flesh, flavedo and juice of citrus fruit, giving rise to colours ranging from light pale yellow to deep orange. For non-blood-type orange, most of the pigments are carotenoids. The citrus carotenoids are by far one of the most complex and numerous carotenoid groups reported of any fruit (Gross, 1987). In addition to commonly known provitamin A carotenoids (e.g. α-carotene, β-carotene, β-cryptoxanthin), citrus also contains many other lesser-known provitamin A carotenoids, as well as a number of unique carotenoids such as C₃₀apocarotenoid (a genus-specific carotenoid found only in citrus), citrus apocarotenenal, trollizanthin, citraurin and α-cryptoxanthin, which is a derivative of α-carotene, to name just a few. Relatively few comprehensive reviews of citrus carotenoids have been conducted since Gross (1987), as summarized by Ladaniya (2008). Additional works in the past decade have focused on carotenoids of specific hybrids or new varieties formed from citrus mutations (Goodner et al., 2001; Lee, 2001; Lee and Castle, 2001; Dhuique-Mayer et al., 2005; Xu, C.J. et al., 2006; Xu, J. et al., 2006; Matsumoto et al., 2007; Meléndez-Martínez et al., 2007a; Wang et al., 2007, 2008; Xu et al., 2008). In summary, these studies show that the concentration and distribution of carotenoids in citrus are affected by variety, maturity, tissue type, climate and season. Most carotenoids are concentrated in the fruit peel and the distribution of carotenoids in juice and in flavedo are very similar, except for a few varieties of citrus (Matsumoto et al., 2007). A review by Meléndez-Martínez et al. (2007b) of carotenoid composition data in citrus fruit revealed that the carotenoid diversity in cultivated citrus was linked more to the global evolution process of the cultivated citrus rather than to the recent mutation or human selection process (Meléndez-Martínez, et al., 2007b). It was also observed that the citrus varieties grown in Mediterranean climates tended to accumulate more carotenoids than those grown in other geographic areas. This is because of the unique stress conditions found in the Mediterranean. Among ten citrus species and varieties (Salustiana, Hamlin, Maltaise, Shamouti, Sanguinelli, Valencia, Pera, Cara Cara, mandarin and clementine) used in the study, mandarin and clementine contained the most provitamin A activity, with activity ranging between 900 and 1000 retinol equivalents (Dhuique-Mayer et al., 2005). Additional new carotenoid works have focused on gene expression in different varieties (Shamouti orange, Sanguinelli orange, Cara Cara navel orange and Huang pi Chen orange) as related to fresh fruit colour (Fanciullino et al., 2008), role of specific enzymes during maturation (Satsuma mandarin, Valencia orange and Lisbon lemon) (Kato et al., 2006), biosynthetic pathway of carotenoids in citrus (25 species) (Fanciullino et al., 2007) and regulation of colour break in mandarin fruit (Álós et al., 2006). These studies reveal that carotenoid distribution in citrus is highly complex and can be explained by many factors, such as diversity of the genes, mutation and changes in plant hormones, among other things.

Ladaniya (2008) summarized research on the distribution of various carotenoids in orange and other citrus fruit and reported that the carotenoids increased in both peel and pulp as the fruit ripened. The same author also reported specific content of some apocarotenoids in the peel of hybrid citrus, although these compounds were only a minor component of the peel of C. sinensis (Ladaniya, 2008). The presence of lycopene was also observed in some citrus species, including pink and red grapefruit and some mutants of sweet orange, such as red navel (Lee, 2001; Xu, C.J. et al., 2006; Xu, J. et al., 2006; Liu et al., 2007). Although citrus species are listed among more than 500 carotenoid sources (Babosa-Filho et al., 2008), very limited work has been done evaluating the activities of the carotenoids in citrus because the contribution of citrus carotenoids to antioxidative activity is negligible in the edible portion of orange juice (Gardner et al., 2000) and also because most of the citrus carotenoids (non-provitamin A carotenoids) are located in the fruit peel, which typically is discarded. In addition, a recent review by Hooper and Cassidy (2006) did not include carotenoids in its list of bioactive compounds with health care potential. Therefore, relatively speaking, carotenoids in citrus may appear to have less prominent health-promoting roles than other classes of citrus bioactive compounds, yet it is
impossible to disregard their importance because of other possible synergistic effects of citrus to human health as a whole.

Anthocyanins (members of the phenolic grouping of compounds) are another class of pigments found in pigmented or blood orange (sweet orange). These include glycosides of pelargonidin, peonidin, delphinidin and petunidin (Robards and Antolovich, 1997), and the concentration of these pigments increases when the fruit reach maturity. The Mediterranean growing conditions of hot days and cool nights facilitate the development of the anthocyanins (Davies and Albrigo, 1994). The blood orange is commercially important in Mediterranean countries. It has a deep colour in the flesh, and occasionally in the peel (Fig. 6.1).

### 6.2.2 Phenolics

As secondary metabolic products, phenolics are a class of organic compounds with one or more hydroxyl (OH) groups attached to an aromatic ring (benzene ring). Ladaniya (2008) has summarized the presence and changes of phenolic compounds which, to a certain extent, affect the taste and colour of orange juice during processing. According to Kanes et al. (1993), four different classes of phenolic compounds absorbing at 285 nm (flavones/ols, flavanones, coumarins/cinnamic acid derivatives and psoralens) are found in Rutaceae species and cultivars; these include glycosides and the highly methoxylated flavones called polymethoxylated flavones (Manthey and Guthrie, 2002). Others (Shahidi and Ho, 2005; Ladaniya, 2008) classify phenolics into three groups based on the complexity of the chemical structures: (i) simple phenolics (monocyclic) such as catechol and hydroquinone; (ii) dicyclic phenolics with two benzene rings such as flavones; and (iii) polyphenolic compounds such as cyanins and anthocyanin pigments found in the blood-type oranges. Because there is no consistent way to group phenolic compounds, they often appear in the literature under different headings. It is well known that plant phenolic levels increase after infection by pathogens (Ladaniya, 2008). Many phenolics in citrus peels are often discarded as waste products after processing for juice. Recent research has attempted to recover some of these bioactive compounds from discarded citrus peel, as a source of natural antioxidants (Li et al., 2006).

![Fig. 6.1](image_url) From top left: Flame red grapefruit,1 Marsh white grapefruit,1 blood orange2 and navel orange.2 From bottom left: Pineapple orange,2 Hamlin oranges,2 pomelo fruit3 and pomelo juice vesicles.3 Photo credits: 1Mark Ritenour, 2Frederick S. Davies, 3Amarat Simonne.
6.2.3 Flavonoids

Flavonoids are a subclass of polyphenols or polyphenolic compounds and they consist of two aromatic rings, each containing at least one hydroxyl (–OH) group, which are connected via a three-carbon 'bridge' and exist as a six-member heterocyclic ring (C₆-C₃-C₆). Citrus flavanones have a B-ring connected to the C-ring at position 2 (on the C-ring) without unsaturation on the C-ring and with another functional group on position 4-Oxo (Benavente-Garcia et al., 1997; Robards and Antolovich, 1997; Mouly et al., 1998; Beecher, 2003; Fig. 6.2). Furthermore, the citrus flavonoids (flavanones) can exist in conjugated forms with sugar (flavanone glycosides) or without sugar, but with one or more methyl groups (aglycones or polymethoxylated flavones), causing much complication in identification and analyses (Robards and Antolovich, 1997). Early works on the flavonoid content of citrus were somewhat fragmented, without a full representation of the citrus species, tissue types, or types of flavonoid, and this might have been due to the lack of suitable analytical methods (Miyake et al., 1997; Mouly et al., 1998; Nogota et al., 2006). For example, Robards and Antolovich (1997) reported that major flavonoids found in grapefruit included naringin, narirutin, hesperidin and neohesperidin, as well as others such as tangeretin and polymethoxylated flavones. Major flavonoids found in sweet orange include hesperidin, narirutin, eriocitrin and narirutin-4' glucoside, as well as other flavones (sinensetin, nobiletin, tangeretin and isosinensitin) (Robards and Antolovich, 1997). Another study reported that orange (C. sinensis L.) contained mainly flavonoid glycoside, hesperidine, and its flavone analogue, diosmin, which have been shown to have anticarcinogenic activities (Manthey and Guthrie, 2002). In citrus plants, flavonoids have been attributed to the protection against some infectious plant diseases such as green mould caused by Penicillium digitatum (Ortuno et al., 2006).

Nogata et al. (2006) evaluated concentrations of flavonoids in various tissues (whole fruit, peel, juice vesicles, flavedo, albedo and segment epidermis) of 45 citrus species classified by Tanaka’s system (Tanaka, 1969). This is one of the most comprehensive studies on citrus flavonoids in various cross sections of citrus species. According to the Tanaka system, citrus is classified into the following sections or groups: I – Papeda (e.g. C. macroptera or Cabuyao); II – Limonellus (e.g. lime, Bergamot and Biroro); III – Citrophorum (e.g. citron, lemon and lumie); IV – Cephacitrus (e.g. Marsh grapefruit, pummelo); V – Aurantium (e.g. sour orange, Valencia); VI – Osmocitrus (e.g. Yuzu, Sudachi); VII – Acrumen (e.g. Ponkan, Satsuma, Clementine and Dancy tangerine); and VIII – Pseudofortunella (e.g. C. madurensis), Fortunella-Eufortunella (e.g. Kumquat), and Poncirus (trifoliolate orange) (Tanaka, 1969; Nogata et al., 2006). Nogata et al. (2006) found that the flavonoid composition of citrus was in agreement in each section, with the exception of the Aurantium (V) section and others with a peculiar flavonoid composition. The profiles may be different if they are classified by a different system (Nogata et al., 2006). Distribution of the predominant flavonoids in various tissues of selected common citrus species, as reported by Nogota et al. (2006), is provided in Table 6.1.

Peterson et al. (2006a,b) reviewed the available analytical data on flavonoids in many common citrus species including grapefruit and orange, and their relatives. They reported that, overall, grapefruit had a distinct flavanone profile, with naringin as a dominant compound, but was similar to the sour orange. These studies confirmed some of the findings of Nogata et al. (2006), with
Table 6.1. Distribution of flavonoids in various tissues from some selected citrus species based on the Tanaka grouping system.

<table>
<thead>
<tr>
<th>Citrus group</th>
<th>Whole fruit</th>
<th>Peels</th>
<th>Juice vesicles</th>
<th>Flavedo</th>
<th>Albedo</th>
<th>Segment epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - <em>Papeda</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabuyao</td>
<td>PON, NHP</td>
<td>PON, NHP</td>
<td>PON</td>
<td>SNT</td>
<td>PON</td>
<td>PON</td>
</tr>
<tr>
<td>II - <em>Limonellus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican lime</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Tahiti lime</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP, ERC</td>
<td>HSP, ERC</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Bergamot</td>
<td>PON, NHP</td>
<td>PON, NRG, NHP</td>
<td>PON, NHP, NRG</td>
<td>NRP, NRG</td>
<td>PON, NHP, NRG</td>
<td>PON, NER, NHP</td>
</tr>
<tr>
<td>Biroro</td>
<td>HSP</td>
<td>HSP</td>
<td>NDM, NRG</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>III - <em>Citrophorum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citron</td>
<td>HSP</td>
<td>ERC, DSM</td>
<td>HSP, RTN, ERC</td>
<td>RTN</td>
<td>ERC, DSM</td>
<td>HSP, DSM</td>
</tr>
<tr>
<td>Eureka lemon</td>
<td>HSP</td>
<td>HSP</td>
<td>ERC, HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Sweet lemon</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Lumie</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP, ERC</td>
<td>ERC, HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>IV - <em>Cephacitrus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirado buntan</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
</tr>
<tr>
<td>Shaten yu</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG, RTN</td>
<td>NRG, PON</td>
<td>NRG</td>
<td>NRG</td>
</tr>
<tr>
<td>Marsh grapefruit</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
</tr>
<tr>
<td>Kinukawa</td>
<td>NHP, NRG</td>
<td>NHP, NRG</td>
<td>NRG, NHP</td>
<td>NHP</td>
<td>NHP, NRG</td>
<td>NRG, NHP</td>
</tr>
<tr>
<td>Hassaku</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG, NRT</td>
<td>NHP</td>
<td>NRG</td>
<td></td>
</tr>
<tr>
<td>V - <em>Aurantium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natsudai dai</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
<td>NRG</td>
</tr>
<tr>
<td>Sour orange</td>
<td>NRG, NHP</td>
<td>NRG, NHP</td>
<td>NRG, NHP</td>
<td>PON, NHP, NRG</td>
<td>NRG, NHP, PON</td>
<td>NRG, NHP, PON</td>
</tr>
<tr>
<td>Valencia</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Morita navel</td>
<td>HSP</td>
<td>HSP</td>
<td>NRT, HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Shunkokan</td>
<td>NRT, HSP</td>
<td>HSP, NRT</td>
<td>NRT</td>
<td>HSP</td>
<td>NRT, HSP</td>
<td>NRT, HSP</td>
</tr>
<tr>
<td>VI - <em>Osmocitrus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yuzu</td>
<td>NRT, HSP</td>
<td>HSP, NHP, NRG</td>
<td>NRT, HSP</td>
<td>NRT, HSP</td>
<td>HSP</td>
<td>NRT, HSP</td>
</tr>
<tr>
<td>Sudachi</td>
<td>NHP, NRT</td>
<td>NHP, RTN, NRT</td>
<td>NRT, HSP, NHP</td>
<td>NHP</td>
<td>NHP, RTN</td>
<td>NRT, NRG</td>
</tr>
<tr>
<td>Kabosu</td>
<td>HSP, NRT</td>
<td>HSP, NRT, RTN</td>
<td>NRT, HSP</td>
<td>NRP, HSP</td>
<td>HSP, RTN, NRT</td>
<td>NRT, HSP</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Citrus group</th>
<th>Whole fruit</th>
<th>Peels</th>
<th>Juice vesicles</th>
<th>Flavedo</th>
<th>Albedo</th>
<th>Segment epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VII – Acrumen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>King</td>
<td>HSP</td>
<td>HSP</td>
<td>NRT, HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>NRT, HSP</td>
</tr>
<tr>
<td>Satsuma</td>
<td>HSP</td>
<td>HSP</td>
<td>NRT, HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP, NRT</td>
</tr>
<tr>
<td>Yatsushiro</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Ponkan</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP, NRT</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Dancy tangerine</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Clementine</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td>Kishu</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
<td>HSP</td>
</tr>
<tr>
<td><strong>VIII – Pseudofortunella</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shikikitsu</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
</tr>
<tr>
<td>Fortunella-Eufortunella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oval kumquat</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
</tr>
<tr>
<td>Meiwa kumquat</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
<td>NRT</td>
</tr>
<tr>
<td><strong>Poncirus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trifoliate orange</td>
<td>PON</td>
<td>PON</td>
<td>PON</td>
<td>NRG</td>
<td>PON</td>
<td>PON</td>
</tr>
</tbody>
</table>

Notes: *Adapted from Nogata et al. (2006). Within each cell of the table, the compounds are listed in order of magnitude. ERC = eriocitrin; NRT = narirutin; NRG = naringin; HSP = hesperidin; NHP = neohesperidin; PON = poncirin; SNT = sinensetin; RTN = rutin; DSM = diosmin; N/F = not found.
additional details on the flavonoid distribution in different types of grapefruit, orange and other related citrus species. As for lemon and lime, the flavanone profiles were dominated by hesperidin and eriocitrin, and were similar to sweet orange. Peterson et al. (2006a) reported total mean flavanone glycone contents of 27 mg/100 g (white grapefruit) and 18 mg/100 g (red grapefruit). Naringin was the major (>60%) of the reported total flavanones in the different types of grapefruit studied. Pink and red grapefruit appeared to contain a lower amount of flavonoids than white grapefruit or grapefruit in general. Other detectable flavanones in grapefruit (in general) include didymin (<1%), eriocitrin (1.7%), hesperidin (10%), narirutin (18%), neohesperidin (1.3%), neohesperidin (5%) and poncirin (<1%). For white grapefruit, after naringin, the distribution of flavonoids is didymin (<1%), eriocitrin (<1%), hesperidin (14%), narirutin (20%), neohesperidin (<1%) and poncirin (<1%). Flavanoids in red and pink grapefruit include hesperidin (1.5%), narirutin (19%) and neohesperidin (2%). Overall, red and pink grapefruit contain lower flavanones than white grapefruit (Peterson et al., 2006a). Total flavanones in lemon and lime were reported to be 26.58 and 17.29 mg aglycone/100 g fresh fruit or juice, respectively, and the major flavonone was hesperidin, with 59% and 90% in lemon and lime, respectively. Eriocitrin was the second major flavanone in lemon and lime, representing approximately 36% and 8% of the total flavonones, respectively. Other detectable flavanones in lemon were naringin and narirutin, and in lime were eriocitrin, narirutin and neohesperidin (Peterson et al., 2006a). According to Peterson et al. (2006b), the major flavonoids in sweet (C. sinensis) and sour orange (C. aurantium) are flavanones. In general, sour orange has a distinctive flavonone profile, with naringin and neohesperidin as dominant flavones, and this finding is consistent with Nogota et al. (2006). Peterson et al. (2006b) found that, among all the oranges included in their study, sour oranges (C. aurantium varieties Bergamot, Chinotto, Daidai and Seville) had the highest flavanone content, with an average of 48 mg aglycones/100 g FW. Lowest levels of flavanone content and similar compounds (19–25 mg aglycones/100 g ranges) were found in sweet oranges (blood, Hemlin, Navel and Valencia), tangors (a cross of C. reticulata and C. sinensis) and tangerines. Tangelos (a cross of C. reticulata and C. paradisi) have moderate levels of flavanones, with a mean content of 29 mg aglycones/100 g (Peterson et al., 2006b). Overall, the flavanone content of the tangelo was different from the sweet and sour orange, tangerine and tangor. The Peterson et al. (2006a,b) data were incorporated into the USDA flavonoid database (Bhagwat et al., 2006). Additional information on flavonoids in citrus fruit continue to be elucidated for various citrus species, including C. bergamia Risso (Gattuso et al., 2007).

6.2.4 Limonoids

Limonoids are chemically related triterpene derivatives typically found in the Rutaceae and Meliceae families (Roy and Saraf, 2006; Manners, 2007). The significant amounts of highly oxygenated triterpenoid compounds (limonoids) to be found in citrus have not been used to their potential (Manners, 2007). Limonin was first identified as a citrus constituent in 1841, but all other limonoids have been isolated in the past 50–60 years. Limonoids have been well documented for the control of many species of insects (Patil et al., 2006a,b). Although the nutritional roles of limonoids have not been defined (Manners et al., 2003; Manners, 2007), much research has been conducted on this family of compound because limonin causes bitterness in many citrus juices. Manners et al. (2003) examined the bioavailability of pure limonin glucoside from citrus juice in healthy humans and found significant variations in plasma concentrations among the subjects, but they found that the average time to reach the highest concentrations (1.74–5.37 nmol/l) was 6 h. Manners (2007) indicated that the association of triterpenoid (limonoid) with bitter taste in orange juice was first established in the 19th century. It was not until 1938 that a specific compound, limonin, was isolated from orange juice. Early research focused on understanding the mechanism of bitterness formation and how to eliminate or prevent the bitterness in citrus.
juices (Nagy et al., 1977a; Drewnoski and Gomez-Cameros, 2000). The first documented antioxidative properties of citrus limonoids (limonin, limonin glucoside and neoeriocitrin) were reported by Yu et al. (2005). A recent review (Ejaz et al., 2006) indicated that citrus limonoids, in either natural fruit or purified form, might provide substantial anticancer effects. Limonoids have been screened and tested for anticancer properties in both laboratory animals and human cancer cells because of their structures. Citrus limonoids are absorbed and metabolized in humans, but the mechanisms of absorption and metabolism are still unclear (Breksa et al., 2009). Furthermore, citrus limonoids are complex triterpenoid compounds and are found in different forms (aglycones, glucosides or A-ring lactone). Thus, isolation and identification of these compounds are important in relation to the evaluation of health benefit in human or animal studies (Jayaprakasha et al., 2006; Patil et al., 2006b; Breksa et al., 2009).

6.2.5 Pectic substances

Although pectic substances can be found in many plant tissues, they are found in large quantities only in citrus fruit tissues. Pectic substances belong to a class of complex polysaccharides that serve as hydrating agents and cementing materials for the cellulosic network in plants (Nagy et al., 1977a; Liu et al., 2001). Commercial pectins are derived mostly from lime, lemon, grapefruit, orange and apple, for the production of jam and jellies. Citrus pectins have also been shown to have health benefits and have been used as wound treatment ingredients, homeostatic agents, immune complement activators, for the treatment of chronic diseases such as diabetes and high blood cholesterol, and for cancer inhibition (Liu et al., 2001, 2002; Salman et al., 2008). In recent years, additional health benefits associated with citrus pectins, used alone or in combination with other citrus bioactive compounds, continue to have evolved, including use in heavy metal detoxification (Eliaz et al., 2007), treatment of advanced solid tumours (Azémar et al., 2007) and lowering cholesterol in eggs (Lien et al., 2008). As pectic substances are predominantly present in citrus peel and typically are discarded as waste, many research efforts have focused on recovering these pectic substances, as a source of dietary fibre (Figueroa et al., 2005; Ubando-Rivera et al., 2005; Marin et al., 2007), and other bioactive compounds, as functional food ingredients or supplements (Schieber et al., 2001; Ubando-Rivera et al., 2005).

6.2.6 Other phytonutrients

In addition to the previously mentioned phytonutrients (carotenoids, phenolic compounds, flavonoids, limonoids, polysaccharides and pectic substances), citrus contain other compounds which may be beneficial or detrimental to human health that are as yet unknown. These include furocoumarins, which are found in grapefruit and other citrus species (Stanley and Jurd, 1971; Gattuso et al., 2007). On the one hand, the furocoumarins (psoralens) have been found to have strong antioxidative properties and to protect against vascular injury caused by catheter-directed arterial intervention procedure (also known as balloon injury-related neointima formation) (Mollace et al., 2008) but, on the other hand, they have been linked recently to increased cutaneous melanoma (Sayre and Dowdy, 2008).

6.3 Chemopreventive Activity and Bioavailability

Citrus is considered to be one of the largest suppliers of vitamin C, as well as other basic nutrients (sugars, folate, provitamin A, carotenoids, other vitamins and minerals) to the human diet (Nagy et al., 1977a; Nagy, 1980; Manners, 2007; Ladaniya, 2008). In the 1980s and 1990s a plethora of epidemiological studies and reviews revealed the positive benefits of consuming fruit and vegetables for reducing the risk of some chronic diseases (Peterson et al., 2006a,b; Table 6.2). Citrus fruit consumption was shown to have positive protective effects on chronic diseases (Economos and Clay, 1999) and to have anticancer (Silalahi, 2002; Cuthrell and Le Marchand,
Citrus fruit consumption with the risks of breast cancer (Gaudet et al., 2004) or prostate cancer (Bae et al., 2008). Based on a comparative study of carotenoid intake, it was found that β-cryptoxanthin was obtained primarily from citrus in France, the UK, Republic of Ireland, Spain and the Netherlands (O’Neill et al., 2001). New research also shows that serum β-cryptoxanthin concentration and circulating bone metabolic markers exist in healthy individuals following prolonged consumption of orange juice containing β-cryptoxanthin (Yamaguchi et al., 2005). Other research revealed preferential uptake of β-carotene and free β-cryptoxanthin from the ester forms of β-cryptoxanthin by Caco-2 cells (Dhuique-Mayer et al., 2006). More recently, citrus and citrus-derived bioactive compounds have been linked to many biological functions, including antioxidative, anti-inflammatory, anti-allergic, antiviral, antiproliferative, anti-mutagenic and anticarcinogenic, to name just a few (Jayaprakasha et al., 2006; Patil et al., 2006a; Tripoli et al., 2007; Benavente-Garcia and Castillo, 2008; Table 6.2). Furthermore, several citrus bioactive compounds (naringenin, hesperetin, hesperidin, eriocitrin, naringin, meoceriocitrin, natrutiun, p-coumaric acid, caffeic acid and ferulic acid) have been identified as having a role in the prevention of cardiovascular disease (Joshipura et al., 2001; Mennen et al., 2004) and cancers (Benavente-Garcia et al., 1997; Kris-Etherton et al., 2002). After the positive associations between citrus bioactive compounds and human health were reported, many researchers examined the plasma kinetics of these compounds. Erlund et al. (2001) studied plasma concentrations or plasma kinetics of flavonones (naringenin and hesperetin) in humans after ingestion of orange and grapefruit juice, in order to see if flavonone concentrations in urine could be used as biomarkers of intakes. However, they found that the urine concentration of flavonones was not a good biomarker of dietary intake. Miyake et al. (2006) examined the fate of flavonoids in humans after ingestion of flavone glycosides and aglycone forms and found that the absorption of both forms was affected by the coexisting solutions, and the aglycone form was absorbed faster than the glycoside form. At this point, limited information is available on the absorption of these compounds from juice compared with whole fruit. Specific reports on prevention or curing of specific diseases are summarized in Table 6.2, respectively.

6.3.1 General health benefits as related to citrus

The pharmacological properties of citrus have been well documented in various regions of the world since medieval times. The early literature from the Mediterranean region revealed the use of citron and lemon as antidotes for ‘poison and venom’. However, in the modern literature citron and bitter orange are documented to have anticancer activity, lime to have immunomodulatory effects in humans, pomelo to be of use in treating circulatory problems and lemon to be useful in easing hangover symptoms (Arias and Ramón-Laca, 2005). The protective benefits against chronic diseases (such as cardiovascular diseases, hypertension, cataract, diabetes, Alzheimer’s disease, stroke and cancer) of diets rich in vegetables and fruit including citrus have been documented extensively in recent years (Joshipura et al., 1999; Silalahi, 2002; Sun et al., 2002; Bazzano et al., 2003; Hung et al., 2004; Mennen et al., 2004; Norman et al., 2004; Ladaniya, 2008). Citrus bioactive components, such as flavonoids, limonoids and dietary fibre, have been linked to the reduction of degenerative disease risk or to a protective effect. Despite the documentation of certain medicinal and nutritional values of citrus in ancient times, the specific basic nutritive values of citrus have only been well documented in the last 50 years (Ladaniya, 2008). For example, since the 1560s, citrus fruit has been known to prevent and cure scurvy (Nagy, 1980). However, it was not until the 1920s and 1930s that ascorbic acid (vitamin C) was identified as the compound that prevented scurvy. Gardiner et al. (2000) examined the relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potentials of fruit juices.
Table 6.2. Health-promoting action of citrus fruit and fruit products including orange (*C. sinensis*), grapefruit, lemon and lime, as well as pure chemical extracts.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Disease conditions</th>
<th>Commodities</th>
<th>Population</th>
<th>Type of study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective effect</td>
<td>Cardiovascular, cancer, neural tube defects, anaemia, cataracts, bone metabolism and osteoporosis, kidney stone disease, cognitive function and asthma</td>
<td>Citrus fruit</td>
<td>N/A</td>
<td>Review (25 refs)</td>
<td>Economos and Clay (1999)</td>
</tr>
<tr>
<td>Protective effect</td>
<td>Association with reduced risk of ischaemic stroke</td>
<td>Citrus fruit and juice</td>
<td>Nurses and health professional (men and women)</td>
<td>Large population study</td>
<td>Joshipura et al. (1999)</td>
</tr>
<tr>
<td>Anticancer No-anticancer</td>
<td>Reduce risk of degenerative diseases Among pre- or postmenopausal women</td>
<td>Citrus fruit</td>
<td>N/A</td>
<td>Review (35 refs)</td>
<td>Silalahi (2002)</td>
</tr>
<tr>
<td>Anticancer No-anticancer</td>
<td>No association of citrus fruit consumption with breast cancer Among pre- or postmenopausal women</td>
<td>Citrus fruit</td>
<td>Pre- and postmenopausal women</td>
<td>Analysis of large population case control study</td>
<td>Gaudet et al. (2004)</td>
</tr>
<tr>
<td>Anticancer No-anticancer</td>
<td>Positively affect serum antioxidant status and bone strength Among pre- or postmenopausal women</td>
<td>Citrus juice</td>
<td>Senescent rat model of osteoporosis</td>
<td>Original research</td>
<td>Deyhim et al. (2006)</td>
</tr>
<tr>
<td>Pure chemical or extracts derived from citrus</td>
<td>Reduce risk of degenerative diseases Among pre- or postmenopausal women</td>
<td>Citrus fruit</td>
<td>N/A</td>
<td>Review (54 refs)</td>
<td>Bae et al. (2008)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer</td>
<td>Inhibit the proliferation of a number of cancer cell lines</td>
<td>Human cancer cell lines</td>
<td>IC50 below 10 μM</td>
<td>Pure citrus flavonoids (orange peel and other sources)</td>
<td>Manthey and Guthrie (2002)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Induce cancer cell death</td>
<td>Neuroblastoma cells (undifferentiated human SH-SY5Y)</td>
<td>0.1–10 mmol/l</td>
<td>Four citrus limonoid glucocides</td>
<td>Poulouse et al. (2005)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Inhibit growth and apoptosis-inducing activity (cell death)</td>
<td>Human breast carcinoma cell lines (MCF-7)</td>
<td>Varied</td>
<td>Sweet orange peel extract containing hydroxylated polymethoxyflavones (PMFS) and non-PMFS</td>
<td>Sergeev et al. (2007)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------</td>
<td>------------------------------------------</td>
<td>--------</td>
<td>---------------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Antineoplastic by reduction of tumour burden</td>
<td>Hamster cheek pouch model</td>
<td>2.0–2.5%</td>
<td>Pure citrus flavonoids</td>
<td>Miller et al. (2008)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Reduce the formation of intestine oedema in mice model</td>
<td>Mice model for induced colitis</td>
<td>Naringin 15.8 mg/kg/day, 5 days; naringenin 5 mg/kg/day</td>
<td>Pure citrus naringin and naringenin</td>
<td>Amaro et al. (2009)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Inhibition of pro-inflammatory cytokinin IL-1B secretion</td>
<td>Human peripheral blood mononuclear cells</td>
<td>0.25–1.0 mg/ml</td>
<td>Citrus pectin (30–90% esterified)</td>
<td>Salman et al. (2008)</td>
</tr>
</tbody>
</table>

**Orange** (*C. sinensis*)

- **Antioxidative stress**: Increase plasma antioxidant concentrations
- **Antioxidative stress**: Improve antioxidant status and suppress peroxidation

**Lime**

- **Antiplatelet activity**: Inhibit platelet aggregation induced by ADP and epinephrine

**Lemon**

- **Antitumour**: Degradation of DNA in cancer cells

---

(Continued)
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer</td>
<td>Inhibit human liver cancer cell proliferation</td>
<td>Human liver cancer cell EC50 = 30.56 mg/ml (HepG2)</td>
<td>Fruit (acetone) extract</td>
<td>Sun et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>Lowering blood lipids</td>
<td>Reducing total cholesterol, VLDL, LDL, triglyceride and phospholipid</td>
<td>Rats</td>
<td>0.35 and 0.70%</td>
<td>Eriocitrin (eriodictyol 7-O-beta-rutinoside) from lemon fruit</td>
<td>Miyake et al. (2006)</td>
</tr>
<tr>
<td>Grapefruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticancer</td>
<td>Inhibit human liver cancer cell proliferation</td>
<td>Human liver cancer cell (HepG2)</td>
<td>EC50 = 130.09 mg/ml</td>
<td>Fruit (acetone) extract</td>
<td>Sun et al. (2002)</td>
</tr>
</tbody>
</table>

Notes: VLDL = very low-density lipoprotein; LDL = low-density lipoprotein.
and found that vitamin C accounted for 65–100% of the antioxidant potential of beverages derived from citrus fruit; they also found that the contribution of carotenoids to antioxidant potential was negligible. As research from in vitro as well as population-based studies revealed the association of dietary flavonoid intake and human cancer risk, many researchers started examining the sources of flavonoid in human diets (Table 6.2). Lowe et al. (2003), based on a population survey, reported that low intake of citrus fruit possibly might play a role in low vitamin C level and a predisposition to low-grade inflammation and thrombosis, cardiovascular diseases and tooth loss. Mukhopadhyay (2004) summarized several reported therapeutic properties of citrus fruit constituents such as pectins and flavonoids. For example, tangeretin (a flavonoid) can prevent invasion of normal tissue by cancer cells. Hesperidin, naringin, tangeretin and nobiletin (also flavonoids) have anti-inflammatory and antiallergic activities. In addition, limonoids and flavonoids have been shown to have some synergistic effects in preventing certain types of cancers and cardiovascular diseases (Patil et al., 2007). Somerset and Johannot (2008) examined sources of dietary flavonoids in Australian adults and found major contribution of flavonoids from various citrus sources, including orange (hesperetin and naringenin), lemon (eriodictyol), mandarin (hesperetin) and grapefruit (naringenin). Several types or classes of bioactive compounds have been identified in citrus fruit and they include non-nutritive fibre (cellulose, hemicelluloses, lignin and pectin), phenolic compounds, terpenoids and steroids, and pigments, which currently have no known vitamin activity (Ladaniya, 2008). Recent evidence has also attributed citrus fruit to reducing the risk of certain types of cancers, or have suggested that they could be used to treat cancers (Del Rio et al., 2004; Vanamala et al., 2006; Huang et al., 2007; Tripoli et al., 2007; Cutler et al., 2009; Lim et al., 2009). However, a recent systematic review by Bae et al. (2008) did not show any association between citrus intake and incidence/severity of prostate cancer. Studies that have shown the protective effects of citrus against cancer have attributed them to flavonoids, limonoids and other phenolic compounds (e.g. coumarins). A recent comprehensive literature review (Tripoli et al., 2007) revealed that citrus flavonoids exerted anticancer effects in various ways, including selective cytotoxicity, antiproliferative action and apoptosis; the conclusions were reached based on results of in vitro (using human and animal cell systems) and in vivo studies. Flavonoids can exert their effects at several stages of cancer formation, including induction (DNA damage), promotion (tumour development) and proliferation (invasion). Although the mechanisms of cancer prevention by flavonoids, or citrus flavonoids in particular, have been studied extensively and reviewed by

6.3.2 Cancer studies

Many researchers have attributed various components of citrus fruit to reducing the risk of certain types of cancers, or have suggested that they could be used to treat cancers (Del Rio et al., 2004; Vanamala et al., 2006; Huang et al., 2007; Tripoli et al., 2007; Cutler et al., 2009; Lim et al., 2009). However, a recent systematic review by Bae et al. (2008) did not show any association between citrus intake and incidence/severity of prostate cancer. Studies that have shown the protective effects of citrus against cancer have attributed them to flavonoids, limonoids and other phenolic compounds (e.g. coumarins). A recent comprehensive literature review (Tripoli et al., 2007) revealed that citrus flavonoids exerted anticancer effects in various ways, including selective cytotoxicity, antiproliferative action and apoptosis; the conclusions were reached based on results of in vitro (using human and animal cell systems) and in vivo studies. Flavonoids can exert their effects at several stages of cancer formation, including induction (DNA damage), promotion (tumour development) and proliferation (invasion). Although the mechanisms of cancer prevention by flavonoids, or citrus flavonoids in particular, have been studied extensively and reviewed by
many authors (Middleton et al., 2000; Ogata et al., 2000; Le Marchand, 2002; Huang et al., 2007; Morley et al., 2007; Rossi et al., 2007; Tripoli et al., 2007; Akao et al., 2008; Linseisen and Rohrmann, 2008; Miller et al., 2008; Miyata et al., 2008; Park et al., 2008), the results remain inconsistent and most reports recommend further research. These inconsistent results or outcomes may be due to other factors such as the forms, concentrations and interactions of the chemicals and the types of systems used in the studies, among others. After citrus limonoids were first linked to potential anticancer properties (Lam et al., 1994), the subject of citrus limonoids and cancer has been studied extensively (Tanaka et al., 2000; Manners et al., 2003; Miller et al., 2004; Vanamala et al., 2005; Poulose et al., 2005; Manners, 2007). Proposed mechanisms of cancer prevention of limonoids include antioxidative properties and the ability to detoxify carcinogens and harmful chemicals, stimulate the immune system, effect cell differentiation, block formation of nitrosamines, alter oestrogen metabolism, preserve the integrity of intracellular matrixes, maintain normal DNA repair, increase programme cell death (apoptosis) and decrease cell proliferations (Ejaz et al., 2006). In 2007, a patent (US Patent 7201928) was awarded to a group of scientists for use of extracts of orange peel for the prevention and treatment of cancer (Huang et al., 2007). Prince et al. (2009) compared different citrus coumarin effects on carcinogen-detoxifying enzymes in Nrf2 knockout mice and the results suggest different modes of actions for these compounds. In coming years, it is expected that much more research will further our understanding of the effects of citrus bioactive compounds on cancers. Additional information on cancer studies is provided in Table 6.2.

6.3.3 Cardiovascular diseases (CVD)

A body of research has revealed a clear association between increased consumption of vegetables and fruit, including citrus, and reduced risk of major chronic diseases such as cardiovascular disease (Middleton et al., 2000; Joshipura et al., 2001; Kris-Etherton et al., 2002; Verhaar et al., 2002; Vinson et al., 2002; Bazzano et al., 2003; Lowe et al., 2003; Hung et al., 2004; Whitman et al., 2005; Takachi et al., 2007; Tripoli et al., 2007; Jayprakash et al., 2008). Citrus components initially reported to have protective effects against CVD include antioxidative nutrients such as the carotenoids, vitamin C and folate as well as bioactive compounds which have no nutritional value (e.g. limonoid and phenolic compounds). Possible modes of action of folate in the prevention or protection against cardiovascular disease include homocysteine–folate interaction, antioxidative action, cofactor bioavailability or direct interactions of folate with enzyme endothelial NO synthase (Verhaar et al., 2002). Among the bioactive compounds with protective effects on CVD, flavonoids have been researched substantially. The potential modes of action of citrus flavonoid include cholesterol-lowering potential (Kurowska and Manthey, 2004), antioxidative properties and anti-inflammatory antithrombosis activities (Tripoli et al., 2007). While citrus bioactive components such as phenylephrine from C. aurantium, or bitter orange, have been reported to reduce fat absorption, thereby preventing obesity and reducing the risk of CVD indirectly (Klein et al., 2004), other compounds may help reduce damage in blood vessels during some intervention blockage procedures (Mollace et al., 2008). Other research showed Phellodendron and citrus extracts to be beneficial on lipid levels, blood pressure and fasting glucose levels in osteoarthritis patients (Oben et al., 2009). To date, it is impossible to isolate any one component of a particular fruit or vegetable as a ‘magic bullet’ for preventing CVD; it appears to be a combination of many factors.

6.3.4 Other beneficial effects

In addition to the previously mentioned benefits, citrus bioactive compounds have been reported to have many other health-promoting effects. For example, citrus flavonoids (tangeretin, naringenin) were reported to have a neuroprotective effect in rat models for Parkinson’s disease (Datla et al., 2001; Zbarsky et al., 2005), Alzheimer’s disease (Heo et al., 2004), neuroblastoma cells
Citrus 105

(Poulose et al., 2005), neurodegenerative diseases (Cho, 2006) and H$_2$O$_2$-induced cytotoxicity in PC12 cells (Hwang and Yen, 2008). Additionally, citrus has also been documented to benefit weight loss, reducing the risk factor for metabolic syndrome (e.g. obesity, lowering cholesterol) (Patil et al., 2006a), and to improve resistance to oxidative stress in streptozotocin-induced diabetic rat liver (Sugiura et al., 2006). Furanocoumarins have also been tested for treating skin disorders such as vitiligo and psoriasis (Conforti et al., 2009). Furthermore, citrus and citrus by-products (e.g. other phenolic compounds) have been associated with anti-inflammatory activities and antimicrobial activities (Patil et al., 2006a; Malhotra et al., 2008). Additional information on the health-promoting action of citrus fruit and fruit products is provided in Table 6.2.

6.3.5 Detrimental effects

The most reported detrimental effect associated with citrus is an interaction of grapefruit and grapefruit juice with the availability of specific medications (Patil et al., 2006a,b). According to a recent review by Farkas and Greenblatt (2008), grapefruit juice is known to interact with more than 30 prescription drugs by increasing their bioavailability. Although such interaction was discovered two decades ago, the mechanisms behind it continue to be elucidated. Other citrus juices, such as orange, Seville orange, pomelo, lime and lemon, and tangerine, also seem to have some degree of influence on drug disposition (Farkas and Greenblatt, 2008). Specifically in grapefruit, it is the furocoumarins and their derivatives such as furanocoumarins that appear responsible for this drug interaction (Fukuda et al., 1997). Furocoumarins and related compounds are formed by only four plant families, including Rutaceae, especially in grapefruit (Fukuda et al., 1997; De Castro et al., 2006; Peroutka et al., 2007) and pomelo (Egashira et al., 2004). The average furocoumarin content of white grapefruit juice was reported to be higher than that of red grapefruit juice (Fukuda et al., 2000). Furocoumarins specifically inhibit CYP3A4 and cytochrome P450 (Guo and Yamazoe, 2004), resulting in drug interactions. Furano- coumarins are toxic secondary metabolites, which may demonstrate antifungal activities and are phototoxic (Peroutka et al., 2007; Larbat et al., 2009). Although the content of furanocoumarins in citrus fruit is low (Peroutka et al., 2007), many researchers have demonstrated that grapefruit and pommelo juice can interact with many types of prescription drugs (Egashira et al., 2004; Dahan and Altman, 2004; De Castro et al., 2006; Mertens-Talcott et al., 2006; Farkas and Greenblatt, 2008; Boobis et al., 2009; Pillai et al., 2009). Another study by Yoo et al. (2007) revealed that a flavonoid glycoside, diosmin, might interact with P-gp-mediated efflux in Caco-2 cells, causing increased absorption of medications that are P-gp substrates. Although citrus limonoids have many potential health benefits, one study revealed that high levels of limonoid exposure in diets might cause a problem with weight gain, as seen in pregnant rats (Miller et al., 2006).

6.4 Effect of Preharvest and Postharvest Continuum

It is clear that bioactive compounds in citrus are important to the human diet. However, there are many pre- and postharvest factors that influence these concentrations. The concentration of bioactive compounds can be influenced by cultivar (both rootstock and scion); climate and cultural practices during production; position of the fruit on the tree; fruit maturation; postharvest treatments; handling, storage and transportation of the fruit; juice processing and storage conditions; and the type of container used for juice packaging. For those involved in growing, packing and marketing citrus in its various forms, it is important to understand how these factors influence the nutritional value of the product ultimately delivered to the consumer, with the aim of maximizing the content of health-promoting compounds (Mukhopadhyay, 2004; Patil et al., 2006a,b; Ladaniya, 2008).
6.4.1 Genotype

As might be expected, different citrus genotypes have been found to contain different levels of bioactive compounds. Nagy (1980) developed an excellent table listing the vitamin C content for a large number of orange, grapefruit, mandarin, lemon and lime cultivars reported in the literature. Overall, he found that orange generally had the greatest vitamin C content, followed by grapefruit, lemon, mandarin and finally lime. He found agreement from several sources that juice from early (cvs. Navel and Hamlin) and midseason (cv. Pineapple) orange varieties contained greater ascorbic acid concentrations than did late (i.e. Valencia) orange varieties. He also found that there were minor, usually insignificant, differences in the vitamin C content among grapefruit varieties, and that the vitamin C content among mandarin varieties was more variable but tended to be lower than either orange or grapefruit varieties. Differences in flavonoid composition and concentrations within citrus species were reported by Nogata et al. (2006) and discussed previously in this chapter (Table 6.1). The concentrations of flavonoids in whole fruit, peel, juice vesicles, flavedo, albedo and segment epidermis can vary among species by several orders of magnitude, but within citrus groups, member species tend to contain similar predominant flavonoids.

6.4.2 Fruit maturity and size

While total vitamin C content in citrus increases as the fruit matures, the actual concentration of vitamin C and flavonoid (hesperidin) decreases as the fruit size and volume increases (McDonald and Hildebrand, 1980; Nagy, 1980; Vandercook and Tisserat, 1989; Aparicio et al., 1990). Vitamin C content also declines late in the season after the fruit begin to experience a decrease in water content (Harding et al., 1940).

6.4.3 Production condition

Climatic conditions such as field temperature, light exposure, etc., have long been known to exert a profound effect on the external and internal quality attributes of fruit and vegetables, and have also been shown to affect the concentrations of bioactive compounds in citrus. For example, higher field temperatures have been shown to reduce citrus vitamin C content, so that fruit grown under cooler climates tend to have more vitamin C than those growing in warmer climates (Nagy, 1980). Furthermore, greater exposure to light during fruit growth and development results in greater vitamin C accumulation in the fruit. Izumi et al. (1992) found that when citrus trees received 80% or more shading for 3 months, the fruit developed significantly reduced ascorbic acid content, in both the juice and flavedo, compared with the unshaded control. Shaded fruit in the tree canopy also develop lower concentrations of vitamin C than do fruit located on the outside of the canopy (Sites and Reitz, 1951). Even the direction of sun exposure affects vitamin C content, with outer canopy fruit on the north side of the tree (in the northern hemisphere) accumulating lower levels of vitamin C than fruit on the south side. Citrus usually is grown on scions budded on to various rootstocks and these rootstocks often exert a strong influence on fruit quality. Nagy (1980) summarized the effects of rootstock on the vitamin C content of several citrus varieties, showing that the effects depended on the scion. For example, whereas Valencia orange, Marsh grapefruit and Orlando tangelo budded on rough lemon and Orlando tangelo budded on rough lemon often resulted in fruit with the least vitamin C content compared with the results budding on sour orange, the results were reversed for Temple orange (Nagy, 1980). Rootstock was also found to influence total flavonoid content of lemon juice (Gil-Izquierdo et al., 2004), the influence of rootstock on juice total flavonoid content being greater than that of the influence of the interstock.

In terms of tree nutrition, increased levels of nitrogen fertilization generally result in decreased ascorbic acid content in the juice of citrus fruit (Nagy, 1980). The same happens when phosphorus is added in excess of what is needed for optimum fruit yield (Sinclair, 1961). Conversely, higher rates of potassium fertilization generally increase ascorbic acid content (Sites, 1944). Deficiencies in minerals such as zinc, magnesium, manganese and
Copper will also result in reduced ascorbic acid content compared with soils without these deficiencies.

There is much interest in stimulating natural plant defence mechanisms to combat various plant diseases, and salicylic acid (SA) has been shown to act as an important signalling molecule in the process. Huang et al. (2008) found that ‘Cara Cara’ navel orange peel and pulp concentrations of lycopene, α-carotene, ascorbic acid, glutathione, total phenolics and total flavonoids were all increased during storage if the trees were sprayed with SA five times over a period of 71 days before harvest.

6.4.4 Postharvest treatments and storage

Postharvest treatments and storage affect the general quality of citrus fruit greatly, as well as their levels of basic nutrients and bioactive compounds. Acidic conditions help preserve ascorbic acid content in fruit and vegetables and the loss of acid is correlated with the loss of vitamin C, and these are both affected greatly by storage temperature (Nagy, 1980). For example, while storage of oranges at low temperatures (~3°C) results in little or no loss in total acids or vitamin C content, the loss of these compounds increases as storage temperatures increase (Nagy, 1980; Verma and Dashora, 2000; Al-Zubaidy and Khalil, 2007). The effect of temperature on the rate of vitamin C loss depends on the type of citrus fruit (Nagy, 1980). During storage, flavanone content tends to increase (Patil, 2004; Patil et al., 2004).

In general, vitamin C losses can also be reduced by storing fruit and vegetables under controlled atmospheres with low O2 or CO2 levels up to 10 kPa (Lee and Kader, 2000). Ultrasound (0.05 kPa) treatments were evaluated as a quarantine treatment and found to promote higher concentrations of β-carotene, lycopene and vitamin C compared with control ‘Rio Red’ grapefruit (Patil and Shellie, 2004).

Low doses of irradiation (<1 kGy) are believed generally to have no effect on the vitamin C content of fruit and vegetables (Lee and Kader, 2000). However, Mahrouz et al. (2002) found that exposure of Moroccan C. clementina (Nour) to 300 Gy resulted in significantly higher vitamin C content than found in untreated fruit during subsequent storage for 49 days at 3°C. Exposure of citrus fruit to ≤300 Gy also stimulated an increase in total phenolics (including flavonoids) and other health-promoting compounds during subsequent storage (Oufedjikh et al., 2000; Mahrouz et al., 2002; Vanamala et al., 2003, 2005, 2007; Patil et al., 2004). Higher irradiation doses, between 400 and 700 Gy, tended to result in decreased flavanone concentrations in grapefruit, especially in early season fruit (Patil et al., 2004).

In terms of processed orange juice products, frozen concentrated orange juice contained 24–55% more ascorbic acid than did ready-to-drink juice (Johnston and Bowling, 2002). After reconstituting frozen concentrates or opening ready-to-drink orange juices, all lost about 2% of their reduced ascorbic acid content per day. However, Johnston and Hale (2005) found that the ascorbic acid content of reconstituted juice decreased 24% after 8 days of storage at 4°C, but did not decrease significantly in juice not-from-concentrate (NFC).

The use of high-pressure treatments on Valencia orange juice, instead of pasteurization, resulted in increased carotenoid content (including provitamin A), but decreased ascorbic acid content compared with that in freshly squeezed juice (Sanchez-Moreno et al., 2003). After subsequent storage at 4°C for 10 days, both total ascorbic acid and carotenoid contents were lower in high-pressure treated juice compared with untreated juice. On the other hand, Butz et al. (2003) did not find reductions in vitamin C, carotenoids, or other antioxidants after high-pressure treatment of orange or lemon juices.

As in fresh fruit, acids help prevent the breakdown of vitamin C in juice products (Nagy, 1980). Phenolic compounds also help prevent the loss of vitamin C in citrus juices, by protecting against oxidative destruction (Miller and Rice-Evans, 1997). Conversely, higher concentrations of fructose in the juice result in accelerated vitamin C loss (Nagy, 1980).

The loss of vitamin C in citrus juices increases as storage duration and temperature increase (Robertson and Samaniego, 1986; Robertson and Samaniego-Esguerra, 1990).
Vitamin C is lost primarily in processed juice due to non-enzymatic aerobic and, to a much lesser extent, anaerobic reactions (Nagy, 1980). Thus, oxygen is excluded as much as possible from the juice during processing. Vitamin C loss in juices is slower when packed in plain tin cans, compared with enamel-lined cans or bottles, because tin reacts with some of the residual oxygen that would otherwise react with vitamin C (Moore et al., 1944; Nagy, 1980). However, orange juice packed in glass still lost vitamin C content substantially slower than juice packed in hermetically sealed polyethylene containers, which in turn lost vitamin C content slower than juice packed in polystyrene bottles or waxed cartons (Bissett and Berry, 1975). About 5%, and usually no more than 10%, of the vitamin C content of citrus is lost during the manufacturing of juice products (Moore et al., 1944; Nagy 1980). Knowledge of the stability and interaction of bioactive compounds in citrus and citrus products, such as flavonoids, in relation to pre- and postharvest treatment and storage, has begun to evolve in recent years thanks to the development of new analytical methods (Del Caro et al., 2004; Dhuique-Mayer et al., 2007; Miguel et al., 2009).

### 6.5 Future Research Needs

In order to evaluate the health effects of citrus fruit and citrus products in humans, additional research is needed to address the analytical challenges with the bioactive compounds themselves (sensitivity, fine structural differentiation, detection limit) and then the effect in various human tissues. The isolation and characterization of citrus bioactive compounds must include the development of effective methods for the extraction, separation, identification and quantification of the compounds. Furthermore, accurate consumption data from various populations of the world need to be assessed so that more precise public health information can be obtained. Effects of pre- and postharvest treatments have been investigated for previously known or identified compounds. However, since citrus trees may have a long lifespan, a database on long-term changes in bioactive compounds, as affected by age and other environmental factors (i.e. disease pressure) and other cultural practices (i.e. pruning), would help in evaluating the health benefits of these compounds (individually and in combination).

### 6.6 Conclusion

The apparent health benefits of citrus bioactive compounds are promising yet inconsistent, due to many factors. Despite the wealth of information from previous research, much is still unknown. Therefore, it is important that collaborative research continues across disciplines ranging from horticulture, postharvest technology, food science, chemistry, pharmacy, plant and human physiology, epidemiology and human nutrition, to name just a few, in order to understand better and to optimize the potential health benefits from citrus bioactive compounds.

### References


A.H. Simonne and M.A. Ritenour


7 Cucurbits
[Cucumber, Melon, Pumpkin and Squash]

D. Mark Hodges and Gene E. Lester

7.1 Introduction

The focus of this chapter is on the edible members of the Cucurbitaceae family, namely cucumber, sweet melon, pumpkin, squash and watermelon. The Cucurbitaceae family is comprised of a taxonomic group of closely related genera with diverse origins, with the term cucurbit (or cucurbits) denoting all species within the Cucurbitaceae. The three important food-grade cucurbit genera, Citrullus, Cucumis and Cucurbita, include the species Citrullus lanatus (watermelons), Cucumis melo (cantaloupes and other sweet melons), Cucumis sativus (cucumbers and pickles) and Cucurbita maxima, Cucur mixta, Cucur moshata and Cucur pepo (squashes, gourds and pumpkins); all have edible fruit, with the exception of gourds (Bailey and Bailey, 1976).

All species of Citrullus, Cucumis and Cucurbita have many similar morphological characteristics. Cucurbit plants are mostly monoecious, the flowers are insect pollinated and the fruit are many-seeded berries (pepos) of various exotic shapes, sizes and beautiful colours. A complete morphological and taxonomic description along with nomenclature, evolution and genetics of cucurbits can be found in Robinson and Decker-Walters (1997) and Janick et al. (2007). Two exquisite photography books are available; one on melons (Goldman, 2002) and the other on gourds, pumpkins and squash (Goldman, 2004), which capture beautifully the artistic morphology of these fruit, as well as their food attributes.

According to Maynard and Maynard (2000), cucurbits have centres of origin throughout the tropics and subtropics of Africa, South-east Asia and the Americas. Most cucurbit species are adapted to moist, warm conditions, with some adapted to warm, arid climates. All are frost sensitive, requiring protection in temperate areas or production during annual warm cycles. Currently, China is the leading producer of nearly all cucurbits (Table 7.1), although other major producing countries include Brazil, Cameroon, Cuba, Egypt, India, Iran, Russia, Spain, Turkey and the USA (USDA-ERS, 2007). The significance of cucurbits in human commerce is demonstrated by an abundance of references (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997; Maynard and Maynard, 2000; Janick et al., 2007; USDA-ERS, 2007), highlighting their major economic value.

In the USA alone the annual (2007) retail value of Citrullus, Cucumis and Cucurbita species is greater than US$2.8 billion (Table 7.2). In addition to cucurbits providing much needed economic livelihood worldwide, humans have come to depend on marketable food-grade cucurbit fruit for critical daily nutrition.
Table 7.1. Leading countries and overall world cucumber production, 2007 data.<sup>a</sup>

<table>
<thead>
<tr>
<th>Cucurbit commodity</th>
<th>Country</th>
<th>World commodity (hectares x 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantaloupe and melons</td>
<td>China (608)</td>
<td>1289</td>
</tr>
<tr>
<td>Cucumber and pickles</td>
<td>China (1604)</td>
<td>2531</td>
</tr>
<tr>
<td>Pumpkins and squash</td>
<td>India (378)</td>
<td>1546</td>
</tr>
<tr>
<td>Watermelon</td>
<td>China (2314)</td>
<td>3804</td>
</tr>
</tbody>
</table>

Note: USDA-ERS (2007).

Table 7.2. Retail values of US cucumber production and imports, 2007 data.<sup>a</sup>

<table>
<thead>
<tr>
<th>Cucurbit commodity</th>
<th>US production (US$ million)</th>
<th>US imports (US$ million)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantaloupe melon</td>
<td>313</td>
<td>156</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>228</td>
<td>471</td>
</tr>
<tr>
<td>Cucumbers (pickling)</td>
<td>167</td>
<td>77</td>
</tr>
<tr>
<td>Honeydew and other melons</td>
<td>82</td>
<td>35</td>
</tr>
<tr>
<td>Pumpkins</td>
<td>117</td>
<td>61</td>
</tr>
<tr>
<td>Squash</td>
<td>227</td>
<td>213</td>
</tr>
<tr>
<td>Watermelon</td>
<td>476</td>
<td>158</td>
</tr>
</tbody>
</table>

Note: USDA-ERS (2007).

The intent of this chapter is to provide a brief description of the botany and physiology of edible cucumber species and to discuss their phytochemical (human health interactive compound) composition and the bioavailability of some major nutrients. Pre- and postharvest factors that may influence the availability of specific major phytonutrients of fresh cucumber, cantaloupe and honeydew melon, squash, pumpkin and watermelon will also be discussed.

7.2 Botany/Physiology of Cucurbitaceae

There are many botanical similarities among the cucumber species (Robinson and Decker-Walters, 1997). Cucurbits have a strong taproot penetrating the soil to a depth of 1 m or more, with secondary roots occurring near the soil surface extending beyond the spread of aboveground stems. Stems are angled in cross section, centrally hollow, sap-filled and branched, herbaceous and often suffrutescent (softly woody), prostrate, trailing or climbing. Palmately veined leaves are usually simple with no leaflets, are three- to seven-lobed, have one leaf per node and are arranged along the stem in a helical fashion. Most cucurbits have solitary, unbranched tendrils, originating from the leaf axis, which are coiled, allowing stems to cling to supports.

Flowers are large and showy, usually orange-yellow, yellow or yellowish-white, and have symmetrically fused calyx and corolla which are cup- to bell-shaped and predominately five-lobed. Staminate and pistillate flowers are originally bisexual. During ontogeny, the pistil is retarded in staminate flowers, and undeveloped stamens can be seen in mature pistillate flowers. Stamen number is five and pistillate flowers have inferior ovaries with a fused style and lobed stigma. Floral nectaries are borne inside and at the base of both staminate and pistillate flowers.

Cucurbit fruit are extremely diverse and have signature characteristics in size, shape, colour and ornamentation familiar to all as a cucumber, melon, pumpkin, squash or watermelon. Fruit growth is sigmoidal (Sinnott, 1945), with expansion occurring continuously, but with a growth rate greater at night than during the day (Lester, 1998). Cucurbit fruit are indehiscent ‘pepos’ (pepo is a fleshy fruit with a leathery, non-septate rind derived from an inferior ovary), with one or three ovary sections or locules. Although the fruit have hard, lignified rinds, some squash cultivars have been bred to have a tender, edible rind (i.e. summer squash). Fruit contain tens to hundreds of seeds, rarely winged, usually flat with an oily embryo, rich in tocopherols, especially the larger seeds typically found in pumpkin and squash (DellaPenna and Pogson, 2006). Seed maturation, optimal for human consumption and germination, occurs after fruit senescence; mature seed germination occurs under low light at 25–30°C, with adequate non-soaking moisture, within 2–3 days.
7.2.1 Cucumber

Fruit generally are oblong or narrowly cylindrical, with small warts (tubercles) and spines, and enlarge (mostly elongate) with maturation. Spine colour is associated with maturation (Robinson and Decker-Walters, 1997). Fruit having white spines are light green to yellow at maturity and not netted. Black-spined fruit become orange or brown and often develop netting at maturity. Immature fruit have dark green internal flesh (yet are edible), whereas at maturity cucumbers have white or colourless flesh. Cucumbers have the highest moisture content (95%) of all cucurbits and contain 15 kcal, 1.0 g protein, 0.1 g fat, 3.4 g carbohydrates and 0.6 g fibre/100 g fresh weight (FW) (USDA-ARS, 2007). The biochemistry of the cucumber fruit is being studied in the USA to decipher the identity of compound Q, an extract used in China and credited with remedial and relief properties in AIDS sufferers (Hoareau and Da Silva, 1999).

7.2.2 Cantaloupe and honeydew melon

Fruit are generally round or oval, although some of the less common cultigens may be oblong (Robinson and Decker-Walters, 1997). Some cultigens have a reticulated (netted) rind (reticulatus types, e.g. cantaloupes), with various levels of aroma, while others are odourless, smooth-skinned (inodorous types, e.g. honeydew). Some less common inodorous types have a furrowed rind (e.g. Casaba); concave vein tracks (sutures) define other cultigens (e.g. Charentais). Mature rind colour varies from green, with or without whitish stripes, to yellow, tan or white. Flesh colour may be orange (β-carotene), green or white. An abscission zone at attachment of the peduncle and fruit occurs in some cultigens at physiological maturity. Melons have a moisture content of 91% and contain 32 kcal, 0.7 g protein, 0.2 g fat, 7.6 g carbohydrates and 0.5 g fibre/100 g FW (USDA-ARS, 2007). Melon seed extract, in some ‘traditional’ cultures, is used as an antidiabetic and for treating chronic or acute eczema (Yanty et al., 2007).

7.2.3 Pumpkins and squash

Plants usually have large, orange-coloured, edible flowers and the fruit come in a vast variety of sizes, shapes, colours and surface characteristics. Pumpkin is a corruption of the Old English word ‘pompion’, which stems from the Latin ‘pepo’ meaning large, ripe, round melon, but it has no botanical meaning (Robinson and Decker-Walters, 1997). Thus, all pumpkins are squashes, but are differentiated from ‘squash’ in use. Almost all pumpkins are used for pies, jack-o’-lanterns or stock feed.

Squash is a corruption of a Native American word ‘askutasguash’, meaning to be eaten raw or cooked, depending on whether the fruit is immature (summer squash) or mature (winter squash). Squash species are distinguished on the basis of peduncle, stem, leaf and seed characteristics (Robinson and Decker-Walters, 1997). Peduncles can be hard, soft, smooth and/or angular. Stems can be hard, soft, round, smooth and/or angular, whereas leaves can be lobed, deeply cut, round, palmate, soft and/or prickly. Seeds can be white, black, brown, white to tan, white to brown, smooth, wrinkled, pitted and with or without smooth or ragged margins. Pumpkins have a moisture content of 92% and contain 26 kcal, 0.1 g protein, 0.1 g fat, 6.5 g carbohydrates and 1.1 g fibre/100 g FW (USDA-ARS, 2007). Immature (summer) squash have a moisture content of 94% and contain 17–20 kcal, 0.9–1.2 g protein, 0.1 g fat, 3.6–5.1 g carbohydrates and 0.6 g fibre/100 g fresh weight (USDA-ARS, 2007). Mature (winter) squash have a moisture content of 84–88% and contain 39–54 kcal, 1.5 g protein, 0.2 g fat, 9.4–14.0 g carbohydrates and 1.4 g fibre/100 g FW (USDA-ARS, 2007). Pumpkin and squash traditionally have been useful, in terms of human health, for blood cleansing, an aid to constipation and digestion and a source of energy (Rahman et al., 2008).

7.2.4 Watermelon

Watermelon is differentiated from its family members by having pinnatified leaves, stems...
that are hairy, thin, angular and grooved, and branched tendrils (Robinson and Decker-Walters, 1997). Flowers are less showy than other cucurbits. Fruit are large, round to oblong or cylindrical, measuring as long as 60 cm. The rind is light to dark green, either solid in coloration, striped or marbled. The flesh can be bland to extremely sweet and is usually red (cis-isomers of lycopene), but there are also orange (β-carotene), salmon, yellow, green and white cultivars. The salmon and yellow cultivars of watermelon result from differing concentrations of a variety of carotenoids. Watermelon seeds can be black, brown, red, green or white; size and shape, along with colour, can be an aid in varietal identification. Seedless watermelons were developed for disease resistance, earliness of maturity, high yield, improved flesh characteristics (e.g. greater sugar content) and a durable rind. Watermelon fruit have a moisture content of 93% and contain 26 kcal, 0.5 g protein, 0.2 g fat, 6.4 g carbohydrates and 0.3 g fibre/100 g FW (USDA-ARS, 2007). In some 'traditional' cultures, watermelon fruit is used as a cooling agent to allay thirst, a source of energy, an aphrodisiac or blood purifier, and is considered good for sore eyes, scabies and itches (Rahman et al., 2008).

### 7.3 Phytochemicals

An inclusive listing of phytochemicals and their health bioactive attributes, important to humans, in cucurbits has been provided recently by Kader et al. (2004). Bioactive components/nutrients appearing in appreciable concentrations in cucurbits include carotenoids, ascorbate, folate (B₉), potassium, citrulline and phenolics.

#### 7.3.1 Ascorbate

The ascorbate dietary reference intake (DRI) for adult males is 90 mg/day (USDA-ARS, 2009). Cucurbit fruit range in total ascorbic acid (ascorbate) from as little as 9 mg/100 g FW in pumpkin to as much as 37 mg/100 mg FW in cantaloupe (Table 7.3). Less than one-half of a cantaloupe is an excellent source (supplying more than 100%) of the DRI of ascorbate. The water-soluble compound ascorbate, commonly known as vitamin C, is related structurally to C₆ sugars (C₆H₈O₆), being an aldonono-1,4-lactone of a hexonic acid (Davey et al., 2000). Ascorbate has three primary functions in plants and animals, namely: (i) as an enzyme cofactor, (ii) as an antioxidant and (iii) as donor/acceptor in plasma membrane or chloroplastic electron transport. As such, in plants it can function in a number of physiological processes such as cell division and growth, re-reduction of α-tocopherol, photosynthesis, transmembrane electron transport and stress tolerance (Hodges, 2001). In humans, ascorbate can promote health in its role as an antioxidant (and thus defend against oxidative stress-related diseases such as cancer, various neurological disorders and cardiovascular disease), and as an enzyme cofactor it can participate in collagen hydroxylation (collagen being important in the synthesis/maintenance of cartilage, skin, teeth, bones and gums) and carnitine synthesis, among others. It has been noted that ascorbate can exhibit pro-oxidant activity in the presence of metal ions; however, there is no evidence that this is a significant problem in vivo.

#### 7.3.2 Carotenoids

The β-carotene (provitamin A) DRI for adult males is 1800 µg/day (USDA-ARS, 2009). Cucurbit fruit range in β-carotene (Fig. 7.1) from as little as 30 µg/100 g FW in green-flesh honeydew melon to as much as 3100 µg/100 mg FW in pumpkin (Table 7.3). Thus, cantaloupe, orange-fleshed honeydew melon and pumpkin are an excellent source of carotenoids (i.e. provitamin A) as they provide more than 100% DRI of vitamin A. The lipophilic carotenoids are essentially long chains of conjugated double bonds and are differentiated by cyclization of the end group or by addition of oxygen (Rao and Rao, 2007). Carotenoids often provide the cucurbits with their distinctive colours; for example the orange-yellow pigment of cantaloupe and orange-fleshed honeydew melon is attributable to β-carotene, while the red hue of certain
Table 7.3. Bioactive/nutrient content per 100 g fresh weight of various cucurbits. Unless otherwise noted, data are from the USDA Nutrient Database (http://www.nal.usda.gov/fnic/foodcomp/search/).

<table>
<thead>
<tr>
<th>Phytonutrient</th>
<th>Cantaloupe</th>
<th>Green-fleshed honeydew melons (orange-fleshed)</th>
<th>Cucumber</th>
<th>Pumpkin</th>
<th>Summer squash</th>
<th>Winter squash</th>
<th>Watermelon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ascorbate (mg)</td>
<td>36.7</td>
<td>18 (20.7)a</td>
<td>2.8</td>
<td>9</td>
<td>17</td>
<td>12.3</td>
<td>8.1</td>
</tr>
<tr>
<td>β-Carotene (µg)</td>
<td>2020</td>
<td>30 (3000)a</td>
<td>45</td>
<td>5100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>α-Carotene (µg)</td>
<td>16</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lycopene (µg)</td>
<td>1</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4532</td>
</tr>
<tr>
<td>β-Cryptoxanthin (µg)</td>
<td>0</td>
<td>0</td>
<td>2145</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>78</td>
</tr>
<tr>
<td>Lutein/zeaxanthin (µg)</td>
<td>26</td>
<td>27</td>
<td>1500</td>
<td>2125</td>
<td>38</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>L-citrulline (mg)</td>
<td>ND</td>
<td>ND</td>
<td>16</td>
<td>29</td>
<td>24</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total folate (µg)</td>
<td>21</td>
<td>19</td>
<td>7</td>
<td>16</td>
<td>29</td>
<td>24</td>
<td>240c</td>
</tr>
<tr>
<td>Total phenolics (mg)</td>
<td>24a</td>
<td>25 (30)a</td>
<td>20d</td>
<td>7e</td>
<td>83.3f</td>
<td>83.3f</td>
<td>6.4g</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>267</td>
<td>228</td>
<td>147</td>
<td>340</td>
<td>262</td>
<td>350</td>
<td>112</td>
</tr>
</tbody>
</table>

Fig. 7.1. Carotenoids were separated by HPLC-DAD on a C-18 column by the method of Okuno et al. (1998). \( \beta \)-Carotene was extracted from Cucumis melo (cv. Cruiser) mesocarp tissue with heptane and monitored at 454 nm. \( \beta \)-Apo-8'-carotenal (internal standard) elutes at \(-3.5\) min and \( \beta \)-carotene elutes at \(-15\) min. \( \beta \)-Carotene levels determined for this melon sample (per g fresh weight) were 26.3 \( \mu \)g/g.

cultivars of watermelon is due to lycopene (Fig. 7.2). In plants and humans, carotenoids are essential as accessory pigments in light harvesting, function as photoprotectants (e.g. skin cancer; Heinrich et al., 2003) and, as active oxygen scavengers (particularly singlet oxygen), serve as precursors for such compounds as abscisic acid and serve to quench the excited triplet state of chlorophyll. Approximately 600 carotenoid compounds have been characterized, and of these about 50 are consumed in the human diet, though only 12 account for the majority of the intake (Voutilainen et al., 2006). The most commonly consumed primary carotenoids from cucurbits include \( \beta \)-carotene and lycopene, with additional secondary carotenoids including \( \alpha \)-carotene, \( \beta \)-cryptoxanthin and lutein/zeaxanthin.

Consumption of carotenoids has been associated with reduced incidences of various forms of cancer, AMD (advanced macular degeneration), cardiovascular diseases and osteoporosis; linkages have also been made between carotenoids and diseases such as hypertension, various neurodegenerative diseases (e.g. Alzheimer’s, Parkinson’s, amyotrophic lateral sclerosis) and emphysema (Voutilainen et al., 2006; Rao and Rao, 2007).

Cucurbits can be an abundant source of various carotenoids (Table 7.3). The health functionality of carotenoids has been attributed primarily to their antioxidant activities (e.g. protection of low-density lipoproteins, scavenging of active oxygen). In addition, they have also been shown to play roles in such processes as immune response, modulation of particular drug-metabolizing enzymes, regulation of cell growth and gap junction communications (Rao and Rao, 2007). Moreover, \( \alpha \)- and \( \beta \)-carotene, as well as \( \beta \)-cryptoxanthin, function as precursors to vitamin A. However,
124

D.M. Hodges and G.E. Lester

HPLC of carotenoids of red watermelon

Fig. 7.2. Carotenoids were separated on a C-30 column by the method of Craft (2001). The carotenoids were extracted from a ripe watermelon (cv. Sangria) with hexane before injection on to the column. Eluting carotenoids were monitored at 503 nm. Cis-isomers of lycopene elute at ~ 45, 46 and 51 min. The carotenoid levels determined for this particular watermelon (per g fresh weight) were 31.7 µg/g trans-lycopene, 1.2 µg/g cis-isomers of lycopene and 4.9 µg/g β-carotene. (Chromatogram courtesy of W. Fish, USDA-ARS, Lane, Oklahoma).

as the intervention study with smokers famously demonstrated (Alpha-Tocopherol, Beta-Carotene Cancer Prevention Group, 1994), pharmacological levels (as opposed to normal dietary levels) of supplementation with β-carotene can lead to pro-oxidant activities, thus promoting active oxygen species-related dysfunctions such as cancer and heart disease. The results of this and other trials have led to the postulation that β-carotene promotes health when consumed at normal dietary levels, but may have detrimental affects when taken at higher levels. There is now a moratorium on similar intervention studies with β-carotene and the focus has switched to lycopene.

7.3.3 Folate

The folate DRI for adult males is 400 µg/day (USDA-ARS, 2009). Cucurbit fruit range in folate from as little as 9 µg/100 g FW in cucumber to as much as 29 µg/100 mg FW in pumpkin (Table 7.3), making cucurbit fruit a good, but not an excellent, source of the DRI for folate. Tetrahydrofolate (composed of a pterin, a p-aminobenzoic acid and a glutamate chain with a number varying from 1 to 14 moieties) and its derivatives (which differ in the oxidation state of the pterin ring, the number of glutamate moieties and in the oxidation state of the transfer C1 unit) are commonly grouped under the name of folates (Rêbeillé et al., 2006). These compounds are involved in ‘carbon one’ (C1) transfer reactions, which occur in important plant and animal cycles: (i) the DNA biosynthesis cycle where C1 is donated during synthesis of purines and pyrimidines; and (ii) the methylation cycle whereby the folates participate in methylation reactions via S-adenosyl methionione (Scott et al., 2000). They are also involved in the biosynthesis of such compounds as methionine, pantothenate, glycine and serine. Additionally, in plants, folates are involved in lignin formation and photorespiration (Basset et al., 2005).

Humans cannot synthesize folate, thus plant foods are the most important sources of folate (Scott et al., 2000). Folate deficiency in humans has been associated with neural tube defects, higher risk of cardiovascular diseases, neurodegenerative diseases such as Alzheimer’s and increased incidences of colorectal, breast, pancreatic, bronchial and cervical cancers, as well as leukaemia (Lucock et al., 2003, as referenced in Rébeillé et al., 2006).

7.3.4 Potassium

The potassium (K) DRI for adult males is 3500 mg/day (USDA-ARS, 2009). Cucurbit
fruit range in K from as little as 147 mg/100 g FW in cucumber to as much as 350 mg/100g FW in winter squash (Table 7.3), making cantaloupe, pumpkin, and squash good sources of potassium DRI. There are many reviews that describe the role of potassium (K) in plant metabolism. Suffice to say for this chapter that, in plants, K plays important roles in enzyme activation, nutrient/assimilate transport into sink organs, photosynthesis and other energetic processes, cell-wall elasticity and osmotic potential (Jordan-Meille and Pellerin, 2008). A potassium deficiency can alter plant metabolism; examples include reduced photosynthetic CO₂ fixation and impairment in partitioning/utilization of photosynthates (Cakmak, 2005). With regards to human health, potassium is a highly significant mineral. Potassium, along with sodium, can regulate the water and the acid-base balance in blood and tissues. It helps with normal kidney function and, through its participation in electrical potential gradient generation, plays a role in nerve impulses and cardiac, smooth and skeletal muscle contraction. Potassium is required in protein synthesis and in carbohydrate metabolism. Potassium deficiencies have been associated with high blood pressure, stroke, inflammatory bowel disease and asthma. Low K diets lead to increased calcium losses through excretion (thereby influencing osteoporosis) (Lanham-New, 2008).

7.3.5 L-Citrulline

Few natural foods are enriched in L-citrulline but this amino acid is found in abundance in the edible tissues of watermelon (Ci. lanatus) (Rimando and Perkins-Veazie, 2005) and at lower levels in most members of the cucurbit family. In adults, L-citrulline is converted to arginine, a substrate for endothelial production of nitric oxide (NO; a vasodilator), an agent considered to be important in the regulation of blood pressure and cardiovascular health. Studies with L-citrulline have also shown that this amino acid can increase the capacity of red blood cells to transport oxygen, and thus can act as a treatment in sickle cell anaemia (Waugh et al., 2001). A recent study indicated that rats dosed with synthetic L-citrulline/arginine reduced glucose uptake and weight gain, and improved aortic flexibility (Wu et al., 2007). Currently, human intervention trials, feeding watermelon, are under way to determine the impact of pre-operation ingestion of L-citrulline/arginine on aiding patients' recovery from general anaesthesia (Mark Arney, National Watermelon Promotion Board, 2009, personal communication).

7.3.6 Phenolics

The phenolic structure is characterized by at least one aromatic ring bearing one or more hydroxyl groups; various subgroups are distinguished by their number of carbons and the basic structure of the carbon skeleton (Hodges and Kalt, 2003). The structure of the phenolic compound influences its bioactivity with regards to human health, and a myriad of health-promoting activities has been ascribed to phenolics and their derivatives. Hydroxyl groups and conjugated double bonds allow the phenolic to assume an antioxidant function through electron donation, metal ion (e.g. Cu³⁺, Fe²⁺) chelation and active oxygen quenching.

Induction of antifungal control using cucumber phenolics (ferulic acid and p-hydroxybenzoic acid) has been effective in controlling microbial populations; but little is known of cucurbit phenolic antifungal properties with humans (Yu, 2001). However, as a last general example of the claimed bioactive effects of phenolics, high phenolic content has been associated with hypocholesterolaemic and hypotriglyceridaemic activities in hamsters (Lin et al., 2008).

7.4 Cucurbits and Human Health

The majority of studies on the direct health-promoting effects of cucurbit consumption have focused on carotenoid bioavailability comparisons with other horticultural products. For example, a study compared bioavailability of lycopene and β-carotene between watermelon and tomato in non-smoking humans (Edwards et al., 2003).
Four treatments were used: (i) control (base diet); (ii) base diet plus 20.1 mg/day lycopene and 2.5 mg/day β-carotene from fresh-frozen watermelon juice; (iii) base diet plus 40.2 mg/day lycopene and 5.0 mg/day β-carotene from fresh-frozen watermelon juice; and (iv) base diet plus 18.4 mg/day lycopene and 0.6 mg/day β-carotene from canned tomato juice. Increases in plasma concentrations of lycopene (100-200%) were similar between the ~20 mg/day treatments of watermelon and tomato juices; increases in lycopene bio-absorption were not dependent on heat treatment of the fresh-frozen watermelon juice. A dose-response effect did not occur in either lycopene or β-carotene plasma levels following the doubling of the watermelon dosage, though there was a large degree of variation; the background diet included <0.4 mg/day lycopene but 0.84 mg/day β-carotene, and the statistical power was weakened as only half the subjects consumed the double dose.

In another carotenoid bioavailability study, spinach (Spinacia oleracea) serving portions containing 3.0 mg/day and pumpkin squash (Cucur moschata) serving portions containing 1.4 mg/day β-carotene were fed to 7- to 12-year-old children for 3 weeks following a 3-week run-in period of 0.8 mg/day β-carotene from long yard beans (Vigna unguiculata) (Van Lieshout et al., 2003). β-Carotene bioavailability from pumpkin was found to be 1.7-fold greater than that from spinach when measured in the serum, emphasizing the importance of the food matrix/composition with regards to bioavailability. In comparing the bioavailability of β-carotene between spinach leaves and pumpkin, the ease of freeing this compound from pumpkin chromoplasts (where it is dissolved in oil droplets) relative to extracting it from leaf chloroplasts, along with the possible presence of dietary fibre in the spinach which may bind to the β-carotene molecules, may account for the greater bioavailability from pumpkin (de Pee et al., 1998).

### 7.5 Preharvest and Postharvest Effects on Bioactive Content

Market quality characteristics, maturity indices, grades, sizes, packaging and postharvest storage protocols and disorders have been summarized for cucumber (Saltveit, 2004), melon (Lester and Shellie, 2004; Shellie and Lester, 2004), squash and pumpkin (Brecht, 2004; McCollum, 2004) and watermelon (Rushing, 2004). These important parameters, having been well documented recently, along with genetic diversity, physiology and biotechnology features of melon (Nunez-Palenius et al., 2008), will not be addressed here. Instead, consideration will be given to pre- and postharvest environmental and some genetic factors influencing production and retention of human health bioactive compounds.

#### 7.5.1 Cucumber fruit

A 240 ml serving of cucumber puree (peel included, weighing 150 g FW) provides 3.8% of the DRI for provitamin A (as β-carotene) and 4.7% of the DRI for ascorbate. Although there is a considerable amount of published literature on pre- and postharvest factors that influence the bioactive content of cucumbers, the vast majority of this work focuses on seedlings, roots or leaves versus fruit. In a postharvest study, cucumber fruit were stored for 15 days in 5, 21 or 100% kPa O₂ at 5, 10 or 20°C (Srilaong and Tatsumi, 2003). Among the parameters measured were antioxidant enzyme (superoxide dismutase (SOD), catalase) activities and compound (ascorbate, glutathione) concentrations. At 5°C catalase activities were higher in fruit stored in 21% kPa O₂; however, the typical ascorbate decline in postharvest products was not affected by storage atmosphere.

#### 7.5.2 Cantaloupe and honeydew melon

Orange-fleshed varieties provide more than 100% (per 236 g FW) of the DRI of β-carotene and more than 33% of the DRI for ascorbic acid. Green-fleshed varieties provide slightly less than one-third of the daily requirement of ascorbic acid; but both melon types provide an extensive list of bioactive nutrients and minerals (Lester, 1997). Melons are a good source of two additional important human health bioactive components, potassium (K)
and 5-methyl-tetrahydrofolic acid (folate or B9), even though concentrations may not satisfy the recommended daily allotment (Lester and Crosby, 2002). Concentrations of vitamins (ascorbic acid, β-carotene, folate) and K in melon fruit are affected by environmental factors such as soil texture (sand versus clay), production season (autumn versus spring) and production year (Table 7.4). Compared with sandy soils, clay produces fruit with generally significantly higher concentrations of ascorbic acid, β-carotene, folate and K. High mineral soils (clay) are naturally higher in the cation exchange capacities necessary for the biosynthesis of many biochemical processes, especially vitamins, found in melon fruit (Marschner, 2002). In the USA, autumn-grown melon fruit, when compared with spring-produced, have higher levels of vitamins and minerals due to cooler autumn temperatures, allowing for a greater retention of phytonutrients. Comparison of production years also had an impact, likely due to a combination of photosynthetic flux (light) and temperature variations from year to year. Although environmental factors impact vitamin and K concentrations in melon fruit, genetics appear to have an even greater influence. The ranges in ascorbic acid, β-carotene, folate and K concentrations are greatest when mature fruit are harvested based on marketable size classifications. The range in phytonutrient concentrations due to fruit size is even greater when comparing cultivars. A strong genetic effect is a positive finding because it is easier to control for cultivar and fruit size in any given production site than it is to maintain consistent natural light and temperature variations on a seasonal and annual basis.

The edible tissue of Cucum melo cultivars is known to be sweeter the closer it is to the seed cavity (Lester and Dunlap, 1985). Human health-promoting compounds, ascorbic acid (vitamin C), β-carotene, (provitamin A) and folic acid, also are more concentrated the closer the edible tissue is to the seed cavity, as are enzymatic antioxidants (ascorbate peroxidase, catalase and SOD) and the health-promoting mineral K (Lester, 2008). Enzymatic antioxidants like the aforementioned usually are destroyed in the human gut on ingestion. However, the biopolymeric matrix of muskmelon (Cucum melo) fruit, i.e. the edible tissue, protects active molecules like SOD against the digestive process by interacting with the intestinal epithelial barrier, allowing for bioabsorptivity (Vouldoukis et al., 2004).

Postharvest decay of melon fruit is reduced when melons are stored between 4 and 10°C (Lester and Shellie, 2004; Shellie and Lester, 2004). Various melon cultivars stored as whole fruit at 5 or 10°C for 17 or 24 days and then sampled for ascorbic acid, β-carotene, folate and K content showed the greatest retention of vitamins at the coldest temperature (5°C) over the shortest period (17 days) (Table 7.5). Storage duration and temperature have no effect on K concentrations. However,

### Table 7.4. Preharvest environmental/genetic influence on melon fruit vitamin and K concentrations.
Data from Lester and Eischen (1996), Lester and Crosby (2002) and Lester and Hodges (2008).

<table>
<thead>
<tr>
<th>Environmental/genetic factors</th>
<th>Ascorbic acid (µg/g FW)</th>
<th>β-Carotene (µg/g FW)</th>
<th>Folate (µg/g FW)</th>
<th>Potassium (mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay soil</td>
<td>160–250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21–26</td>
<td>0.38–0.68</td>
<td>1.6–2.0</td>
</tr>
<tr>
<td>Sandy soil</td>
<td>140–220&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17–21</td>
<td>0.29–0.57</td>
<td>1.3–1.7</td>
</tr>
<tr>
<td>Autumn</td>
<td>160–220&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12–19</td>
<td>0.36–0.45</td>
<td>2.1–3.8</td>
</tr>
<tr>
<td>Spring</td>
<td>140–180&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10–16</td>
<td>0.57–0.69</td>
<td>2.1–2.9</td>
</tr>
<tr>
<td>Year</td>
<td>150–260&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15–21</td>
<td>0.28–0.68</td>
<td>1.6–2.0</td>
</tr>
<tr>
<td>Cultivar</td>
<td>140–260&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17–21</td>
<td>0.28–0.69</td>
<td>1.6–2.0</td>
</tr>
<tr>
<td>Fruit size</td>
<td>70–250&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12–21</td>
<td>0.20–0.68</td>
<td>1.2–2.0</td>
</tr>
</tbody>
</table>

Notes: *Range of all cultivars at the same size across seasons, and years; †range of all cultivars at the same size across soil types, seasons, and years; ‡range of all cultivars at the same size across seasons; §range of all cultivars across sizes, soil types, seasons and years; ¶range of all sizes, across cultivars, soil types, seasons and years.
Table 7.5. Postharvest storage temperature and duration influence on melon fruit vitamin and K concentrations. Data from Lester and Hodges (2008).

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Ascorbic acid (µg/g FW)</th>
<th>β-Carotene (µg/g FW)</th>
<th>Folate (µg/g FW)</th>
<th>Potassium (mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17°C</td>
<td>5</td>
<td>124-181°C</td>
<td>9-16</td>
<td>0.48-0.59</td>
</tr>
<tr>
<td>17°C</td>
<td>10</td>
<td>122-173°C</td>
<td>7-15</td>
<td>0.28-0.35</td>
</tr>
<tr>
<td>24°C</td>
<td>5</td>
<td>123-179°C</td>
<td>9-16</td>
<td>0.30-0.53</td>
</tr>
<tr>
<td>24°C</td>
<td>10</td>
<td>90-142°C</td>
<td>7-12</td>
<td>0.26-0.29</td>
</tr>
</tbody>
</table>

Notes: a Fruit were stored 14 days at 5 or 10°C, followed by 3 days at 20°C; b fruit were stored 21 days at 5 or 10°C, followed by 3 days at 20°C; c range across all cultivars.

Some minerals such as calcium do decrease in melon with storage duration (Lester and Grusak, 1999, 2001). Of the vitamins (ascorbic acid, β-carotene, folate) that decrease in content during storage, folate demonstrates the greatest rate of decline, followed by ascorbic acid then β-carotene.

Volatile or aroma compounds generally are not considered as having direct health-promoting properties. However, 3-methylthiopropionic acid ethyl ester (MTPE), found in certain Cucum melo varieties, has anticancer properties (Nakamura et al., 2008). Japanese pickling melon (Cucum melo var. conomon) at the overly ripe fruit stage and orange-fleshed muskmelon (Cucum melo var. reticulatus) are the only Cucumis species to have significant concentrations of MTPE. As an anticancer compound, MTPE targets human colon cancer cells. The only down side is that the average MTPE concentrations found in orange-fleshed melon and overly ripe Japanese pickling melon are 2.5 and 3.8 µg/100 g FW, respectively; thus ~ 1000-fold too dilute. These melon varieties, therefore, would have to be processed and the volatile concentrated to have a pharmacological effect.

7.5.3 Pumpkin and squash

A 240 ml serving of pumpkin squash puree (~ 140 g FW) offers about 250% of the DRI of β-carotene and about 10% of the required K. Pumpkin also represents an appreciable source of α-carotene, β-cryptoxanthin and lutein/zeaxanthin. A 140 g FW portion of winter squash (e.g. acorn squash, buttercup squash) generally contains 65 and 10.4% of the recommended intakes of β-carotene and K, respectively. Summer squash (e.g. zucchini/courgette) provides 8% of the daily requirements of β-carotene, 22.7% of ascorbate and 6.6% of K in a 120 g (FW) cup.

Not only can production/techniques, growth environment (elevated temperature and light favour carotenoid synthesis) and maturity/ripening stage affect carotenoid levels drastically (Rodriguez-Amaya et al., 2008), but carotenoid content can also be highly dependent on the often overlooked cultivars within a genotype. For example, α-carotene levels ranged from 0.42 to 7.5, β-carotene from 1.4 to 7.2 and lutein/zeaxanthin from 0.8 to 17 mg/100 g FW in 12 varieties of Cucur maxima grown under similar conditions (albeit only for 1 year) (Murkovic et al., 2002). However, a detailed listing of specific cultivars and their individual carotenoids was not included.

Processing and storage can also have dramatic effects on the bioactive content of postharvest commodities (Hodges and Toivonen, 2008). Concentrations of β-carotene were found to increase dramatically (0.065-0.733 mg/100 g FW) in whole pumpkins stored for 3 months, concomitant with darkening flesh colour, although the storage conditions were not specified in this study (Chavasit et al., 2002). With respect to processing/food preparation, total phenolics and total antioxidant activity (as measured by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method) were assessed in squash (type not reported) prior to cooking and following microwaving (100 g squash in 6 ml water microwaved for 1 min), steaming (7.5 min) or
boiling (100 g squash in 150 ml water boiled for 5 min) (Turkmen et al., 2005). All three processing protocols resulted in decreases in total phenolic contents of about 34%, with no significant difference in losses noted between cooking procedures. Although these cooking protocols induced an apparent increase in total antioxidant capacities (by approximately 132%), post-cooking values were not significantly different from the control data.

Pumpkin and gourd seeds are an excellent source of health-promoting oils (Stevenson et al., 2007). Nearly 100 different pumpkin and gourd varieties were assayed for seed oil content, fatty acid composition and tocopherol content. Oil content ranged from 11 to 31% of the seed weight, and the oil total unsaturated fatty acids content ranged from 73 to 81%. α-Tocopherol, the predominant tocopherol in plant tissues and the molecular form with relatively all the vitamin E activity (DellaPenna and Pogson, 2006), ranged from 75 to 500 µg/g oil. This study extracted oil from fresh-frozen seeds and concluded pumpkin seeds were a good commercial source of highly unsaturated oil and tocopherol, with the potential to improve the nutrition of human diets. The authors did not assay seeds as a roasted product, or study how seed harvesting conditions, storage period, storage environment and processing procedures influenced oil characteristics.

7.5.4 Watermelon

Watermelon fruit is an excellent source of antioxidant vitamin C and provitamin A. A 240 ml serving of watermelon puree (156 g FW) provides 13.5% of the DRI for vitamin C, 25.2% of vitamin A (from β-carotene), 11% of vitamin B₆ (pyridoxine), 8% of vitamin B₁ (thiamine), 3.6% of potassium and 4% of magnesium. Red and pink coloured watermelon cultivars are excellent sources of lycopene and the predominant tocopherol in plant tissues and the molecular form with relatively all the vitamin E activity (DellaPenna and Pogson, 2006), ranged from 75 to 500 µg/g oil. This study extracted oil from fresh-frozen seeds and concluded pumpkin seeds were a good commercial source of highly unsaturated oil and tocopherol, with the potential to improve the nutrition of human diets. The authors did not assay seeds as a roasted product, or study how seed harvesting conditions, storage period, storage environment and processing procedures influenced oil characteristics.

7.6 Future Research Needs

Cucurbits, a family of diverse species, are in fact limited in germplasm (i.e. parental lines) used in breeding current commercial cultivars (Kevin Crosby, cucurbit breeder, Texas A&M, 2008, personal communication). Selection of potential commercial cultivars is based almost entirely on disease and insect resistance, suitable phenotype and sugar content (e.g. sweet melon), whereas postharvest keeping quality or human health-promoting properties are largely ignored. Exotic cucurbit germplasm, likely available from national/international seed bank repositories and commercial seed companies' heirloom selections, represent a potentially untapped phytonutrient source and should be investigated for variability in increases plasma arginine concentration in adults (Collins et al., 2007).

Minimal processing (fruit cutting, packaging and chilling) of watermelon pieces does not affect their nutritional content significantly even after 6, and up to 9, days' storage at 5°C; no loss in carotenoids occurred and vitamin C declined only 5% (Gil et al., 2006). A study of carotenoid changes in three types of watermelon (open-pollinated seeded, hybrid seeded and seedless) stored at 5, 13 or 21°C for 14 days found carotenoid levels increased in fruit stored at 21°C versus fresh-harvested fruit (Perkins-Veazie and Collins, 2006). Watermelons stored at 21°C gained between 11 and 40% in lycopene, and β-carotene content increased by between 50 and 139%; fruit stored at 5°C and 13°C, however, showed very small changes in carotenoid content.

Lycopene, the carotenoid that gives the characteristic red colour in watermelon, degrades even at freezing (~20°C) temperatures (Fish and Davis, 2003), whereas β-carotene, the carotenoid that gives the characteristic orange colour in muskmelon, squash and orange-fleshed watermelon, is more heat stable and is increased with cooking time and temperature (Park, 1987). It is hypothesized that the enzyme, lypoxygenase, which is associated with carotene degradation, is degraded with heating, thus preserving β-carotene (Reeve, 1943).
the critical human health compounds such as ascorbic acid, carotenes, folates and other B vitamins, tocopherols, phyloquinone and potassium. Other, lesser known health compounds such as essential amino acids (e.g. citrulline/arginine), phenolics and enzymatic antioxidants (e.g. SOD; the mesocarp matrix unique to the Cucum melo species provides a protective layer keeping SOD viable during the digestive process, and, therefore, is able to elicit in vivo pharmacological effects (Vouldoukis et al., 2004)), also require further investigation in current and future cucurbit germplasm. Besides conventional breeding and molecular engineering approaches (e.g. to alter regulatory factors that influence bioactive content), numerous preharvest production factors as well as postharvest protocols can influence bioactive content (for reviews, see Lee and Kader, 2000; Hodges et al., 2004; Hodges and DeLong, 2007). Once cucurbit cultivars inherently enriched in bioactives are identified/developed, production and postharvest technologies designed to enhance optimally bioactive content can be characterized.

7.7 Conclusions

Although cucurbits are an excellent source of a variety of bioactive compounds, it is the outstanding carotenoid content of certain cucurbits that places them in the top tier of fruit and vegetables with health-promotion properties. Pumpkin, cantaloupe melon, orange-fleshed honeydew melon and watermelon, in particular, are commonly consumed members of the Cucurbitaceae containing good to excellent carotenoid concentrations (β-carotene for pumpkin, cantaloupe and orange-fleshed honeydew melon and lycopene for watermelon); in all cases, a 240 ml serving contains enough carotenoids to meet the recommended DRI. The netted rind of cantaloupe can harbour illness-related enteric bacteria such as Salmonella Lignieres, Shigella Chatellani and Dawson and Escherichia coli O157:H7; however, there are a number of cultivars or an alternative cucurbit, orange-fleshed honeydew melon, that contain equal or greater amounts of carotenoids and other bioactives (Lester and Hodges, 2008). The concept of growing crops for health rather than just for food or fibre is slowly diversifying the focus of plant biotechnology and medicine and has been well reviewed by Raskin et al. (2002). There are a number of other avenues to enhancing bioactive content besides conventional/molecular breeding and cultivar/species selection. For example, growing practices, as demonstrated by the relatively few well-conducted studies comparing organic versus conventional and sustainable cropping systems on plant phytonutrients, have demonstrated a production system effect, and this should be investigated further in cucurbits. Additionally, now that the US Food and Drug Administration is clearing more and more fruit and vegetables for food safety irradiation, updated investigations into the effect of ionizing irradiation on cucurbits must occur, given ‘irradiation treatments can stimulate the biosynthesis of bioactive compounds’ (Lacroix and Vigneault, 2007). These and other methods of enhancing the already notable bioactive content of cucurbits will result in consumer access to cucurbits such as melon, squash and pumpkin enriched in health-promoting compounds. As bioabsorption of bioactive compounds within a natural food matrix has often been considered to be more effective than with supplements/nutraceuticals (e.g. Kader et al., 2004), the enrichment of cucurbits already high in bioactive complements will almost certainly improve consumer health and well-being.

References


Reeve, R.M. (1943) Microscopy of the oils and carotene bodies in dehydrated carrot. *Food Research* 8, 137–141.


8 Exotics
[Litchi, Longan, Rambutan, Pomegranate, Mangosteen, Kiwifruit, Passion Fruit, Persimmon, Carambola]

Nettra Somboonkaew and Leon A. Terry

8.1 Introduction
Exotic fruit are usually subtropical to tropical in origin. They are becoming more important fresh commodities in both producer and overseas countries. Exotic fruit are a significant source of potential functional dietary agents, for example, phenolic compounds, ascorbic acid and provitamin A (Wall, 2006). However, detailed information about the potential health benefits of fresh exotic fruit is limited. As a consequence, and for the purpose of this chapter, the nutritional and medicinal benefits of nine exotic fruit, namely litchi, rambutan, longan, mangosteen, kiwifruit, persimmon, passion fruit and carambola, are discussed.

8.2 Identity of Bioactive Compounds
Fruit are an important source of bioactive substances, e.g. provitamin, plant pigments and polyphenolic compounds (Zhang et al., 2001). Increasingly, edible plant products rich in phytochemicals are recognized worldwide for their potential to reduce the risk of several diseases, including cancer, diabetes and cardiovascular disease (Charoensiri et al., 2009), and to promote human health. The extraction of fruit and the isolation and purification of active substances are dependent on the type of plant material, extraction solvent and isolation methodology. The extraction solvents are often acetone, methanol, ethanol and water, while acetonitrile, methanol, ethanol, acid and base and water are common chemicals used in isolation methods (Table 8.1).

8.3 Litchi, Longan and Rambutan
Litchi (Litchi chinensis Sonn.; Fig. 8.1) and longan (Dimocarpus longan Lour. or Euphoria longana Lam.; Fig. 8.2) are subtropical to tropical fruit, while rambutan (Nephelium lappaceum Linn.; Fig. 8.3) is a warm-tropical fruit. They belong to the family Sapindaceae. Litchi and longan originated in China, whereas rambutan was derived from Malaysia and the Sumatra region. However, the fruit are now grown widely in North Australia, South-east Asia, South Asia, Israel, South and equatorial Africa, Madagascar, Central America and the USA (O’Hare, 1997).

The edible portion of litchi and longan is sweet and very fragrant. Rambutan pulp is similar in flavour to the litchi but less aromatic, while the texture is firmer and not as juicy. The pericarp colour of litchi and rambutan varies between a yellow and crimson, whereas the pericarp of longan is light brown.
Table 8.1. Analytical methods for health-related compounds in exotic fruit.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method: column</th>
<th>Extracting</th>
<th>Mobile phase</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>and other carotenoids</td>
<td>HPLC: C-18</td>
<td>KOH</td>
<td>CH₃CN: THF: CH₃OH: TEA + ammonia acetate</td>
<td>Charoensiri et al. (2009)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>HPLC: C-18</td>
<td>H₂PO₄</td>
<td>NaH₂PO₄</td>
<td>Wall (2006)</td>
</tr>
<tr>
<td></td>
<td>HPLC: RP-18</td>
<td>H₃PO₄</td>
<td>Bu₄NOH: CH₃OH: KH₂PO₄</td>
<td>Nishiyama et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>HPLC: LC-NH₂</td>
<td>OxaH₂DCPIP</td>
<td>−</td>
<td>Al-Maiman and Ahmad (2002)</td>
</tr>
<tr>
<td></td>
<td>HPLC: Supercosil HC-18</td>
<td>H₂O</td>
<td>75% Formic acid: 25% Acetonitrile (pH 5.0)</td>
<td>Vinci et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Titrimetric method</td>
<td>CH₃COOH</td>
<td>−</td>
<td>AOAC (2000)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>HPLC: C-18</td>
<td>KOH</td>
<td>CH₃OH</td>
<td>Charoensiri et al. (2009)</td>
</tr>
<tr>
<td>Organic acids</td>
<td>HPLC: C-18</td>
<td>H₂O</td>
<td>0.2% H₂PO₄</td>
<td>Somboonkaew and Terry (2010)</td>
</tr>
<tr>
<td></td>
<td>Capillary electrophoresis</td>
<td>−</td>
<td>CTA8 (pH 2.8)</td>
<td>Tezcan et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>HPLC: C-18</td>
<td>H₃PO₄</td>
<td>H₂SO₄ (A), CH₃OH (B)</td>
<td>Ozgen et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Capillary electrophoresis</td>
<td>−</td>
<td>C₆H₄N₂O₄ (pH 12.85)</td>
<td>Tezcan et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>HPLC: C-18</td>
<td>CH₃COOH: H₂O</td>
<td>H₂O</td>
<td>Somboonkaew and Terry (2010)</td>
</tr>
<tr>
<td></td>
<td>Exsil amino</td>
<td>H₂O</td>
<td>CH₃CN: H₂O</td>
<td>Ozgen et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>HPLC: LC-NH₂</td>
<td>−</td>
<td>−</td>
<td>Al-Maiman and Ahmad (2002)</td>
</tr>
<tr>
<td></td>
<td>AOAC method</td>
<td>−</td>
<td>−</td>
<td>Yapo and Koffi (2008)</td>
</tr>
<tr>
<td>Fibre</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>FAMEs</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Phenolics</td>
<td>HPLC: C-18</td>
<td>50% C₃H₆O: 50% H₂O: 2% Acetic acid (A), 10% Acetonitrile: 13%</td>
<td>Prasad et al. (2009)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPLC: Nucleosil</td>
<td>CH₃OH: HCl</td>
<td>H₂SO₄ (A), CH₃CN: H₂O: H₂SO₄ (B)</td>
<td>Sami-Manchado et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>GC-MS: capillary column</td>
<td>−</td>
<td>−</td>
<td>Zadernowski et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>HPLC: RP Amide C16</td>
<td>CH₃OH</td>
<td>H₂O: CH₃CN: H₂SO₄</td>
<td>Mahattanatavee et al. (2006)</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>HPLC: LC-18</td>
<td>CH₃CN: C₃H₆O</td>
<td>−</td>
<td>Talcott et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>HPLC: C-18</td>
<td>CH₃OH</td>
<td>H₂O: CH₃OH: H₂PO₄</td>
<td>Chen et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>HPLC: silica gel</td>
<td>C₂H₅OH</td>
<td>Petroleum ether: C₂H₅O₂</td>
<td>Somboonkaew and Terry (2010)</td>
</tr>
<tr>
<td>Xanthones</td>
<td>HPLC: RP-18</td>
<td>C₂H₅OH</td>
<td>CH₃CN: H₂O</td>
<td>Teetrakul et al. (2009)</td>
</tr>
</tbody>
</table>

Note: FAMES = fatty acid methyl esters.
Fig. 8.1. Litchi (*Litchi chinensis* Sonn.).

Fig. 8.2. Longan (*Dimocarpus longan* Lour. or *Euphoria longana* Lam.).

Fig. 8.3. Rambutan (*Nephelium lappaceum* Linn.).
The skin of litchi and longan is thin, while the skin of rambutan is thicker and is covered with hair-like spinterns.

8.3.1 Health-promoting compounds in Sapindaceae fruit

*Sapindaceae* fruit are used traditionally as a tonic to the heart, brain and liver, and they also allay thirst (Souza et al., 2007). Modern medicine has studied the health-promoting compounds in *Sapindaceae* fruit. Ascorbic acid, or vitamin C (L-ascorbic acid and L-dehydroascorbic acid form), is only a minor constituent in fresh fruit and vegetables but is of major importance in human nutrition for the prevention of several diseases such as scurvy. Litchi, longan and rambutan pulp are good sources of ascorbic acid. Longan flesh had the highest ascorbic content (63.3–88.8 mg/100 g fresh weight (FW)) among these three fruit, while litchi pulp and rambutan flesh had 17.9–27.6 mg/100 g and 36.4–56.0 mg/100 g FW, respectively (Jiang and Fu, 1999; Srilaong et al., 2002; Song et al., 2006; Wall, 2006). Litchi pulp extract (in water or alcohol and analysed by HPLC) was reported to show promising hepatoprotective activity, which was influenced by the antioxidant activity of ascorbic acid (Souza et al., 2007).

Carotenoids are water-insoluble and isoprenoid lipid molecules which normally are found in membrane structures and result in the yellow, orange or red colour of the fruit (Britton and Hornero-Méndez, 1997). The carotenoid backbone is either linear or contains end cyclic end groups. The most abundant end is the β-ionone ring of β-Carotene (β,β-carotene) and its derivatives. β,β-carotene is a major provitamin A due to the fact that it can provide two molecules of retinol, which is a precursor for vitamin A. However, other carotenoids such as Ε-carotene (β,Ε-carotene), lycopene, lutein and zeaxanthin also supply vitamin A from fruit but they can contribute only one molecule of vitamin A. A lack of vitamin A causes impaired iron mobilisation, growth retardation and blindness, to depressed immune response and increased susceptibility to infectious disease (Sommer and Davidson, 2002). Vitamin A may help to prevent many types of cancer, cardiovascular disease and diabetes (Maughan, 2005). However, the edible part of *Sapindaceae* fruit is not a good source for carotenoid i.e. β-carotene and lycopene with 0–2 µg/100 g FW concentration (Holden et al., 1999; Setiawan et al., 2001; Charoensiri et al., 2009).

Polyphenols are a large group of compounds which contribute to the organoleptic and nutritional qualities of fruit and vegetables (Macheix et al., 1990) found in varied ranges, for instance, flavonoids, cinnamic acid and coumarins. They have been reported to prevent degenerative diseases, and are anti-allergenic, antiatherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective and vasodilatory agents (Parada and Aguilera, 2007). However, very small concentrations of polyphenols have been reported in *Sapindaceae* fruit pulp. Only 3.19–3.51 and 1.57–1.77 mg/100 g FW of total phenolic compounds were detected by HPLC in litchi and rambutan, respectively (Gorinstein et al., 1999; Somboonkaew and Terry, 2010). Several studies have reported high concentrations of total polyphenols from the seed and pericarp of *Sapindaceae* fruit. The extracts from seed and pericarp, therefore, have been applied to cosmetic, nutraceutical and pharmaceutical applications. For instance, ethanolic rambutan pericarp extract consisted of high phenolic concentration (> 700 mg GAE/g extract), low pro-oxidant capacity and effective antioxidant activities (strong scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH), with 0.26 1/IC50 (µg/ml), galvinoxyl and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with 1.7 IC50 (µg/ml) and superoxide anions and inhibition of induced lipid autooxidation) (Palanisamy et al., 2008). These results showed higher antioxidant activities than vitamin C and commercial grape seed supplements. Ethanolic extracts of rambutan peel inhibited extremely high value of IC50 to scavenged DPPH free radical (>100 µg/ml) and showed non-toxic property to the Caco-2 cells (derived from human colon adenoma and used for drug absorption screening) and peripheral blood mononuclear cells (PBMC), they could be a potential source of
natural antioxidants for food or drugs (Okonogi et al., 2007). However, Thitilertdecha et al. (2008) documented a higher total phenolic content in a methoanolic fraction of rambutan peel (542.2 mg catechin/g dry extract) than in ether and aqueous extractions, with corresponding antioxidation activity (50% DPPH inhibition concentration (4.94 μg/ml)) and antibacterial activity against five pathogenic bacteria, namely Pseudomonas aeruginosa, Vibrio cholera, Enterococcus faecalis, Staphylococcus aureus and S. epidermidis, measured using a disc diffusion assay.

Furthermore, the three main polyphenols in pulp, pericarp and seed of 70% (v/v) methaonolic longan extracts are gallic acid, ellagic acid and corilagin, with the highest concentrations found in the seed: gallic acid 0.8-2.3 mg/g dry weight (DW), ellagic acid 1.4-4.5 mg/g DW and corilagin 3.7-8.6 mg/g DW (Rangkadilok et al., 2005). The high content of ellagic acid in longan seed was found to inhibit Plasmodium falciparum (in vitro) (Banzouzi et al., 2002), while corilagin from seed extracts reduced blood pressure of spontaneously hypertensive rats by blocking noradrenaline and/or direct vasorelaxation (Cheng et al., 1995). The antioxidative activities and polyphenolic contents in dry litchi pericarp have also been reported (Hu et al., 2010); the levels of phenolics, total flavonoids and proanthocyanidins among eight cultivars (cvs. Nuomici, Feizixiao, Edanli, Yuhebao, Wuye, Guiwei, Dingxiang and Lanzhu) varied between 10-24, 15-38 and 16-44 mg/g pericarp DW, respectively, while the antioxidative activities (free-radical scavenging activities) were 18-72% in a DPPH decoloration test and 22-57% using a ferric-reducing antioxidant assay.

Dietary fibre (DF), for example cellulose, pectin substance, lignin and gum, is partially digestible in the human colon. DF intake appears to be negatively correlated with the incidence of colon cancer, ischaemic heart disease and diabetes mellitus (Burkitt et al., 1972). Jenkins et al. (1978) described how water-soluble DF decelerated sugar digestion and absorption, resulting in insulin and hormone reduction (Monnier et al., 1982; Mann et al., 2004). The contents of total DF in litchi fruit and rambutan were 2.20 and 1.64 g/100 g aril FW, respectively (Gorinstein et al., 1999) and only 0.19 g/100 g FW was found in longan aril (Nititham et al., 2004).

Phytate (phytic acid or inositol hexakisphosphate; IP6) was investigated in DF. The amounts of IP6 found in litchi, rambutan and longan pulp were 1.84-4.30, 2.04 and 0.04-0.116 mg/100 g FW, respectively (Nititham et al., 2004; Charoonsiri et al., 2009). IP6 provides the antioxidant effect and starch digestibility (Jenkins et al., 1978), prevents colon cancer (in vitro) by increasing cell apoptosis and differentiation, affects colon morphology favourably, and protects against a fatty liver (in vitro) resulting from elevated hepatic lipogenesis (Onomi et al., 2004).

### 8.3.2 Effect of preharvest and postharvest treatments on bioactive compounds in Sapindaceae fruit

Although the effects of preharvest factors, for example climate, growing area, agricultural practices and biofortification (genetic and breeding), on the quantity and quality of litchi, longan and rambutan fruit have been widely studied, few of the reports have been linked to the potential effect on health-promoting compounds.

The requirement of potassium (K) in litchi fruit development is much higher compared with nitrogen (N) and phosphorus (P). K enhanced photosynthesis activity, CO2 uptake (Debnath et al., 2006), stomatal conductance and water use efficiency (Pathak et al., 2007), which led to better quantity and quality of litchi. However, the rate of K application varies with fruit cultivar, environment and agricultural practices. Pathak and Mitra (2008) stated that the application of 600 g K2O/ plant/year, split into two treatments (15 days after fruit set and 30 days before flowering) increased leaf K content and resulted in higher total soluble solids (TSS):acid ratio (64:1) and ascorbic acid concentration (49.67 mg/100 g fresh aril) in litchi fruit. Baomei et al. (2008) also reported that the use of NPK plus K fertilizer increased yield (38.8%), fruit size (by 0.4 g) and soluble solid contents (0.9%) in litchi but gave no details on the health-promoting compounds in the fruit.
Potassium chlorate \((\text{KClO}_3)\) has been found to induce flowering in longan and litchi and to accelerate cytokinin (CK) synthesis (Bangerth, 2008; Hegele et al., 2008) in buds. Cks play an important role in fruit development. Stern et al. (2006) described how applying cytokinin \(N\)-(2-chloro-4-pyridy1)-N‘-phenylurea (CPPU) at 5–10 mg/1 to litchi tree extended litchi harvesting time by 2–3 weeks. CPPU treatment also produced more than 50% higher soluble solid contents:titrable acidity (SSC:TA) ratios than seen with the control fruit and gave a red colour (anthocyanins) comparable with that seen in the untreated group. A high ratio of SSC:TA resulted in less browning, lower decay development and less aril discoloration. Sprays of ethrel (0.25 ml/1) to litchi fruit cv. Bombay at aril development stage also increased the ratio of total soluble solids (TSS) to acid, while enhancing the anthocyanin content in pericarp at harvest stage (Dhua et al., 2005).

Hu et al. (2010) suggested that mulching the tree basins with fallen litchi leaves, combined with sprinkle irrigating the orchard at 40% pan coefficient during fruit development, with shade nets 30 days before harvesting, minimized pericarp cracking and sunburn disorder in litchi cvs. Bombay, Bedana and Elaichi. Control of fruit disorders reduces the loss of health-promoting compounds. Fruit bagging is another preharvest practice that influences health-promoting compounds in several fruit due to the effect of microclimate changes around the fruit. However, there was no significant difference in \(\beta\)-carotene, anthocyanin and phenolic concentrations between bagged and unbagged longan fruit, but significant differences in all three compounds between maturation stages were reported in the same study (Jaroenkit et al., 2008).

Appropriate postharvest handling can prolong the postharvest life of fruit and preserve the amount and quality of health-promoting compounds. The effects of postharvest treatment on phytochemical compounds in litchi, longan and rambutan fruit have been widely studied over the past decades, but little emphasis has been placed on monitoring the temporal changes in bioactives. Rather, most postharvest work has concentrated on the role of phenolics in the postharvest browning of pericarp tissue and rarely has considered phenolic compounds in the edible aerial tissue.

### 8.4 Pomegranate

Pomegranate \((\text{Punica granatum} \text{ L.}; \text{Fig. 8.4})\) belongs to the family 

\text{Punicaceae}, which originates from the Near to Middle East (Roy and Waskar, 1997). However, the fruit is now grown extensively in Iran, Afghanistan, India, Turkey, Egypt, Tunisia, Morocco and Spain (Saxena et al., 1987; Vardin and Fenercioglu, 2003). The fruit can be grown in a wide range of climates (temperate to desert conditions) and growing areas (Patil and Karale, 1985). The edible portion of pomegranate is juicy, sweetly acidic yet slightly astringent, with small angular hard seeds. The pomegranate aril varies between crimson and yellowish-white in colour, depending on the cultivar, whereas the fruit skin ranges from a glossy reddish-yellow to red colour.

#### 8.4.1 Health-promoting compounds in pomegranates

The fruit arils hold high concentrations of sugars, acids, polyphenols, vitamins and minerals (Al-Maiman and Ahmed, 2002), while the skin is an important source of tannin and other phenols (Roy and Waskar, 1997). Vitamin contents in pomegranate vary with cultivar, season and growing area. Vitamin C, or ascorbic acid \(10.2–69.0 \text{ mg/100 g fresh aril}\), is especially high in concentration in pomegranate aril tissue (USDA, 2008). High contents of vitamin B, especially pantothenic acid \((\text{vitamin B}_5)\), tocopherol \((\text{vitamin E})\) and phyloquinone \((\text{vitamin K})\) have also been reported in pomegranate aril tissue (Tezcan et al., 2009). The main sugars in pomegranate are fructose and glucose \(5.80–7.06\) and \(5.80–7.62 \text{ g/100 g fresh aril FW, respectively}\), with low content of sucrose \(0.02–0.04 \text{ g/100 g fresh aril}\) (Tezcan et al., 2009). Total sugar in fruit aril is \(11.60–14.3 \text{ g/100 g aril FW}\). However, organic acids are relatively low in pomegran-
Exotics

141

ate. Total organic acids are c.0.36–3.34 g/100 g aril FW. Citric acid is the most important acid in aril, with small concentrations of malic and ascorbic acid (0.2–3.2, 0.09–0.15 and 0.07–0.14 g/100 g aril FW, respectively).

Pomegranate aril contains high concentrations of phenolic compounds, particularly anthocyanins (328–815 mg/l). Delphinidins (3-glucoside and 3,5-diglucoside) are predominant, with lower levels of cyanidins (3-glucoside and 3,5-diglucoside) and pelargonidins (3-glucoside and 3,5-diglucoside). Ellagic acid and tannins are other polyphenols that have been detected in aril tissue. Commercial pomegranate juice, prepared from aril, peel and seed, contains high levels of gallotannin, quercetin, kaempferol, lutrolin glycoside, catechin and epicatechin; however, punicalagin, a member of the tannin family, and ellagic acid are the most abundant phenols in pomegranate juice.

The high content of bioactive compounds in pomegranate results in strong antioxidant properties (total monomeric anthocyanin: 6.1–219.0 mg cyanidin 3-glucoside/l; Trolox equivalent antioxidant capacity (TEAC): 4.58–7.70 mmol Trolox equivalents (TE)/l; and ferric ion reducing antioxidant power (FRAP): 4.6–10.9 mmol TE/l)). Recent studies have shown potential antioxidant effects of pomegranate in reducing heart disease and cancer risk, eliciting antimicrobial, antidiarrhoeal and antiulcer activities, increasing epididymal sperm concentration and reducing abnormal sperm rate. Cuccioloni et al. (2009) documented the potential role of bioactive metabolites in pomegranate components in the regulation of physiopathological processes involving thrombin. Pomegranate extract may also be of therapeutic use for the treatment of inflammatory diseases, by suppressing human mast cells/ basophils (Rasheed et al., 2009). Such an extract can reverse proatherogenic effects induced by shear stress perturbation (in vivo and in vitro) (de Nigris et al., 2007).

Daily consumption of pomegranate juice improved stress-induced myocardial ischaemia in patients who had coronary heart disease (Sumner et al., 2005). Syed et al. (2009) summarized data involving the chemoprotective and chemotherapeutic potentials of pomegranate against various cancers, including skin cancers. Ellagitannin from pomegranate juice was reported to inhibit androgen-dependent and androgen-independent prostate cancer cell growth in humans (Hong et al., 2008). Therefore, pomegranate juice has attracted increasing interest recently for its health benefits.

8.5 Mangosteen

Mangosteen (Garcinia mangostana Linn.; Fig. 8.5) is a tropical fruit belonging to the family Guttiferae. Although mangosteen is found mainly in South-east Asia, specifically Thailand, Indonesia, the Philippines, Malaysia, Myanmar, Vietnam, Cambodia and Papua New Guinea, the fruit has spread to other warm and humid tropical areas, namely Madagascar, Sri Lanka, India, Honduras, Brazil and Australia (Osman and Milan, 2006).
The edible part of the mangosteen is juicy, with a slightly acidic sweet taste, soft, fragrant and has a cream- to white-coloured flesh, whereas the peel is dark purple.

8.5.1 Health-promoting compounds in mangosteen

Mangosteen fruit have been used for over a century as a traditional medicine in the Southeast Asian region, namely in the treatment of chronic ulcer, dysentery, diarrhoea, cystitis, thrush, eczema, gonorrhoea and infected wounds (Osman and Milan, 2006; Pedraza-Chaverri et al., 2008). Current research has been centred on understanding these effects and identifying the causal bioactives.

In general, vitamin concentrations in mangosteen are relatively low compared with other exotic fruit, yet both folate and ascorbic acid levels are comparatively high (USDA, 2008). Dangcham and Siriphanich (2001) reported that citric, succinic and malic acids (approximately 2 mg/g FW) were the main organic acids in the edible part of mangosteen. Total sugar is 0.175 g/g FW aril (Charoensiri et al., 2009). The major sugars in the fruit aril are fructose, glucose and sucrose (Morton, 1987; Osman and Milan, 2006; Charoensiri et al., 2009) and a small content of dextrose and kerrelose (Jayaweera, 1981), while D-galacturonic acid and a small amount of neutral sugars (L-arabinose as the major one and L-rhamnose and D-galactose) were reported as carbohydrates in mangosteen peel (Chararath et al., 1997). Total dietary fibre in mangosteen is 0.02 g/g FW aril.

Similarly to most exotic fruit, pericarp phenolic compounds are significantly more abundant than in the aril. Zadernowski et al. (2009) documented that the total phenolic content of the peel and rind was 288.3 g/kg DW, whereas it was only 6.4 g/kg in aril. The main phenolic acid in aril is p-hydroxybenzoic. The pericarp is a rich source of phenolic compounds such as xanthones, phenolic acids, tannin and anthocyanins (Jung et al., 2006; Fu et al., 2007). However, xanthone is the only phenolic compound in mangosteen that has been widely studied. Fifty xanthones have been found recently in mangosteen (Pedraza-Chaverri et al., 2008). Walker (2007) reported a rapid analysis method for xanthones in mangosteen rind. Dry rind powder was extracted with 80:20 (v/v) acetone: water and six xanthones quantified, namely α-mangostins, β-mangostins, gartanin, 9-hydroxy calabaxanthone, 3-isomangostin and 8-desoxygartanin, using standard HPLC-photodiode array detector. Xanthones have been documented as having antioxidant (Jung et al., 2006), antimalarial (Likhitwitayawuid et al., 1998), antimicrobial (Sundaram et al., 1983; Mahabusarakam et al., 1986; Rukachaisirikul et al., 2003) and anti-inflammatory activities (Chen et al., 2008), antimyocardial toxicity and oxidative stress (Sampath and Vijayaragavan, 2008), ROS scavenging capacity (Pedraza-Chaverri et al., 2008), antileukemic activity (Chiang et al., 2004) and antiacne properties (Chomnawang et al., 2007).
8.6 Kiwifruit

Kiwifruit, or Chinese gooseberry, belongs to the Actinidaceae family. There are more than 50 species in the genus Actinidia but the commercial fruit is *A. deliciosa* (Ferguson, 1999). Although kiwifruit originated from China, it has been developed commercially in New Zealand, and subsequently elsewhere. The fruit is egg-shaped, with pale-brown skin and covered with downy hairs. The fruit flesh is a translucent green in colour with a massive amount of little black seeds around the fruit core.

8.6.1 Health-promoting compounds in kiwifruit

Kiwifruit is a rich source of ascorbic acid (68.24–96.00 mg/100 g FW), vitamins A, E and K and folate (Kvesitadze et al., 2001; Guldas, 2003). Apart from ascorbic acid, citric, quinic and malic acids are the major organic acids in kiwifruit pulp (Boyes et al., 1996; Marsh et al., 2003). Major sugars are fructose and glucose (4.35 and 4.11 g/100 g FW pulp, respectively), with minor contents of maltose, galactose and sucrose (USDA, 2008). Amino acids (e.g. aspartic and glutamic acids) were shown to be present in the fruit aril, whereas α-linolenic and linoleic acids (fatty acids) have been reported in fruit seed (Donaldson and Quirin, 2008). Fresh fruit contains 2.6–3.0 g total dietary fibre in 100 g FW (USDA, 2008). Phenolic compounds in kiwifruit are low in comparison with other exotic fruit. Total phenols in fresh kiwifruit were 1–7 mg/1 juice (Dawes and Keene, 1999). Cinnamic acids were the only phenolic compounds detected in kiwifruit, and caffeic acid was the main cinnamic acid present (Wijngaard et al., 2009). However, chlorogenic acid, protocatechuic acid, hydroxybenzoic acid, epicatechin, catechin, procyanidins and kaempferol have also been reported in kiwifruit (Dawes and Keene, 1999; Mattila et al., 2006). Du et al. (2009) stated that the concentrations of phenols and vitamin C in eight Actinidia genotypes were highly correlated with total antioxidant capacity (as measured in DPPH, ABTS, ORAC, FRAP, SASR and MCC tests; see chapters 18 and 19 of this volume). Kiwi seed oil contains more than 65% omega-3 fatty acid in the α-linolenic acid (ALA) form, providing a vegetable alternative to fish oil (Donaldson and Quirin, 2008).

The health benefits of kiwifruit have been widely discussed. The fruit may increase the antioxidant status of plasma and lymphocytes (Collins et al., 2001, 2003). Kiwifruit may also have a beneficial effect in reducing the levels of carcinogens absorbed from the diet, by promoting laxation (Kestell et al., 1999). Rush et al. (2006) documented that the fruit might provide a sustainable population intervention that could decrease risk factors associated with cancer. Although the health benefits of kiwifruit have been widely reported, people can be allergic to the fruit due to the protein-dissolving enzyme, actinidin, and calcium oxalate crystals. These two chemicals can cause itching and soreness of the mouth.

8.7 Passion Fruit

Passion fruit originated from Brazil and Ecuador and is classified in the Passifloraceae family. *Passiflora edulis* Sims. is the most well-known species, which contains two forms, purple and yellow. Yellow passion fruit has a thick and hard yellow rind, brown seed and aromatic and acidic pulp, while purple fruit is smaller, with purple peel, black seed and a sweeter taste (Bora and Narain, 1997; Talcott et al., 2003).

8.7.1 Health-promoting compounds in passion fruit

High levels of vitamins have been detected in passion fruit, including vitamin C and vitamin A (40–65 mg and 1272 IU/100 g fresh fruit, respectively) (Vinci *et al.*, 1995; Suntornsuk *et al.*, 2002; USDA, 2008). Mercadente *et al.* (1998) documented 13 provitamin A in passion fruit, including β-carotene, α-carotene, β-cryptoxanthin, lycopene and xanthophyll. Total sugar in 100 g fresh passion fruit is about 7.21–14.45 g, which includes glucose, fructose and sucrose (USDA, 2008; Vera *et al.*, 2009). The fruit contains high content of organic acids,
resulting in an acidic flavour (c.3.2–4.2 pH; Godoy and Rodriguez-Amaya, 1994; USDA, 2008). Vera et al. (2009) reported that the predominant organic acids in passion fruit were citric (5.41 g/100 g FW) and malic (0.33 g/100 g FW). Six different sugar residues, xylose, glucose, galactose, galactosamine, unknown and fucose, were found in extracts of passion fruit peel with a relative ratio of 1:0:2.0.06:0.05:trace (Tommonaro et al., 2007). Passion fruit (100 g) contains about 9.14–28.33 g total dietary fibre (DF) (USDA, 2008). Passion fruit seed oil consists of up to 89.43% unsaturated fatty acid which is 72% linoleic acid (Liu et al., 2009). Seed oil also contains passifin, which is a novel distinctive dimeric antifungal protein (Lam and Ng, 2009). HPLC analysis indicated that passion fruit contained a number of phenolic compounds including syringic acid, tryptophan, flavonoids glycoside, quercetin, luteolin, cyanidins, catechin and epicatechin (Talcott et al., 2003; Foo et al., 2010). Total phenols in passion fruit are about 43.5–61 mg GAE/100 g FW, with antioxidant capacity FRAP: 17.5–50.0 μmol Trolox/100 g FW and DPPH: 94.0% (Talcott et al., 2003; Vasco et al., 2008) and TEAC: 0.32 μmol/mg FW (Tommonaro et al., 2007).

The health benefits of passion fruit have been documented widely. Barbosa et al. (2008) reported that an extract of passion fruit ( cvs. Alata and Edulis) induced anxiolytic-like effects in rats without disruption to the memory process. Administration of oral P. incarnata as a premedication (in outpatient surgery) reduced anxiety without inducing sedation (Movafegh et al., 2008). Brown et al. (2007) also described the anxiolytic properties of chrysin (passion fruit extract) in rats. Rebello et al. (2008) documented that passion fruit extract affected the biodistribution of sodium 99mTc in male rats but did not influence the shape of red blood cells. The fruit peel is a rich source of health-promoting compounds. Rebello et al. (2007) illustrated that the pectin in fruit peel extract decreased cholesterol levels and increased glucose tolerance in rats and humans. Extracts from purple fruit peel decreased systolic blood pressure and serum nitric oxide levels significantly in rats and systolic and diastolic blood pressure in humans (Zibadi et al., 2007).

8.8 Persimmon

Persimmon (Fig. 8.6) is a subtropical fruit and is grown commercially in China, Japan, Korea and the USA. Diospyros kaki, oriental or Japanese persimmon, is the most well-known worldwide, while D. virginiana is widely grown in the USA. The fruit is pumpkin-shaped, a bright to dark orange colour and varies in size from 1.5–9 cm.

The major vitamins in persimmon fruit include vitamins A and C. Total vitamin A

![Fig. 8.6. Persimmon (Diospyros kaki).](image_url)
(1627 IU; USDA, 2008) includes β-carotene, β-cryptoxanthin, zeaxanthin, lutein and lycopene. Vitamin C contents in persimmon fruit ranged from 35 to 66 mg/100 g FW according to cultivar and fruit maturity (Homnava et al., 1990; USDA, 2008). Organic acids in the fruit include succinic, malic, citric and quinic, with total acids of 0.88–1.36 g/100 g DW (Senter et al., 1991). Persimmon contains about 12.53 g sugar/100 g FW, with a majority of fructose, glucose and sucrose and a minor concentration of arabinose, galactose, sorbitol and inositol (Senter et al., 1991). Total DF in persimmon ranges between 1.5 and 3.6 g/100 g fresh fruit (Gorinstein et al., 2001; USDA, 2008).

Persimmon fruit can be divided into two varieties, astringent and non-astringent. The non-astringent type contains fewer tannin cells and less water-soluble tannins, resulting in a sweeter flavour. However, both varieties are rich in phenolic compounds, namely phenolic acid (e.g. p-coumaric, ferulic and protocatechuic acids) and proanthocyanidins (Haslam et al., 1988; Gorinstein et al., 1994; Jung et al., 2005). Chen et al. (2009) reported that persimmon tannins consisted mainly of (-)-catechin, (-)-epicatechin, (-)-epigallocatechin, chlorogenic acid and caffeic acid, with 32.31 mg total phenols in 100 g fresh fruit. Kato (1984) found a high correlation between astringency level and tannin concentration, with fruit containing about 0.25% tannin being slightly astringent, while those having less than 0.1% were non-astringent. Astringent varieties contain phenolic compounds at concentrations 4–6 times higher than those in non-astringent fruit (Suzuki et al., 2005). Concentrations of volatile compounds such as acetaldehyde and ethanol, produced by seeds, also affect the degree of astringency in persimmon fruit (Taira et al., 1986). Sugiuira et al. (1979) reported that non-astringent fruit accumulate less volatile compounds.

Persimmon pulp phenols, in methanolic extracts, contain high molecular weight tannins (epigallocatechin, epigallocatechin-3-O-gallate, epicatechin-3-O-gallate and unknown polymers) with powerful antioxidant activities (Gu et al., 2008). Phenols in fruit peel reduced glucose-induced cytotoxicity, intracellular reactive oxygen species, nitric oxide, superoxide and peroxynitrite concentrations and the overexpression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), indicating the benefit of persimmon peel phenols to antioxidant activity in the diabetic condition (Yokozawa et al., 2007). Several recent studies have documented health-promoting compounds in persimmon leaves, root and bark extracts, including antimicrobial activity in the leaves (Sakanaka et al., 2005; Lee et al., 2007), antiprotozoal activity in the root (Ganapaty et al., 2006), antioxidant and lipoxygenase inhibitory activity in the root and bark (Maiga et al., 2006) and antiplasmodial properties (Kantamreddi and Wright, 2008). Furthermore, persimmon has been associated with an inhibitory effect on human lymphoid leukaemia cells (Achiwa et al., 1996, 1997), mutagenicity of c-nitro and c-nitroso compounds, incidence of stroke and extension of the lifespan in stroke-prone spontaneously hypertensive rats (Uchida et al., 1995) and the treatment of streptozotocin-induced diabetic rats and, possibly, morphological changes in the livers, kidneys and hearts of such rodents (Azadbakhta et al., 2010).

8.9 Carambola

Carambola, or starfruit (Averrhoa carambola; Fig. 8.7), belongs to the Oxalidaceae family and originates from South-east Asia (Watson et al., 1988). The fruit has been grown recently in South-east Asia, India, China, the USA, Central America and Brazil. It is oblong-shaped, with three to six longitudinal ribs, resulting in a star-shaped cross section. The fruit size ranges from 80 to 250 mm in length and 50 to 100 mm in width, depending on the cultivar (Watson et al., 1988). Starfruit pulp is tart in taste when immature and slightly acidic sweet when ripe, with a smooth to fibrous texture. The skin of the fruit is waxy, thin and firm, and is a pale greenish-yellow to orange colour, and thus a similar colour to the fruit pulp.

Vitamins A and C are the major vitamins in carambola fruit. Gross et al. (1983) reported that ζ-carotene was the highest provitamin A (25% of total carotenoids in carambola),...
while trace levels of β-cryptoxanthin and β-carotene were present. USDA (2008) found 25 μg β-carotene, 24 μg α-carotene and 61 IU vitamin A in 100 g fresh carambola. Vitamin C contents ranged between 14.4 and 53.1 mg/100 g of fresh edible portion, depending on cultivar and maturity stage (Morton, 1987; Cooper et al., 1995; Luximon-Ramma et al., 2003). Fresh green fruit (immature fruit) contained 12.51 mg/g organic acid, consisting of 5 mg oxalic, 4.37 tartaric, 1.32 citric, 1.21 malic, 0.39 α-ketoglutaric, 0.22 succinic and a trace of fumaric, while 13 mg of total acids, made up of 9.58 mg oxalic, 0.91 tartaric, 2.20 α-ketoglutaric and 0.31 fumaric acid, were found in yellow (mature) carambola (Morton, 1987; Maharaj and Badrie, 2006). Total sugar in 100 g fresh carambola is about 4 mg (USDA, 2008); the predominant sugars are glucose and fructose (51.0 and 35.9%, respectively), with a small amount of sucrose (13.1%) (Chan and Heu, 1975). The fruit possess a high level of total DF: 2 g/100 g fresh fruit (USDA, 2008) or 46.0–58.2 g/100 g pomace (Chau et al., 2004). Amino acids and fatty acids are found in carambola, but only in minor quantities (Morton, 1987; USDA, 2008).

Carambola fruit could offer an inexpensive source of antioxidants. Fresh carambola (100 g) contained total phenolics 142.9–209.9 mg gallic acid, flavonoids 10.3–14.8 mg quercetin, and proanthocyanidins 89.6–132.1 mg cyanidin chloride (Luximon-Ramma et al., 2003). Luximon-Ramma et al. (2003) also reported strong correlations between antioxidant activities and total phenol and proanthocyanidin concentrations. The antioxidant activities of 1 g fresh carambola, measured as TEAC and FRAP, were between 11 and 17 μmol Trolox and 9–22 μmol Fe(II), respectively. The antioxidant activities in carambola were mainly attributed to singly linked proanthocyanidins that existed as dimers, trimers, tetramers and pentamers of catechin or epicatechin (Shui and Leong, 2004).

Starfruit was used traditionally for treating restlessness, headache, nausea and coughs (Burkhill, 1935). High DF contents resulted in good swelling properties, water and oil capacities and cation-exchange capacity (Chau et al., 2004a,b). Carolino et al. (2005) reported a neurotoxic fraction from carambola that changed animal behaviour, including tonic-clonic seizures that evolved into status epilepticus, accompanied by cortical epileptiform activity. These effects indicate that carambola may be considered as a new tool for neurochemical and neuroethological research. However, there have been reports of hiccups, confusion and death after fruit ingestion in uraemic patients (Neto et al., 1998, 2003). A neurotoxin is present in carambola that can cross the blood–brain barrier, being excreted by the kidneys (Ise et al., 2003).
References


N. Somboonkaew and L.A. Terry


Dangham, S. and Siriphanich, J. (2001) Mechanism of flesh translucent disorder development of mango- 


Fu, C., Loo, A.E., Chia, F.P. and Huang, D. (2007) Oligomeric proanthocyanidins from mango- 


9 Grape

Pierre-Louis Teissedre and Christian Chervin

9.1 Introduction

The grape is the fruit of the grapevine (Vitis species). This is the second most widely grown fruit in the world. Indeed, according to a report by the Food and Agriculture Organization (FAO) about the world market for fruit (FAO, 2000), grapes represent 14.6% of the global production of fruit, just after oranges; equivalent to nearly 68 million tonnes (Mt) per annum. Grapes are a valuable source of numerous phytonutrients and many studies have suggested cardiovascular benefits, while some work has indicated cancer chemopreventive activity (Pezzuto, 2008).

Grape berries are classified as white (green yellow or golden yellow) or red grapes (pink, purple or black). Grapes are used primarily for making wine from fermented juice (one speaks in this case of wine grapes), but they can also be consumed as fruit, either fresh as table grapes (namely, cvs. Chasselas, Thompson Seedless, Cardinal, Lavalée, Hamburg Muscat, Danlas, Prima, Italia, etc.) or as dried fruit, such as raisins, which are used primarily in baking or cooking. Grapes are also used to produce plain juices. Most research to date has concentrated on establishing the health-promoting properties of wine grapes rather than table grapes, and thus this inevitably will form the basis of this chapter.

More than 5000 grape varieties are listed commercially. The varieties are distinguished by their different shapes of leaf, berries and colours, and have different fragrances and taste profiles. The study of grapes is called ampelography, which has the etymology of the Greek words, ampelos: 'grapevine' and graphein: 'write'. The most important species of grapes are: Vitis vinifera (from Europe, and from which are derived all major varieties for wine and table grapes) and V. labrusca, V. riparia or V. rupesstris (from North America, used mainly as table grapes and relatively few for wines). During the attack of European vineyards by phylloxera in the 19th century, the cultivation of European grape varieties (Cabernet-sauvignon, Merlot, Cabernet Franc, Pinot Noir, Syrah (Shiraz), Grenache, Cinsault, Carignan, Sauvignon Blanc, etc.) was allowed to continue thanks to grafting on to North American Vitis rootstocks.

Grapes are a major source of polyphenols (Waterhouse et al., 1996), which constitute a family of organic molecules characterized by the presence of several phenol groups, leading to molecules of high molecular weight.

9.2 Identity and Role of Bioactives

9.2.1 Polyphenols

Polyphenols are products of secondary metabolism in plants and are becoming increasingly...
important, particularly because of their reported beneficial effects on health (Stanley et al., 2003). Indeed, their role as natural antioxidants is attracting increasing interest in the prevention and treatment of cancer (Chen et al., 2004) and inflammatory (Laughton et al., 1991), cardiovascular (Frankel et al., 1993) and neurodegenerative (Orgogozo et al., 1997) diseases. They are also used as additives in the food, pharmaceutical and cosmetic industries (Anon., 2006). The term ‘polyphenol’ was introduced in 1980 (Anon., 1980), replacing the former term ‘tannin’, and was defined as follows: ‘water-soluble phenolic compounds, molecular weight of between 500 and 3000 Da, which, in addition to the properties of usual phenols, have the ability to precipitate alka- loids, gelatine and other proteins’. In addition to this definition, polyphenols possess high antioxidant properties (Teissedre et al., 1996). The natural polyphenols include a wide range of chemicals, each including at least one aromatic nucleus and one or more hydroxyl groups, in addition to other constituents (Bamforth, 1999). Polyphenols can range from simple molecules such as phenolic acids to compounds that are highly polymerized (more than 30,000 Da), as for tannins. The latter are molecules containing at least one cycle benzene and hydroxy groups. Because some hydroxyl groups bind to salivary proteins, some polyphenols, such as, tannins are defined as astringent, giving a sensation of dryness in the mouth. Some fruit have high concentrations of phenolic compounds, such as plums and persimmons, in which they can reach 1–2 % of fresh weight (Macheix et al., 1990).

Grape berries also contain large amounts of phenolic compounds, including catechins, concentrated mainly in the seeds and skins (Table 9.1). Up to maturity, some quantitative and qualitative changes occur during grape ripening. The harvesting date of grapes (directly correlated with the degree of horticultural maturity) influences their chromatic characteristics. In general, higher intensities of blue or violet tones are detected in black grapes, collected at a later harvest maturity date, in which the ratios of anthocyanins/proanthocyanidins are the lowest. Polyphe- nolic maturity or skin maturity is achieved on a chemical level when the potential for anthocyanin synthesis starts to diminish after reaching a plateau. With increased fruit maturity there is a change in the skin phenols that corresponds to an increase in phenol maturity or polymerization. Skin tannin polymerization parallels a sensory transformation, an evolution from hard and astringent to dusty to soft and supple. Poly- merized skin tannins have a large molecular weight and are smoother on the palate than smaller, low molecular weight tannins, which are considered to be hard and astringent. The pips change from green to a brown or uniform yellow colour; once all traces of green have disappeared, the tannins become less dry, less astringent; the pips become less

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Catechins</th>
<th>Per cent in stalk</th>
<th>Per cent in seeds</th>
<th>Per cent in skins</th>
<th>Per cent in pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alicante-bouschet</td>
<td>551</td>
<td>15</td>
<td>64</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>344</td>
<td>10</td>
<td>83</td>
<td>7</td>
<td>T</td>
</tr>
<tr>
<td>Carignan</td>
<td>94</td>
<td>27</td>
<td>54</td>
<td>19</td>
<td>T</td>
</tr>
<tr>
<td>Cinsault</td>
<td>154</td>
<td>47</td>
<td>37</td>
<td>9</td>
<td>T</td>
</tr>
<tr>
<td>Grenache Blanc</td>
<td>144</td>
<td>17</td>
<td>51</td>
<td>32</td>
<td>T</td>
</tr>
<tr>
<td>Grenache Noir</td>
<td>173</td>
<td>25</td>
<td>64</td>
<td>11</td>
<td>T</td>
</tr>
<tr>
<td>Merlot</td>
<td>601</td>
<td>9</td>
<td>81</td>
<td>11</td>
<td>T</td>
</tr>
<tr>
<td>Mourvedre</td>
<td>171</td>
<td>25</td>
<td>58</td>
<td>17</td>
<td>T</td>
</tr>
<tr>
<td>Pinot Noir</td>
<td>1165</td>
<td>4</td>
<td>94</td>
<td>2</td>
<td>T</td>
</tr>
<tr>
<td>Colobel (hybrid)</td>
<td>862</td>
<td>8</td>
<td>79</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Mean</td>
<td>377</td>
<td>20</td>
<td>65</td>
<td>14</td>
<td>--</td>
</tr>
</tbody>
</table>

Note: T = trace.

Source: Bourzeix et al. (1986).
hard, less brittle, and lose their grassy aromas, which are replaced first by toasted notes and then by roasted, once polyphenolic maturity has been reached (Kennedy et al., 2000). This colour change represents oxidative reactions and corresponds to the degree of extractable tannins. Finally, a limited degree of polymerization, and perhaps depolymerization, occurs in the fruit during maturation. In wines, tannin polymerization continues (although there is some depolymerization) until an anthocyanin molecule binds the terminal end; polymerization is then believed to stop. The ratio of free anthocyanins to tannins is important in impacting polymerization (Teissedre, 2008). The main phenolic molecules (non-flavonoids or flavonoids) are given in Fig. 9.1.

Phenolic compounds are synthesized primarily from carbohydrates via the shikimic acid or acetate pathways; that of shikimic acid leading, via transamination and deamination, to cinnamic acids and their derivatives, and that of acetate leading to polycetoesters or polyacetate (malonate). The structure of phenolic compounds ranges from a simple aromatic nucleus in low molecular weight tannins to complex high molecular weight polyphenols. Such compounds can be ordered by the nature of their carbon skeleton and the length of the aliphatic chain linked to a benzene ring (Cheynier et al., 1997). Phenolic compounds can be divided into two major groups: flavonoids and non-flavonoids.

Phenolic compounds of 14 pomace samples originating from red and white winemaking were identified and quantified by HPLC-MS-DAD (13 anthocyanins, 11 hydroxybenzoic and hydroxycinnamic acids, 13 catechins and flavonols, as well as two stilbenes) in the skins and seeds. Large variability in all of the individual phenolic compounds was observed, and was dependent on cultivar and vintage. Grape skins proved to be a rich source of anthocyanins, hydroxycinnamic acids, flavanols and flavanol glycosides, whereas flavanols were present mainly in the seeds. Both skins and seeds of most grape cultivars constitute a promising source of polyphenols (Kammerer et al., 2004).

**Flavonoids**

Flavonoids are polyphenolic compounds containing 15 carbon atoms forming a C6-C3-C6 structure (Fig. 9.2): two aromatic cores connected by a bridge of three carbons. These compounds are the most abundant among all phenolic compounds. They have a variety of roles in plants as secondary metabolites, being involved in UV protection, pigmentation and resistance to diseases. The C6-C3-C6 structure is the product of two synthesis pathways, the B ring and the three-carbon bridge constituting a phenylpropanoid unit synthesized from phenylalanine, while the A ring comes from the acetic–malonic acid pathway. The merger of these two parties involves condensation of a phenylpropanoid, 4-coumaryl, with three molecules of malonyl CoA, each giving two carbon atoms. The reaction is catalysed by chalcone synthase, giving tetrahydroxychalcone, which in turn will lead to all flavonoids (Crozier, 2003). There are several groups of flavonoids: flavonols, flavan-3-ols, flavones, isoflavones, flavanones and anthocyanidins (Fig. 9.3). It should be noted that isoflavones are not present in grapes. The basic structure of flavonoids may undergo numerous substitutions; the hydroxyl groups generally are in positions 4, 5 and 7. Most flavonoids exist in the form of glycosides; the nature of sugar varies greatly, depending on the species. The substitutions change the solubility of flavonoids; hydroxylations and glycosylations make compounds generally more hydrophilic, while other substitutions, such as methylation, make them more lipophilic.

**Flavonols**

Flavonols are the most widespread flavonoids among fruit. Flavonols such as myricetin, quercetin, isorhamnetin and kaempferol are most often present in the form of O-glycosides. The combination is most often in position 3 of the aromatic ring C (Fig. 9.1), although substitutions in positions 5, 7, 4', 3' and 5' are possible. The number of aglycones is quite low, but there are a very large number of conjugated forms; kaempferol alone can be conjugated in 200 different glycosidic forms. There
**Phenolic acids**

![Phenolic acid structures]

- **Benzoic acids**
  - $p$-hydroxybenzoic acid
  - protocatechuic acid
  - vanillyl acid
  - gallic acid
  - syringic acid
  - salicylic acid
  - gentisic acid

<table>
<thead>
<tr>
<th>Benzoic acids</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-hydroxybenzoic acid</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>protocatechuic acid</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>vanillyl acid</td>
<td>H</td>
<td>OCH$_3$</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>gallic acid</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>syringic acid</td>
<td>H</td>
<td>OCH$_3$</td>
<td>OH</td>
<td>OCH$_3$</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>gentisic acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
</tbody>
</table>

- **Hydroxycinnamic acids**
  - $p$-coumaric acid
  - caffeic acid
  - ferulic acid
  - sinapic acid

<table>
<thead>
<tr>
<th>Hydroxycinnamic acids</th>
<th>R5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-coumaric acid</td>
<td>COOH</td>
</tr>
<tr>
<td>caffeic acid</td>
<td>H</td>
</tr>
<tr>
<td>ferulic acid</td>
<td>OH</td>
</tr>
<tr>
<td>sinapic acid</td>
<td>OCH$_3$</td>
</tr>
</tbody>
</table>

- **Stilbenes**

- **Flavonols**

- **Flavan-3-ols**

- **Anthocyanidins**

**Fig. 9.1.** Examples of phenolic compounds: grape non-flavonoids and flavonoids.

is a high variability in their concentration, depending on season and cultivars (Crozier, 2003). The flavonol structure is flat. Four flavonols are mostly found in grapes: kaempferol, quercetin (5–10 mg/kg), myricetin and isorhamnetin. The derivatives of quercetin are predominant. The average flavanol content in grapes is about 50 mg/kg, but can vary between 10 and 285 mg/kg. The presence of flavonols was investigated in the berry skins...
of 91 grape varieties (V. vinifera), in order to produce a classification based on the flavonol profile (Mattivi et al., 2006). In red grapes, the main flavonol was quercetin (mean = 43.99%), followed by myricetin (36.81%), kaempferol (6.43%), laricitrin (5.65%), isorhamnetin (3.89%) and syringetin (3.22%). In white grapes, the main flavonol was quercetin (mean = 81.35%), followed by kaempferol (16.91%) and isorhamnetin (1.74%).

**Flavanones**

Flavanones are the first products of the flavonoid biosynthesis pathway. They are characterized by the absence of the double bond between C2 and C3 and by the presence of a chirality centre in C2. Most flavanones encountered in nature have the B cycle attached to the C cycle in C2 (Fig. 9.1). The structure of flavanones is very reactive and gives rise to reactions of hydroxylation, O-methylation and glycosylation. Flavanones are present at concentrations of a few mg/kg in grapes.

**FLAVAN-3-OLS.** Flavan-3-ols are the most complex category of flavonoids. These compounds range from simple monomers such as (+)-catechin and its isomer (-)-epicatechin, to oligomers and polymers of proanthocyanidins. Proanthocyanidins are formed of catechin and epicatechin, with oxidative coupling

---

**Fig. 9.2.** Flavonoid skeleton. In light grey, the three-carbon bridge; in dark grey, the moiety arising from the shikimate pathway and in black the moiety arising from the acetate pathway.

**Fig. 9.3.** Structure of the main flavonoids.
between positions C4 and C6 or C8 of the adjacent monomer. The oligomers of procyanidins are formed by two to five units of catechin or epicatechin, the polymers being formed by six or more units. In addition, flavan-3-ols may be esterified with gallic acid or hydroxylated to form gallatecins (epicatechin gallate, epigallatecchin, epigallatecchin gallate) and gallotannins. The flavan-3-ols present in grapes are mostly in the form of polymers. The seed tannins are made up of procyanidins (polymers of catechin and epicatechin), partially galloylated, while those of the skins also contain prodelphinidins (polymers of gallicatechin and epigallocatechin). The average number of monomeric units, defined as the mean degree of polymerization (mDP), may go up to 18 in a fraction from seeds and to around 30 in a skin extract. It has been found that mDP can change between vintages. For grapes of cv. Cabernet Sauvignon from the Bordeaux area, the mDP in seeds was 4.7 while that in skins was 25.7, in 2006 (Chira et al., 2009), and these levels were found to be double in 2007. On the other hand, the same authors found, in cv. Merlot grapes from 2006, an mDP of 3.6 in seeds and one of 35.4 in skins. However, the mDP in skins was found to be only 24.2 in 2007. Soil and climate can create variability in tannin mDP levels in grape skins and seeds. The GPC (gel permeation chromatography) molecular weight (MW) distribution was used by Weber et al. (2007) and indicated components ranging from 1180 to 5000 MW in grape seed. In this work, MALDI-TOF mass spectrometry (MS) analyses showed that grape seed contained oligomers with both odd and even numbers of gallate. Reflector MALDI-TOF MS identified oligomers up to a pentamer and heptamer, and linear MALDI-TOF MS showed a mass range nearly double that of the reflectron analyses. Recent studies have speculated that, as well as their antioxidant role, flavonoids can act by modulating cell signalling pathways and/or gene expression. In streptozotocin-induced diabetic rats (used as an oxidative stress model), grape seed procyanidin extract (GSPE) was used to study regulation of copper/zinc-superoxide dismutase (Cu/Zn-SOD), an enzyme that defends against oxidative stress (Puiggros et al., 2009). The results indicated that the expression profile of Cu/Zn-SOD in diabetic rats was similar to the profile in non-diabetic rats. Nevertheless, the administration of GSPE increased Cu/Zn-SOD activity in both diabetic and non-diabetic rats, and a direct interaction between some small or medium-sized GSPE components and the enzyme was found to be responsible for the increase in Cu/Zn-SOD activity. The levels of catechins and procyancidins (oligomers of catechins) vary depending on the type of grape or wine. Levels of these compounds are between 243 and 1108 mg/kg, of which over 89% generally is located in the seeds (Revilla et al., 1997). Table 9.2 provides a breakdown of the families of polyphenols based on the different parts of the berry of red grapes.

**Flavones**

Flavones are structurally very close to flavonols; the difference is the absence of hydroxyl in C3. There are also numerous substitutions on the flavone skeleton, such as hydroxylation, methylation, O- and C-alkylation and glycosylation. In plants, flavones are present mainly in the form of glycosides (Bohm et al., 1998). The flavone content in grape is very low.

**Anthocyanidins**

Anthocyanidins are widely present in the plant kingdom, mainly in the form of glycosides, and are found in black/red grape skins, where they are responsible for the colours red, blue and purple, depending on the pH of the cell compartment. These compounds are involved in protecting plants against excessive sunlight. The most common anthocyanidins are pelargonidin, cyanidin, delphinidin, peonidin and malvidin, but these compounds are present in glycosylated forms only and are referred to as anthocyanins. Anthocyanidins

<table>
<thead>
<tr>
<th>Table 9.2. Average content of phenolic compounds in different parts of the red grape berry (mg/kg).</th>
<th>Pulp</th>
<th>Skins</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Traces</td>
<td>100-500</td>
<td>1000-6000</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>–</td>
<td>500-3000</td>
<td>–</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>20-170</td>
<td>50-200</td>
<td>–</td>
</tr>
</tbody>
</table>
are also capable of conjugating with hydroxycinnamic acids and other organic acids (e.g. malic and acetic acids). Unlike other species (North American Vitis species) that have significant levels of diglycosylated anthocyanins in positions C3' and C5', V. vinifera contains only traces of these and is characterized by the presence of a majority of anthocyanin monoglucosides, particularly the malvidin 3-O-glucoside and its acylated derivatives. Anthocyanins are present in red grapes at about 500-3000 mg/kg, but can reach higher values in cultivars called ‘dyers’, particularly cv. Alicante Bouschet (5000 mg/kg), in which the anthocyanin concentration in pulp is also high. The delphinidin-like flavonols, myricetin, laricitrin and syringetin, are missing from all white varieties, indicating that the enzyme flavonoid 3',5'-hydroxylase is not expressed in white grape varieties (Mattivi et al., 2006).

Non-flavonoids

The main non-flavonoids important in fruit are phenolic acids, hydroxycinnamic acids and stilbenes. In grapes, although not coloured themselves, the non-flavonoid constituents are known to enhance and stabilize the colour of red wines by intra- and intermolecular reactions. Furthermore, they contribute to wine flavour (volatile phenolic acids), and some of them exhibit potent biological activities.

Simple phenols

Simple phenols are derivatives of the C6 benzene ring, are rare in their natural state and are obtained by decarboxylation of shikimic acid. They include hydroquinol, pyrocatechol and phloroglucinol.

Phenolic acids

An enzyme-assisted release of phenolic antioxidants from grape pomace from wine production was examined (Meyer et al., 1998). The enzymes used were grindamyl pectinase and celluclast. Total phenols released ranged from 820 to 6055 mg/l gallic acid equivalents (GAE) and varied in response to enzyme type, time of enzyme treatment, particle size of the pomace and type of extraction solvent employed. The yield of total phenols was correlated to the degree of plant cell wall breakdown within the pomace. Grindamyl pectinase catalysed degradation of grape pomace polysaccharides, whereas cellulase did not. Reduction of the particle size of grape pomace to 125–250 µm increased enzymatic polysaccharide hydrolysis and the recovery of phenols. The grape pomace extracts retarded human low-density lipoprotein oxidation significantly in vitro. When evaluated at 3.0 μM GAE, phenolic extracts of grindamyl pectinase-treated pomace of small particle size (125–250 µm) appeared to release more active antioxidant phenols than seen with the other types of enzyme treatments tested.

Hydroxybenzoic acids have a C6-C1 structure, composed of a benzene ring on which a one-carbon aliphatic chain is bound. They include vanillic acid, syringic acid, gentisic acid and gallic acid. The main compound in grapes is gallic acid, the content of which is between 100 and 230 mg/kg. The differences in levels of phenolic acids in seeds and skins from grapes of V. vinifera cvs. Merlot and Chardonnay and in seeds from grapes of V. rotundifolia cv. Muscadine were determined, and the antioxidant activities of these components assessed (Yilmaz and Toledo, 2004). The contribution of phenolic acid to the total antioxidant capacity of grape seeds and skins was also determined. Gallic acid concentrations were 99 mg/100 g of dry matter (DM) in cv. Muscadine seeds, 15 mg/100 g of DM in cv. Chardonnay seeds and 10 mg/100 g of DM in cv. Merlot seeds, respectively. This phenolic constituent of grape contributed to the antioxidant capacity, measured as oxygen radical absorbance capacity (ORAC), on the basis of the corrected concentrations of gallic acid.

Cinnamic acid is a C6-C3 compound produced by a deamination of phenylalanine catalysed by phenylalanine ammonia-lyase; para-coumaric acid (p-coumaric) is then produced by hydroxylation of cinnamic acid. Cinnamic acid and hydroxycinnamic acids are also called phenylpropanoids. Their basic skeleton is a benzene ring with a three-carbon aliphatic chain, with one or more hydroxyl groups often esterified as esters of aliphatic alcohols. Common hydroxycinnamic acids are caffeic,
p-coumaric, ferulic and sinapic acids. They are produced by a series of hydroxylations and methylations and they often accumulate in the form of tartaric acid esters: coutaric, caftaric and fertaric acids, which are esters of p-coumaric, ferulic and sinapic acids, respectively. These constituents are present mainly in the flesh of grape berries. The main hydroxycinnamic acid in grapes is caftaric acid (cafeoyl-tartrate ester), which may reach about 200 mg/kg.

Stilbenes

Stilbenes are polyphenolic compounds that have a C6-C2-C6 structure, with two benzenes linked by a methylene bridge. They are produced by plants in response to fungal, bacterial and viral attacks; this has been particularly demonstrated for trans-resveratrol. Resveratrol is synthesized by condensation of 4-coumaryl CoA with three molecules of malonyl CoA, each giving two carbon atoms. The reaction is catalysed by stilbene synthase; the products involved are the same as for the synthesis of flavonoids, the only difference being the enzyme catalysing the reaction. Resveratrol is present in the cis and trans forms, and is present in plant tissue mainly in the form of trans-resveratrol-3-O-glucosides (trans-piceid and trans-astringine). There are oligomers of stilbenes, identified in grapes, such as palloidol and viniferins (Ribeiro et al., 1999; Landrault et al., 2002; Vitrac et al., 2005) and, more recently, a tetramer of resveratrol: hopeaphenol (Guebailia et al., 2006).

In grapes, stilbene synthesis occurs in the skin and is induced by biotic and abiotic stresses. Stilbene biosynthesis has been investigated in healthy grapes, at both biochemical and molecular levels, by measuring the concentration of resveratrols (trans-resveratrol, trans-piceid and cis-piceid) in the ripe berries of 78 V. vinifera varieties for 3 years (Gatto et al., 2008). Significant differences appeared among genotypes, providing the first tentative varietal classification based on resveratrol content. Furthermore, an increasing stilbene accumulation from veraison to ripening phase was also observed. The highest resveratrol-producing varieties found are cvs. Pinot Noir (22 mg/l), Pinot Tete de Negre (18 mg/l), Tarrango (16 mg/l), Franconia (16 mg/l), Marsanne, Roussanne and Malvasia (c.12 mg/l). The lowest resveratrol producers include cvs. Xarello, Refosco and Primitivo (between 3 and 5 mg/l), Petit Manseng and Nebiolo (< 3 mg/l). Moreover, macroarray data analysis revealed that high resveratrol levels were also accompanied by the upregulation of genes involved in plant defence, and the concomitant underexpression of genes related to the ripening process and to indole alkaloid synthesis. Results obtained for the cardiovascular benefits and cancer chemopreventive activity of resveratrol might be relevant to grape consumption, especially responses that could be mediated by low concentrations of the substance (Pezzuto, 2008).

9.2.2 Sugars

Grape is one of the sweetest fruit, with sugar levels reaching 20% of fresh weight at full maturity. In raisins, the sugar content can reach 60%. Obviously, this is important for a nutritionist given the consequences of a lack or excess of sugars in a diet.

Glucose, fructose, galactose, sucrose, maltose, melibiose, raffinose and stachyose were identified in the berries of grape V. vinifera L. cv. Thompson Seedless (Kliewer, 1966). The grapes accumulated mainly glucose and fructose, at equal levels. On average, a grape contains 15-18 g of sugars in 100 g of fresh weight at full maturity. Total soluble solids can reach 20, 22 or even 25% (when in many fresh fruit it does not exceed 12%).

9.2.3 Organic acids, minerals and aromas

Organic acids found in grape berries are: malic, tartaric, citric, isocitric, ascorbic, cis-aconitic, oxalic, glycolic, glyoxylic, succinic, lactic, glutaric, fumaric, pyrrolidone-carboxylic, α-ketoglutaric, pyruvic, oxaloacetic, galacturonic, glucuronic, shikimic, quinic, chlorogenic and caffeic (Kliewer, 1966). The organic acids reach 1-1.5 g/100 g of FW.

In the water reserve of grape berries, which represents over 80% of the fruit fresh weight, many inorganic compounds (K⁺, Ca²⁺, Mg²⁺, Fe²⁺, SO₄²⁻, PO₄³⁻) are also present.
It should be noted that among the minerals present, potassium (especially in the form of bitartrate) dominates, with a level of 250 mg/100 g FW. The level of sodium remains very low (2 mg/100 g FW). There are also trace elements (reducing or oxidizing), essential for carrying out the chemical reactions required for cell multiplication (Se, Ni, Cr, I, Zn, Cu, Mn, F, V, Co,...), which give the grape some original nutritional qualities. The aroma compounds in grapes are either free, such as terpenes, or bound, mainly as glycosides. They are detailed in a recent review on grape berry biochemistry (Conde et al., 2007).

9.2.4 Vitamins

One interesting point with grapes is that they accumulate very low levels of vitamin C, ascorbic acid, in comparison with other fruit. This may be due to the fact that grape berry tissues transform ascorbic acid to tartaric acid, as shown in a recent study (De Bolt et al., 2006). The group B vitamins are all well represented, particularly B₁, B₃ and B₅. The level varies with the degree of maturity of the grapes. Vitamin C (more abundant in the outer grain) varies between 4 and 10 mg/100 g. The mean nutritional profile of a grape, including the percentage of the RDA (recommended daily allowance) of minerals and vitamins represented by a 100g serving, is given in Table 9.3.

9.3 Chemopreventive Activity and Bioavailability

The grape offers some potential health benefits via its phenolic composition, flavonoids and non-flavonoids. It also has sugars, acids and vitamins that may have interesting properties which can be modulated by an adapted nutrition.

9.3.1 Cancer studies

Many grape polyphenols have been shown to have anticancerous effects. The polyphenol, 3,5,4'-trihydroxystilbene, commonly called resveratrol, was shown to be an effective candidate for chemoprevention of lung cancer due to its ability to induce apoptosis (Weng et al., 2009). It is worth noting that polydatin, a glycoside of resveratrol, which is present in grape juice and wine, has been shown to be metabolized rapidly to resveratrol in the small intestine and liver of rats, then metabolized further to glucuronidated resveratrol (Zhou et al., 2009).

Other studies have shown that grape polyphenols play several roles in limiting cancer development. For example, proanthocyanidins have been shown to prevent the metastatic cascade by mediating the inhibitory signals for cancer cell migration, an essential step in invasion and metastasis (Punathil and Katiyar, 2009).

9.3.2 Cardiovascular diseases

The moderate consumption of grapes or grape products (containing polyphenols) may lead to a decrease of platelet aggregation and vasodilatory effects in blood vessels. In the case of atherosclerosis, polyphenols reduce the formation of the plaque, cholesterol, and increase rates of certain enzymatic antioxidant defences against free radicals. Recently, Décordé et al. (2008) were able to demonstrate that the phenolic compounds of red grapes prevented the development of atherosclerosis, induced earlier by an atherogenic diet, in Syrian golden hamsters. In this work, consumption of grapes (cv. Hambourg Muscat) or derived juice by the hamsters, at doses equivalent to 600 g/day or 500 ml/day, respectively, for a man weighing 70 kg, led to a reduction in the surface fat deposition in the aortic arch by 78 and 93%, respectively, compared with that seen in the control animals (atherogenic food only). Likewise, with the consumption of grapes or grape juice, total cholesterol was decreased by 30.4 and 34.6%, and non-high-density lipoprotein cholesterol by 64 and 58.9%, respectively. In parallel, levels of antioxidant capacity of blood plasma in the animals receiving the grapes or grape juice increased by 41 and 61%, respectively. In
Table 9.3. Nutritional profile of grape (for 100 g).

<table>
<thead>
<tr>
<th>Class of nutrients</th>
<th>Specific nutrients</th>
<th>Quantity</th>
<th>Adult EU RDA (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td></td>
<td>67.0 kcal</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>81.3 g</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td>17 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monosaccharides</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disaccharides</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soluble fibre</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insoluble fibre</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other saccharides</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>Arginine</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspartic acid</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histidine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenylalanine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proline</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Fats</td>
<td>Saturated fatty acids</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oleic acid</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linoleic acid</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linolenic acid</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Vitamins</td>
<td>β-Carotene</td>
<td>59.1 μg</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₁</td>
<td>0.09 mg</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₂</td>
<td>0.07 mg</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Niacine</td>
<td>0.30 mg</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₆</td>
<td>0.11 mg</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Biotin</td>
<td>0.30 μg</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Vitamin C</td>
<td>4.00 mg</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>0.35 mg</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td>3.89 μg</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Pantotenic acid</td>
<td>0.02 mg</td>
<td>0.3</td>
</tr>
<tr>
<td>Minerals</td>
<td>Potassium</td>
<td>191.7 mg</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
<td>14.0 mg</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
<td>10.0 mg</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
<td>5.0 mg</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
<td>2.0 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>0.7 mg</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>0.26 mg</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>0.04 mg</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>0.04 mg</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Iodine</td>
<td>1.0 μg</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Selenium</td>
<td>0.2 μg</td>
<td>0.3</td>
</tr>
<tr>
<td>Organic acids</td>
<td>Malic acid</td>
<td>540.0 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>23.0 mg</td>
<td></td>
</tr>
</tbody>
</table>

Note: aAdult European Union Recommended Daily Allowance (%).
agreement with other work (Vinson et al., 2001), a hypolipidaemic effect was also found. Indeed, the antioxidant potential of grape juice polyphenols has been shown to limit atherosclerosis linked to the oxidation of human low-density lipoproteins. A normalization of the systolic pressure, plus a significant reduction of cardiac hypertrophy and of free radical generation in the thoracic aorta, have also been observed.

A study compared the platelet activity ex vivo of human volunteers before and after they had drunk black grape juice, orange juice or grapefruit juice for 7–10 days (Keevil et al., 2000). Drinking red grape juice for a week reduced blood platelet aggregation by 77%, while the consumption of orange juice or grapefruit juice had no effect on this parameter. The red grape juice contained approximately three times more polyphenols than the total citrus juice and had platelet inhibitor potential in the healthy human subjects. The platelet inhibitory effect of grape juice may decrease the risk of coronary thrombosis and myocardial infarction. The bioavailability of other polyphenols, such as resveratrol and quercetin, has also been shown (Meng et al., 2004).

Despite its relatively low level, the vitamin C in grapes is important. Indeed, it reinforces the roles of anthocyanins and other polyphenols at the level of the arterial wall, as it acts as a cofactor. It is therefore worth noting that 4 mg of vitamin C/100 g fresh grapes may have an impact on polyphenol actions.

### 9.3.3 Antibiotic effects

Extracts from grape seeds have been tested for antibacterial activity against Bacillus cereus strains, B. coagulans, B. subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Jayaprakasha et al. (2003) demonstrated that Gram-positive bacteria could be inhibited completely with 850–1000 μl/1 of phenolic extract of grape seed, while Gram-negative bacteria were inhibited by levels of 1250–1500 μl/1 extracts of grape seed.

The antibacterial action of organic acids against oral streptococci, responsible for cavity development, and against Streptococcus pyogenes, responsible for pharyngitis, have been studied (Daglia et al., 2007). The compounds found responsible for such activities were succinic, malic, lactic, tartaric, citric and acetic acids. Findings show that organic acids found in grapes are active against oral streptococci and S. pyogenes and suggest that it enhances oral health. There are, however, concerns over the effect of fruit acids on enamel integrity.

Some antimicrobial activities were observed with water-soluble extracts prepared from cv. Muscadine seeds (Kim et al., 2008). The extracts had strong antimicrobial activity against a cocktail of three strains of E. coli O157:H7. Extracts had high acidity (pH 3.39–3.43), total phenolics (2.21–3.49 mg/ml), tartaric acid (5.6–10.7 mg/ml), tannic acid (5.7–8.1 mg/ml) and gallic acid (0.33–0.59 mg/ml). Heat treatment of the extracts increased antimicrobial activity, possibly because of increased acidity, tartaric acid, total phenolics and individual phenolics.

With regard to the relationship between the structure and activity of triterpene acids (ursolic, oleanolic, gypsogenic and sumareresinolic acids) and derivatives, it was found that both hydroxy and carboxy groups present in the triterpenes were important for their antibacterial activity against several oral pathogens (S. mutans, S. mitis, S. sanguinis, S. salivarius, S. sobrinus and Enterococcus faecalis), potentially responsible for the formation of dental caries in humans (Scalon Cunha et al., 2007).

Flavonoids and related polyphenols also possess promising anti-HIV effects. A study showed that grape seed extracts (GSE) downregulated the expression of HIV-1 entry coreceptors significantly and that GSE-treated cultures showed a significantly lower number of HIV-positive cells (Nair et al., 2002).

### 9.3.4 Metabolic diseases

In order to improve defences against oxidative stress in diabetes and stimulate oxygen secretion, it is necessary to maintain high antioxidant capacity of the plasma. The high levels of fructose and glucose in grapes make
them a useful supply of rapidly available sugars to counteract hypoglycaemia.

The grape, with its wealth of organic acids and minerals, can affect the acid–alkaline balance of the cellular environment. The grape facilitates urinary excretion because of its high water and potassium contents, which promote diuretic action. Grape suspensions in water or ethanol have been shown to prevent obstructive bladder dysfunction, which often affects ageing men (Agartan et al., 2005). The stimulation of intestinal transit is also facilitated by the presence of effective fibres in grapes. This dual activity on elimination (urine and faeces) has sometimes resulted in the prescription of grapes in detoxification diets. The potassium/sodium ratio is high (around 125) in grapes and this may also contribute to the diuretic properties of this fruit (Szentmihályi et al., 1998).

9.3.5 Other beneficial effects

Fresh and dried grapes provide phenolic antioxidants, which are believed to contribute to potential health effects. In a recent study, Parker et al. (2007) checked the antioxidant capacity of grape polyphenols from (fresh, dried) cv. Thompson Seedless and observed the effects of their consumption for 4 weeks in 15 healthy human subjects. The ORAC (oxygen radical absorbance capacity) was increased after 2 weeks of grape consumption (250 g/day) and after 3 weeks of raisin consumption (50 g/day). Even the oxidation of serum was significantly limited by the consumption of sultanas (golden raisins) after 4 weeks (time latency).

The main sugars accumulating in grape berries are glucose and fructose, as mentioned previously. Even if they are not regarded generally as bioactives, they provide readily available energy, thanks to their high bioavailability, which is increased by the fact that these sugars are accompanied by natural organic acids and group B vitamins that ensure their good assimilation at the cellular level. The grape is therefore a recommended natural food for high-energy needs or when the body is subjected to intense muscle activity. The grape provides an average of 72 kcal (301 kJ)/100 g FW, with extremes ranging between 60 and 80 kcal, depending on the sugar content.

Organic acids give grapes a refreshing flavour, which compensates for their pleasant but high sweetness. They also have a slightly stimulating action on digestive secretions, which facilitates good assimilation. The presence of some vitamins in grape berries plays a beneficial role, especially in the functioning of the nervous system, thanks to the presence of group B vitamins (vitamin B deficiency has been shown to be linked with mouse cognitive dysfunction (Troen et al., 2008)), and for the protection of capillary blood vessels, due to ‘vitamin P’ action.

9.4 Effects of Postharvest Continuum

The literature is scarce on postharvest treatments affecting grape bioactives and nutrition. It is likely that there may be more studies in the future, now that bioactive compounds have attracted more attention.

The main physiological change that occurs during the postharvest life of grapes is loss of water. During grape dehydration, Moreno et al. (2008) observed that the soluble solid content increased. The same study showed that the amount of anthocyanin per berry remained unchanged over the dehydration process (weight loss of 15% in 4 days) and that the terpene contents increased in wines made from dried grapes, but the study did not check whether this was due to concentration changes and/or modulation of the glycosylated compound content. Glycosylation may indeed modify compound availability in the digestive process, thus modulating the nutritional value of grapes. However, Bellincontro et al. (2006) showed that, in a different grape cultivar, cv. Aleatico, a longer postharvest period (13 days at a relatively high humidity) induced a decrease in anthocyanins, but that a postharvest treatment with ethylene increased the global polyphenol content. Postharvest ethylene treatment was also shown to increase the concentrations of some alcohols. In another study, the amount of sodium metabisulfite used to preserve table grapes (as an antioxidant and
antifungal agent) was shown to preserve the vitamin C content (Sharayei et al., 2004).

Several studies have demonstrated that postharvest UV treatment and wilting can induce some increase of the stilbene content in various cultivars of grapes (Versari et al., 2001; Cantos et al., 2003). Sanchez-Ballesta et al. (2006) showed that a high CO₂ pretreatment could delay stilbene accumulation over the first part of storage; this compound increasing again during the shelf-life period. Treatment of table grapes, cv. Autumn Seedless, with ozone increased total phenolics, while modified atmosphere packaging maintained their concentration (Artes-Hernandez et al., 2007). These postharvest treatments were tested as an alternative to the ubiquitous industrial SO₂ application, which can cause bleaching, berry drop and flavour taint.

The main postharvest problem affecting table grapes is the development of fungi. Disease is manifested principally as a result of infection by Botrytis cinerea, the causal agent of grey mould. Rots increase waste and reduce grape quality by consuming sugars and producing some acids, and render the clusters unattractive so they loose their commercial value. Although B. cinerea generally is not considered a hazard to human health, some fungi, like Aspergilus spp., may produce ochratoxin A (Hocking et al., 2007), and this is a mycotoxin that has attracted recent attention. Hocking et al. (2007) reviewed the pre- and postharvest factors affecting Aspergilus development and the solutions available to avoid ochratoxin accumulation. One strategy that may be used to counteract these fungi in the coming years is the use of antagonistic yeasts (Bleve et al., 2006) isolated from the associated microflora found on the surface of the berries.

9.5 Conclusions

Grape berries, and the derived products thereof, could play a preventive role in many diseases when consumed regularly and moderately. The phenolic compounds of grapes and wine undeniably have therapeutic properties (Auger et al., 2002; Landrault et al., 2003; Al-Awwadi et al., 2004a,b, 2005), particularly for certain chronic diseases such as atherosclerosis, diabetes, hypertension and some cancers. Among the mechanisms of action of phenolic compounds involved in the prevention of chronic diseases, the following should be recognized:

- a direct effect by trapping free radicals.
- preserving endogenous antioxidants (vitamin E, vitamin C, β-carotene...).
- preserving antioxidant enzymes (SOD: superoxide dismutase, catalase, SeGSH-Px: glutathione peroxidase).
- reducing the re-equilibration of cholesterol and blood lipids (HDL/LDL).
- chelation of cofactors of fatty acid oxidation such as some metals (Fe²⁺, Cu²⁺).
- inhibitory effect on oxidative enzymes such as cyclooxygenases and lipoxygenases.
- effects on the synthesis of endothelial nitric oxide: at the cellular level of the arterial wall leading to vasorelaxation and a hyperpolarization of the membrane by human extracellular potassium.
- inhibit the production of NAPH oxidase-level cells of the vascular wall (thoracic aorta and heart), thus reducing the production of free radicals.

Polyphenols have received a lot of attention in the past two decades regarding their chemopreventive role, but other compounds such as sugars are present at very high concentration in grapes and present interesting or negative nutritional values, depending on the consumer and the quantities ingested; sugars (derived from both natural and synthetic sources) are now under the scrutiny of many nutritionists. Whether future specific health claims will be sought from or allowed by regulatory authorities is not known but, based on existing data, it is clear that grapes should be considered an integral component of the fruit- and vegetable-enriched diets that are widely recommended by health authorities. Further research on the effects and mechanisms of action of compounds in grape and its derivatives on chronic diseases needs to be pursued.
Grape

References


Anon. (1980) Polyphenols [Substance Name]; use the precise structure header, most commonly in the Flavonoids group; this term only refers vaguely to phenolic (aromatic) hydroxyls. Date introduced: 18 August 1980 in MeSH database.


FAO (2000) Fruit production and consumption, data from World Fruit Program.


Leafy Vegetables and Salads

Peter M.A. Toivonen and D. Mark Hodges

10.1 Introduction

The diversity in consumption of leafy vegetable types is significant worldwide (Table 10.1). Most leafy vegetables are consumed in a cooked format. However, consumption in the raw state is becoming a more common practice, partly attributable to expanded production of salad vegetables in regions of the world that historically have not consumed the products in a raw format. The need for cooking in many leafy vegetables may arise from the need to detoxify components present in the raw product (Kuti and Konuru, 2006; Orech et al., 2006). Also, some very bitter indigenous leafy vegetables are very high in polyphenols, but will show significant losses of these bitter compounds with cooking, which thus renders them more palatable (Kuti and Konuru, 2004). Hence, it is important to keep this in mind when evaluating the consumption format (cooked versus raw) for adaptation of indigenous leafy vegetables to conventional Western diets.

In this chapter we will deal with lettuces and other salad greens (e.g. spinach). Lettuces of different types, such as romaine and leafy lettuces, increasingly are cultivated globally, depending on the type and preferences of the population (de Vries, 1997). There are other leafy vegetables, such as spinach and the leafy Asian crucifers, which make up a large dietary distribution in Asia as well as North America and Europe (FAO, 2008). Less ubiquitous are indigenous green leafy vegetables, which can form a significant local requirement for nutrition; some, like tree spinach, are also eaten more widely as part of ethnic diets in the developed world (Kuti and Konuru, 2006). Leafy green vegetables make up a significant component of healthful diets around the world (FAO, 2008).

The production of leafy vegetables intended for salads has expanded significantly and forms a key agrifood sector in North America, Europe, Australia and New Zealand (Hedges and Lister, 2005). The industry is so well developed that now a significant portion (over 30% in the USA) of the fresh vegetable industry is involved in the ready-to-eat format as packaged salads (Cardwell, 2005). Consumption of leafy vegetables such as iceberg and romaine lettuces has shown a large increase over the past several decades, as demonstrated by US data (Putnam and Allshouse, 1999), and this has been attributed partly to the convenience factor of the ready-to-eat (fresh-cut) format in an increasingly busy world (Rocha and Morais, 2007). Consumer awareness of the healthfulness of leafy vegetables in their diet has also contributed to increased consumption of leafy vegetables and related products (Goldman, 2003; Rai et al., 2006; Rocha and Morais,
<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Distribution</th>
<th>Usage format</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceberg lettuce or crisphead lettuce</td>
<td><em>Lactuca sativa</em> L. var. <em>capitata</em></td>
<td>USA, Canada, Europe</td>
<td>Fresh salad</td>
</tr>
<tr>
<td>Romaine (cos) lettuce</td>
<td><em>Lactuca sativa</em> L. var. <em>longifolia</em></td>
<td>Europe, North America, Australia</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Loose-leaf lettuce, green leaf lettuce, red leaf lettuce or oak leaf lettuce</td>
<td><em>Lactuca sativa</em> L. var. <em>crispa</em></td>
<td>Worldwide</td>
<td>Fresh salad</td>
</tr>
<tr>
<td>Butterhead lettuce, Boston lettuce or Bibb lettuce</td>
<td><em>Lactuca sativa</em> L. var. <em>capitata</em></td>
<td>Europe, North America</td>
<td>Fresh salad</td>
</tr>
<tr>
<td>Celtuce, stem lettuce, celery lettuce, asparagus lettuce or Chinese lettuce</td>
<td><em>Lactuca sativa</em> L. var. <em>asperagina, augustana or angustata</em></td>
<td>China</td>
<td>Stir-fried</td>
</tr>
<tr>
<td>Escarole, chicory, witloof, Belgian endive, French endive or frisée</td>
<td><em>Cichorium endivia</em> L.</td>
<td>Europe, North America</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Radicchio, leaf chicory or Italian chicory</td>
<td><em>Cichorium intybus</em> L.</td>
<td>Europe, North America</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Spinach</td>
<td><em>Spinacia oleracea</em> L.</td>
<td>Worldwide</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>New Zealand spinach</td>
<td><em>Tetragonia tetragonioides</em> (Pallas)</td>
<td>Worldwide</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td></td>
<td><em>O. Ktze</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chard, Swiss chard, silverbeet, perpetual spinach and mangold</td>
<td><em>Beta vulgaris</em> L. var. <em>cicla</em></td>
<td>Worldwide</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Pak choi</td>
<td><em>Brassica campestris</em> L. ssp. <em>chinensis</em> or <em>Brassica rapa</em> ssp. <em>chinensis</em></td>
<td>Worldwide</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Chinese leaf mustard, gai choi</td>
<td><em>Brassica juncea</em> Coss</td>
<td>China, Vietnam</td>
<td>Stir-fried</td>
</tr>
<tr>
<td>Yun tai</td>
<td><em>Brassica rapa</em> ssp. *nippisinica var. <em>chinoleiara</em></td>
<td>China</td>
<td>Stir-fried</td>
</tr>
<tr>
<td>Garden cress</td>
<td><em>Lepidium sativum</em> L.</td>
<td>Europe, North America, Asia</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Watercress</td>
<td><em>Nasturtium officinale</em> W.T. Aiton,</td>
<td>Europe, Asia</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td></td>
<td><em>Nasturtium microphyllum</em> (Boenn.) Rchb.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorrel, dock, sour dock</td>
<td><em>Rumex acetosa</em></td>
<td>Worldwide</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td>Tree spinach or chaya</td>
<td><em>Cnidoscolus chayamansa</em> McVaugh or</td>
<td>Guatemala, Belize, Mexico, Cuba, USA</td>
<td>Raw or cooked</td>
</tr>
<tr>
<td></td>
<td><em>Cnidoscolus aconitifolius</em> (P. Mill.) I.M. Johnston</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drumstick tree, horseradish tree</td>
<td><em>Moringa oleifera</em> Lam.</td>
<td>Tropics and subtropics of Asia and Africa</td>
<td>Cooked</td>
</tr>
<tr>
<td>Amaranth, bayam, kulitis, yin choi, lenga lenga, biteku teku, callaloo, eto tete, arowo jeja and vleeta</td>
<td><em>Amaranthus sp.</em></td>
<td>Indonesia, Malaysia, India, China, Congo, Caribbean, Guatemala, East Africa, Nigeria, Greece</td>
<td>Cooked</td>
</tr>
</tbody>
</table>
2007). As a consequence, it is quite likely that consumption of leafy vegetables will continue to grow. Moreover, as the developing world begins to tackle health-based issues relating to diet, there will be the need to adapt indigenous as well as mainstream leafy vegetables to support health-promoting initiatives (Nakahara et al., 2002; Fahey, 2005; Islam, 2006; Smith and Eyzaguirre, 2007).

10.2 Common Commodities

There is great variety in the leafy vegetables that are consumed in different areas of the world, including those that are consumed in indigenous diets (Table 10.1). While the word ‘indigenous’ is used to describe some leafy vegetables, globalization of world cuisine and emigration have broadened the geographic consumption of many of these vegetables. In many cases there is a movement to enhance the consumption of very healthful indigenous leafy vegetables as part of a strategy to ensure good nutrition and health in developing countries (AVRDC, 2004). There is a wide range of plant genera that provide leafy vegetables that can be consumed by humans. Their use and distribution will be discussed briefly, to gain a perspective on consumption rates and impact on human diets. While an attempt has been made to review as many leafy vegetables as possible, the discussions will focus necessarily on the vegetables for which there is a significant amount of bioactive constituent data in the literature.

10.2.1 Lettuce and specialty salad vegetables

Three main families of plants provide the bulk of the commonly consumed lettuce and specialty salad vegetables. The largest variety of lettuce and lettuce-type vegetables consumed is included under the Compositae family of vegetables. These vegetables are generally labelled as lettuces or chicories. Many members of the Brassicaceae family of vegetables have become popular in salad mixes. Finally, Amaranthaceae family leafy vegetables are used extensively, either cooked or as salad vegetables or garnishes.

The largest representation from the Compositae family comes from the leafy vegetables classified as lettuces, and they are all classified as the species Lactuca sativa L. and are generally divided into six edible genotypes: butterhead, cos, latum, crisphead, cutting and stalk lettuce (de Vries, 1997). The common names associated with lettuces include iceberg lettuce or crisphead lettuce, romaine or cos lettuce, loose-leaf lettuce, green leaf lettuce, red leaf lettuce, oak leaf lettuce, butterhead lettuce, Boston or Bibb lettuce, celtuce or stem lettuce, celery lettuce and asparagus or Chinese lettuce (Table 10.1). While most are eaten in a raw format as a salad vegetable, some are eaten
cooked, depending on the food customs of the area in question. Celtuce or stem lettuce, also known as asparagus or Chinese lettuce, for which the stem is the primary edible component, generally is eaten in the cooked form. Lettuce production and consumption are worldwide in scope (de Vries, 1997).

The chicory leafy vegetables are considered specialty salad vegetables and are also members of the Compositae family. There are many common names used within the species Cichorium endivia L., including escarole, chicory, witloof, Belgian endive, French endive or frisée (Lucchin et al., 2008). There are also several common names associated with the leafy portions of a related species, C. intybus L.: radicchio, leaf chicory or Italian chicory (Lucchin et al., 2008). It is consumed in fresh and cooked forms, mostly in Europe, but has gained popularity in other parts of the world.

The Amaranthaceae are represented by vegetables commonly known as chard, Swiss chard, silverbeet, perpetual spinach and mangold (Beta vulgaris L. var. cicla) or beet leaves (B. vulgaris subsp. vulgaris). These two types of leafy vegetables have been cultivated in Europe since antiquity and are now grown worldwide, including parts of Asia (Pyo et al., 2004; Goldman and Navazio, 2008).

The Brassicaceae are represented by the vegetables commonly called cresses. Watercress (Nasturtium officinale W.T. Aiton, N. microphyllum (Boenn.) Rchb.) and garden cress (Lepidium sativum L.) are widely consumed in cooked or salad forms throughout Europe, North America and Australasia (Nuez and Hernández Bermejo, 1994; Fennell, 2006).

Coriander (also known as cilantro, dha-nia, kindza, Chinese parsley, Mexican parsley) is classified under the Apiaceae family. Coriander is consumed in diets around the world (Alberta Agriculture, Food and Rural Development, 1998).

10.2.3 Asian leafy brassicas

These vegetables are species of the genus Brassica and include leafy vegetables commonly termed pak choi, Chinese leaf mustard or gai choi and yun tai. While the consumption of these leafy vegetables is primarily in China, it is increasing in transplanted populations of Chinese and Asians in other parts of the world (Nöthlings et al., 2006; Chen et al., 2008). They are all used in cooked formats, but most especially as part of stir-fried mixtures.

10.2.4 Indigenous greens

The indigenous leafy vegetable grouping includes a broad range of species generally eaten in a cooked form. While some are widely consumed, their consumption is in traditional diets and hence these vegetables are classified in this chapter as indigenous.

Tree spinach or chaya (Cnidoscolus chayamansa McVaugh or C. aconitifolius (P. Mill.) I.M. Johnston) is in the family Euphorbiaceae and is always eaten in the cooked form since thorough cooking destroys most of the toxic cyanogenic glycosides present in the raw leaves. Tree spinach is consumed primarily in Central America, Mexico and southern USA (Kuti and Konuru, 2006).

Several different species of amaranth (Amaranthus sp.) are considered indigenous vegetables, even though they are consumed globally in a cooked format. Amaranth species are cultivated and consumed as a leafy vegetable in such diverse countries as Indonesia, Malaysia, India, China, Congo, Caribbean, Guatemala, East Africa, Nigeria and Greece (Enama, 1994; Costea, 2003).

Young cowpea (Vigna sp.) shoots have been used as a green leafy staple in many tropical and subtropical countries of the world (Imungi and Potter, 1983; Booth et al., 1992; Mosha et al., 1997). Cowpea shoots are
now consumed worldwide in the developed and developing world (Fatokun et al., 2002).

Sweet potato leaves (*Ipomoea batatas* (L.) Lam) are consumed predominantly in African and Asian countries (Nwinyi, 1992; Almazan et al., 1997). The shoots of sweet potato are rich in bioactive content, the tuber of the sweet potato is an important crop worldwide and the crop is tolerant of a range of climatic conditions (including monsoon season) (Islam, 2006). Therefore, it is felt that there is good potential for this leafy vegetable to become an important part of a healthy diet in most of the developing world (Islam, 2006).

Water convolvulus leaves (*I. aquatica*) are widely consumed in Asian countries (Prasad et al., 2005). The vegetable has many common names, including Chinese water spinach, kangkong and swamp cabbage.

### 10.3 Major Phytochemicals

Leafy vegetables are highly regarded as carotenoid-enriched foods and many studies have shown that consumption of green leafy vegetables, such as spinach and collard greens, rich in the carotenoids lutein and zeaxanthin is associated with a substantially reduced risk of cataracts and advanced macular degeneration, one of the leading causes of blindness among the elderly in North America (Seddon et al., 1994). In another carotenoid study, lutein intake from dietary sources that included spinach, lettuce and greens was associated inversely (after adjustment for fibre and folate) with colon cancer in both men and women, with the greatest inverse association observed in patients who were diagnosed when they were young (Slattery et al., 2000). The food matrix itself can have a dramatic effect on bioavailability of leafy vegetable bioactive carotenoids. For example, either a 300 g dose (containing 20.8 µmol *trans-*β-carotene equivalents) of pureed spinach or a 100 g dose (containing 19.2 µmol *trans-*β-carotene equivalents) of carrots that had been deuterated intrinsically was fed to each of a group of men and women, with a standard liquid diet containing 13.5 g fat, for a 21-day period (Tang et al., 2005). The subjects fed the spinach exhibited a blood serum retinol yield of $21 \pm 11$ nmol/dose, whereas those who received the pureed carrot showed a yield of $32 \pm 16$ nmol/dose. The authors explained the yield difference between spinach and carrot as due to the β-carotene in spinach being associated with pigment proteins in the chloroplasts, whereas it was in the form of more easily digestible carotene crystals in carrot chromoplasts.

Bioactive compounds other than carotenoids present in leafy vegetables have also been studied for their efficacy in health promotion. For example, in its role of promoting bone health, a study of phylloquinone (vitamin K) and incidences of hip fractures demonstrated that women who consumed one or more daily servings of lettuce (the study food that contributed most to the dietary phylloquinone intakes) exhibited significantly reduced risk of hip fracture (Feskanich et al., 1999). Out of more than 72,000 studied patients, 65 cases of hip fracture occurred with less than one lettuce serving/week, 52 cases occurred with two to four servings/week and 46 cases with five or six servings/week. A serving in that study was defined as one cup (~227 g) of lettuce.

In a comparison between glycolipid extracts from spinach, parsley, green onion, chive, sweet pepper, green tea, carrot and garlic, spinach extracts demonstrated the strongest inhibition of DNA polymerase α and human cancer cell proliferation (in cell cultures of gastric cancer and promyelocytic leukaemia cell lines), which the authors attributed to spinach having the highest levels of sulfoglycolipids (Kuriyama et al., 2005).

As a final example of the effects of consumption of leafy vegetable products on human health, several polyphenolics isolated from sweet potato leaves were applied to three human cancer cell lines (stomach cancer, colon cancer and promyelocytic leukaemia cell lines) (Kurata et al., 2007). Results indicated that the polyphenolics, especially 3,4,5-tri-O-caffeoylquinic acid, inhibited both the mutation of normal cells as well as the growth of cancer cells by apoptosis induction. Moreover, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid and 3,4,5-tri-O-caffeoylquinic acid exhibited highest sensitivity towards the cancer cell
lines in the order of the promyelocytic leukaemia, stomach cancer, then colon cancer cell lines.

10.3.1 Carotenoids

Most green leafy tissue has significant levels of lipophilic carotenoids and, as such, all leafy vegetables surveyed in this chapter are known to contain quantities of various carotenoids (Table 10.2). In this chapter the word 'carotenoid' is used as a general term to refer to both pure hydrocarbon carotenoids and oxygen-containing xanthophylls. The carotenoids are essentially long chains of conjugated double bonds and are differentiated by cyclization of the end group or by addition of oxygen (Rao and Rao, 2007). Xanthophylls have identical structures to carotenoids, other than specific sites where hydrogen atoms are substituted with hydroxyl groups or oxygen. The compounds considered as carotenoids are β-carotene, α-carotene, lutein, lycopene, neoxanthin, zeaxanthin and violaxanthin. All of these species have significant antioxidant capacities and it is thought this is one of their primary roles with regards to human health benefits. In addition, they have also been shown to play roles in such processes as immune response, modulation of particular drug-metabolizing enzymes, regulation of cell growth and gap junction communications (as referenced in Rao and Rao, 2007). Moreover, α-carotene and β-carotene, as well as β-cryptoxanthin, can be converted to vitamin A, and lutein and zeaxanthin have a specific function in protecting against high-energy blue light in the eye (Johnson, 2002). In general, β-carotene tends to be the predominant species in most leafy vegetables, with lutein plus zeaxanthin making up the majority of the remainder of the carotenoids (USDA, 2008).

The variability in carotenoid content between the leafy vegetables listed in Table 10.2 is much less than for most of the other bioactives that are reported. This may be due to the fact that carotenoids are associated with photosynthesis and protection against high-light injury in the chloroplast membranes (Young, 1991). All plant leafy tissues would have a similar requirement for such a protection mechanism and, as such, the content in leafy tissues from different plants would be expected to be relatively stable.

10.3.2 Tocopherols/tocotrienols

In most cases the data in Table 10.2 have derived from data values representing vitamin E content, which includes various forms of both tocopherols (e.g. α, β and γ) and tocotrienols. As it is well established that tocopherols are found in the vegetative or green portions of a plant, while tocotrienols are localized in the seeds (Munné-Bosch and Alegre, 2002), it can be assumed that all the data referring to vitamin E can be considered to be the same, as referring specifically to tocopherols. The tocopherol levels in leafy vegetables are relatively low, but stable in amounts over all of the vegetables that are listed in Table 10.2.

As discussed for carotenoids, tocopherol contents of the leafy vegetables listed in Table 10.2 are very similar for all. This uniformity can be explained by the role that this lipophilic bioactive plays in leaf cells. Tocopherol is a component of the plant membrane and acts as an important free radical protection system in both the chloroplast and mitochondria of leaf tissues (Munné-Bosch, 2005). Since all plant leaves require a similar protection capability, the contents of this bioactive would be expected to be extremely stable across all species. The consumption of one leafy green vegetable versus another should not result in great differences in tocopherol uptake.

Tocopherol consumption has been associated with a number of mammalian health benefits. For example, α-tocopherol acts as a potent chain-breaking antioxidant and can inhibit vascular smooth muscle proliferation (thus reducing incidences of atherosclerosis and hypertension). γ-Tocopherol reduces prostaglandin E2 synthesis and cyclooxygenase-2 activity (i.e. possesses anti-inflammatory activity) and may reduce the risk of diabetes and Alzheimer's disease (via protection against reactive nitrogen species) (for review, see Saldeen and Saldeen,
<table>
<thead>
<tr>
<th>Vegetable common name</th>
<th>Carotenoids (mg/100 g FW)</th>
<th>Tocopherols/tocotrienols (mg/100 g FW)</th>
<th>Ascorbate (mg/100 g FW)</th>
<th>Phenolics (mg/100 g)</th>
<th>Phylloquinones (ug/100 g)</th>
<th>Folate (ug/100 g)</th>
<th>Sulfur compounds (mg/100 g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td>4–13</td>
<td>0.2–0.3</td>
<td>3–24</td>
<td>105–453</td>
<td>16–173</td>
<td>30–136</td>
<td>ND</td>
</tr>
<tr>
<td>Spinach</td>
<td>4–18</td>
<td>2</td>
<td>5–28</td>
<td>2</td>
<td>380–498</td>
<td>172–302</td>
<td>ND</td>
</tr>
<tr>
<td>Chicory</td>
<td>14</td>
<td>2</td>
<td>24</td>
<td>320–537</td>
<td>298</td>
<td>110</td>
<td>ND</td>
</tr>
<tr>
<td>Endive</td>
<td>5–16</td>
<td>0.5</td>
<td>7–41</td>
<td>432–1093</td>
<td>231</td>
<td>48–142</td>
<td>ND</td>
</tr>
<tr>
<td>New Zealand spinach</td>
<td>16–18</td>
<td>1</td>
<td>30–36</td>
<td>123</td>
<td>~</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Swiss chard</td>
<td>15</td>
<td>2</td>
<td>30</td>
<td>145–1320</td>
<td>830</td>
<td>14</td>
<td>~</td>
</tr>
<tr>
<td>Garden cress</td>
<td>17</td>
<td>1</td>
<td>69</td>
<td>~</td>
<td>542</td>
<td>80–186</td>
<td>120–390</td>
</tr>
<tr>
<td>Watercress</td>
<td>8</td>
<td>1</td>
<td>43</td>
<td>263</td>
<td>250</td>
<td>9</td>
<td>17–145</td>
</tr>
<tr>
<td>Coriander</td>
<td>3–11</td>
<td>3</td>
<td>27–72</td>
<td>580</td>
<td>310</td>
<td>62–196</td>
<td>~</td>
</tr>
<tr>
<td>Bell tree dahlia</td>
<td>3</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>630</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Cowpea (leafy lips)</td>
<td>6</td>
<td>~</td>
<td>9–36</td>
<td>~</td>
<td>101–154</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Tziton</td>
<td>5</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>250</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Tree spinach</td>
<td>0.1–3</td>
<td>~</td>
<td>165–172</td>
<td>122–291</td>
<td>~</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Amaranth</td>
<td>6–8</td>
<td>1–2</td>
<td>43–59</td>
<td>247</td>
<td>72–130</td>
<td>85–332</td>
<td>ND</td>
</tr>
<tr>
<td>Drumstick tree leaves</td>
<td>7</td>
<td>9</td>
<td>36–245</td>
<td>691–1300</td>
<td>~</td>
<td>40</td>
<td>ND</td>
</tr>
<tr>
<td>Sweet potato leaves</td>
<td>3</td>
<td>13</td>
<td>11–35</td>
<td>684–1111</td>
<td>185–427</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Garland</td>
<td>3–5</td>
<td>~</td>
<td>45</td>
<td>257–281</td>
<td>230–350</td>
<td>177</td>
<td>~</td>
</tr>
<tr>
<td>Sorrel</td>
<td>1</td>
<td>0.4–0.6</td>
<td>26–48</td>
<td>1456</td>
<td>~</td>
<td>13</td>
<td>~</td>
</tr>
</tbody>
</table>

(Continued)
Table 10.2.  Continued

<table>
<thead>
<tr>
<th>Vegetable common name</th>
<th>Carotenoids (mg/100 g FW)</th>
<th>Tocopherols/tocotrienols (mg/100 g FW)</th>
<th>Ascorbate (mg/100 g FW)</th>
<th>Phenolics (mg/100 g)</th>
<th>Phylloquinones (µg/100 g)</th>
<th>Folate (µg/100 g)</th>
<th>Sulfur compounds (mg/100 g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water convolvulus leaves</td>
<td>10</td>
<td>~</td>
<td>16–45</td>
<td>726</td>
<td>~</td>
<td>35–225</td>
<td>ND</td>
</tr>
<tr>
<td>Arugula</td>
<td>5</td>
<td>0.4–2</td>
<td>15–254</td>
<td>132–235</td>
<td>109</td>
<td>97–196</td>
<td>95–139</td>
</tr>
<tr>
<td>Beet greens</td>
<td>5</td>
<td>1.5</td>
<td>30</td>
<td>128</td>
<td>400</td>
<td>15</td>
<td>~</td>
</tr>
</tbody>
</table>

Notes: ND = measured but not detected; ~ = no reported measurement found in the literature.
Source: Data are extracted and/or calculated from: Imungi and Potter (1983); Booth and Wickens (1988); Booth et al. (1992, 1993); Makkar and Becker (1997); Mosha et al. (1997); Abenavero et al. (1998); Booth and Suttie (1998); Jiao et al. (1998); Kuti and Kuti (1999); Chu et al. (2000); Alzoreky and Nakahara (2001); Ching and Mohamed (2001); Islam et al. (2003); Iwatani et al. (2003); McNaughton and Marks (2003); Ninfáli and Bacchiocca (2003); Rao (2003); Seshadri and Nambiar (2003); AVRDC (2004); Kuti and Konuru (2004); Poy et al. (2004); Thu et al. (2004); Damon et al. (2005); de Azevedo-Meleiro and Rodriguez-Amaya (2005); Higdon (2005); Innocenti et al. (2005); Krumberg et al. (2005); Alawaz (2006); Kidmose et al. (2006); Kim et al. (2006); Bergquist et al. (2007); Johansson et al. (2007); Kamao et al. (2007); Liu et al. (2007); Chen et al. (2008); Lavelli (2008); Martínez-Sánchez et al. (2008); USDA (2008); van der Walt et al. (2009).
The positive health benefits associated with tocopherol (vitamin E) have led to recommendations to use vitamin E supplements; however, recent clinical research has raised concern that this high-dose supplementation can actually increase mortality in humans (Miller et al., 2005). As a consequence, current medical advice is to rely on dietary tocopherol from fruit and vegetables while avoiding high-dosage supplements until more is known.

10.3.3 Ascorbate

Ascorbic acid is a ubiquitous antioxidant, providing protection against most, if not all, free radical species (Smirnoff, 1996). Unlike carotenoids and tocopherol, ascorbic acid is water soluble and shows a high degree of variation in content between leafy vegetable types, and even within a particular vegetable species (Table 10.2). Since it is a major metabolite in plant cells and has multiple functions, it is found in significant quantities in plant tissues, particularly photosynthetic tissues (Table 10.2; Smirnoff, 1996). Ascorbic acid is quite labile; hence, a significant ingestion is required to prevent deficiency symptoms such as scurvy (Smirnoff, 1996). In humans, ascorbate can promote health in its role as an antioxidant (i.e. defend against oxidative stress-related diseases such as cancer, various neurological disorders and cardiovascular disease) and, as an enzyme cofactor, it can participate in such reactions as collagen hydroxylation (collagen being important in the synthesis/maintenance of cartilage, skin, teeth, bones and gums) and carnitine synthesis.

As indicated above, there is a high degree of variation in ascorbate content in leafy vegetables. Some of this variation is due to differences in metabolism in tissues, but some variation may be related to the fact that ascorbate is so labile and is often the first bioactive compound to show decline under even optimal postharvest handling conditions (Gil et al., 1998). This effect of postharvest handling conditions on bioactives content will be discussed later in this chapter.

10.3.4 Phenolics

The term 'phenolic', for the purposes of this discussion, refers to flavonoids and phenolic acids. Flavonoid components probably have been the most widely studied of all the phenolics in regards to human health effects (Chu et al., 2000; Ekman and Patterson, 2005). The structure of the phenolic compound influences its bioactivity with regards to human health and a myriad of health-promoting activities has been ascribed to phenolics and their derivatives. Hydroxyl groups and conjugated double bonds allow the phenolic to assume an antioxidant function through electron donation, metal ion (e.g. Cu$^{3+}$, Fe$^{3+}$) chelation and active oxygen quenching. The presence of a conjugated sugar appears to enhance specific anticancer activities of anthocyanins (Katsube et al., 2003). Anthocyanins also possess anti-inflammatory activities due to their abilities to reduce chemoattractants, cyclooxygenase activities, chemokine content, platelet aggregation and cell wall adhesion factors in endothelial cells (Youdim et al., 2002; Sreenivasan and Gaffar, 2008). As a last example of the claimed bioactive effects of phenolics, high phenolic content has been associated with hypocholesterolaemic and hypotriglyceridaemic activities in hamsters (Lin et al., 2008). Leafy vegetables generally are very good sources of phenolics (Table 10.2; Ekman and Patterson, 2005).

Plant phenolics in the leaf are usually localized in the cell vacuole, a storage area for cell excreta (Toivonen and Brummell, 2008). In plants they can behave as anti- or pro-oxidants, depending on the environment in which they are present (Sakihama et al., 2002). Their role has been considered previously to be as preformed antifungals and antifeedants, due to their reactivity and bitterness (Sakihama et al., 2002; Ekman and Patterson, 2005). There is now consideration that they may also have protective effects for leaves against light damage, due to their ability to absorb high-energy light strongly (Close and McArthur, 2002). Also, because of their reactivity, phenolics become an important component of browning (healing) reactions after leaf cells have been damaged (Toivonen and Brummell, 2008).
Phenolic concentrations show a wide range of variation between and within the leafy vegetable types listed in Table 10.2. The lettuces generally have a moderate content of phenolics and the levels can range fourfold between types (Table 10.2; Liu et al., 2007); iceberg lettuce has the lowest concentrations; butterhead and Batavia intermediate concentrations; romaine and leaf lettuce have the highest concentrations. Other leafy vegetables that are commonly used in salads (i.e. chicory, endive, chard, coriander and sorrel) can have much higher levels of phenolics than lettuce (Table 10.2), and hence these vegetables add a bitter character to salads, which is associated with their high phenolic levels (Ekman and Patterson, 2005). Some of the indigenous vegetables, such as drumstick tree leaves, sweet potato leaves and water convolvulus leaves, have extremely high levels of phenolics, but this is likely moderated at the consumer level because all of these vegetables generally are cooked in water before consumption and these compounds are leached out of the leaves during cooking (Table 10.1; Kuti and Konuru, 2004).

### 10.3.5 Phylloquinones

Phylloquinones are very abundant in dark green vegetables (Table 10.2; Booth et al., 1993). There are two major forms of phylloquinones from natural sources and they are phylloquinone (vitamin K1) and menaquinone (vitamin K2); only phylloquinone is found in plant tissues (Booth and Suttie, 1998). In lettuces, phylloquinone content has a wide range, with greatest content in green, red and Boston leaf lettuces, followed by romaine and then iceberg lettuce (Damon et al., 2005). However, phylloquinone uptake is considered to be greater from iceberg lettuce in North America since that type of lettuce is consumed most (Damon et al., 2005). While much is known about phylloquinone content in some leafy vegetables, little is known for those not so commonly consumed (Table 10.2). In relation to human health, phylloquinone plays a role in blood coagulation (through action as a cofactor in conversion of specific glutamyl residues to γ-carboxyl glutamyl residues) and in increased bone-mineral density and reduced bone resorption (Shearer et al., 1996; Kamao et al., 2007).

#### 10.3.6 Folate

While folate is considered generally to be a significant nutrient from leafy green vegetables, it is clear from Table 10.2 that there is a wide range of folate concentrations within and between each type of leafy green vegetable. In terms of lettuce, the variation in folate concentration is fourfold, which is similar to the range found for phenolic concentrations in lettuces (Table 10.2). Romaine lettuce has the highest folate content, butterhead has about half that concentration and iceberg and leaf lettuces have a quarter of the concentration in romaine (USDA, 2008). Asian leafy brassicas and spinach have the highest contents of all of the most widely consumed leafy vegetables (Table 10.2). There are also several leafy vegetables that have very low folate concentrations, including Swiss chard, watercress and New Zealand spinach (Table 10.2).

Higher folate levels are known to exist in the leaves of plants than in other plant tissues (Gambonnet et al., 2001) and hypotheses for this higher concentration have been suggested. Gambonnet et al. (2001) found that folate was involved with nucleic acid synthesis in all parts of the plant. However, there are two potentially significant roles for folate (present as methyltetrahydrofolate) in the leaves: (i) as a substrate to enable S-adenosyl homocysteine recycling in mitochondrial photorespiration, a process that requires methylation reactions; and (ii) as a source for methyl groups for the assembly of the highly methylated chloroplast photosynthetic apparatus. Hence, it is expected that folate levels generally would correlate with chloroplast concentrations in a particular leafy vegetable, i.e. darker green vegetables generally should have relatively high folate levels.

Animals cannot synthesize folate; thus, plant foods are the most important sources of folate (Scott et al., 2000). Folate supplementation is most often recommended for pregnant women to reduce the risks of spina bifida and neural tube defects in developing
embryos (Daly et al., 1995). Folate deficiency in humans has been associated with higher risk of cardiovascular diseases, neurodegenerative diseases such as Alzheimer’s and increased incidences of colorectal, breast, pancreatic, bronchial and cervical cancers, as well as leukaemia (Lucock et al., 2003, as referenced in Rébeillé et al., 2006).

### 10.3.7 Sulfur compounds

Although there are several forms of sulfur compound found in plants, the discussion in this chapter will focus on the glucosinolate levels found in leafy vegetables. This group of sulfur compounds can be grouped into three chemical classes, aliphatic, indolyl and aromatic glucosinolates, based on whether their amino acid precursor is methionine, tryptophan or an aromatic amino acid (tyrosine or phenylalanine) (Rosa, 1999). While there are many families of plants that contain glucosinolates (Fahey et al., 2001), most of those listed in Table 10.2 are from the family Brassicaceae (see Chapter 5 of this volume). The only exception may be the drumstick tree. While the leaves of that tree have no measurable levels of glucosinolate (Makkar and Becker, 1997), the alternative common name, horseradish tree (Table 10.1), suggests that there must be some sulfur compounds that confer a pungent character in that leafy vegetable.

The main role of glucosinolate in plant leaves appears to be for defence against herbivory (Shroff et al., 2008). Physical injury to cells causes the native glucosinolates to be hydrolysed by myrosinase, a process which requires that the two become desegregated (Fahey et al., 2001; Shroff et al., 2008). The hydrolysis of glucosinolates results in the formation of acrid isothiocyanates, which are the active defence compounds (Fahey et al., 2001; Shroff et al., 2008). The isothiocyanates are the compounds that provide the sharp mustard- or horseradish-type character to vegetables containing glucosinolates, since mastication will initiate hydrolysis of the glucosinolates (Fahey et al., 2001).

Although glucosinolate breakdown products have been noted to exert a plethora of toxic and antinutritional effects in animals (e.g. detrimental effects on thyroid metabolism, embryotoxicity, growth impairment), epidemiological evidence also indicates that glucosinolate consumption reduces colon, rectum and thyroid cancers, and, when part of a diet enriched with other fruit and vegetables, protects against other cancers as well (Mithen et al., 2000). The anticancer activity of glucosinolates has been attributed primarily to their ability to induce phase 1 (e.g. cytochrome P450s) and 2 (e.g. glutathione-S-transferase) detoxification enzymes that modify and conjugate carcinogens prior to excretion.

### 10.4 Preharvest and Postharvest Effects on Bioactive Content

There are some reports on the effects of pre- and postharvest factors on the bioactive content of some leafy vegetables. However, this information is limited to only a few representative crops within this category. Hence, the following discussion is very limited for many of the leafy vegetables listed in Table 10.1.

#### 10.4.1 Preharvest effects

In the past, many cultivars have been chosen for their shelf-life qualities (Toivonen and DeEll, 2002), but in some cases this selection has also resulted in opting for cultivars having greater bioactive content. For example, Hodges and Forney (2003) associated higher basal ascorbate levels with improved shelf-life potential when comparing two cultivars of spinach. This finding is supported by another study finding that ascorbic acid concentration at harvest could be correlated with post-storage visual quality of spinach, thus indicating that the antioxidative capacity of ascorbic acid protects plant tissue against oxidative stress and ensuing quality loss (Bergquist, 2006). As databases develop for leafy vegetable cultivar differences in bioactive content, the commercial selection of cultivars that provide improved healthfulness to the consumer can be implemented. An additional benefit may also be improved postharvest handling characteristics.
There are a few reports identifying cultivar differences in bioactive content in the current literature. Differences in carotenoid, ascorbate, phenolic and phylloquinone content of lettuce types and cultivars have been reported by various groups (Degl’Innocenti et al., 2005; Johansson et al., 2007; Liu et al., 2007; USDA, 2008). The differences in the total phenolic content among leaf lettuce cultivars can be more than fourfold, from the lowest content cv. Two Star, to the highest content cv. Galactica (Liu et al., 2007). A study comparing the bioactive content of two cultivars of leaf lettuce showed that the cultivar having the highest ascorbate content also had the lowest levels of phenolics (Degl’Innocenti et al., 2005), suggesting that not all bioactive compounds might be optimized simultaneously in single cultivar selection. In regards to phenolics, it must be noted that cultivars having higher phenolic content may be less desirable for fresh-cut processing, since higher phenolic content is associated with greater severity in cut-surface browning (Toivonen and Brummell, 2008). Therefore, there must be a level of compromise between improving the functionality of the vegetable and maintaining its suitability for the market place.

Spinach also shows significant cultivar-dependent variation in bioactive content. Howard et al. (2002) and Pandjaitan et al. (2005) have demonstrated significant differences in phenolic and flavonoid content in numerous commercial spinach cultivars and advanced selections. Hodges and Forney (2003) found large differences in ascorbate content when they compared two commercial cultivars of spinach.

Cultivar evaluations in Swiss chard have focused on the phenolic components in the tissues. Pyo et al. (2004) have reported significantly greater levels of phenolic content in red- versus green-type Swiss chard, with the differences between the Swiss chards due to differences in both the phenolic acid as well as the flavonoid content/profiles. Gil et al. (1998) found that yellow-type Swiss chard had significantly higher flavonoid content than the green type. Differences in phenolic content have also been reported in cultivar comparisons of a vegetable related closely to Swiss chard, beet green (Ninfali and Bacchiocca, 2003).

Chicory cultivars show extremely wide variations in total phenolic content (Innocenti et al., 2005). The reported differences in phenolic content of chicory cultivars were associated largely with chicoric acid content, which accounted for over 50% of the phenolics extracted. Rocket, a leafy vegetable of similar usage as chicory, displays significant variation in both glucosinolate and flavonoid content among the different commercial cultivars (Bennett et al., 2006).

In regard to indigenous leafy vegetables, there has been a reported twofold difference in total flavonoid content between the red and green type of sweet potato leaf (Chu et al., 2000). In another study where ten sweet potato leaf cultivars were evaluated, there was a twofold range in total phenolic content between the lowest and highest content cultivars (Yashimoto et al., 2002). In a later study comparing 1389 sweet potato genotypes, a two orders of magnitude (i.e. 100-fold) difference was found in total phenolic content (Islam, 2006).

Asian leafy vegetables generally have been highly regarded for their health-promoting properties (Jiao et al., 1998; McNaughton and Marks, 2003), but very little is known about cultivar differences for this class of leafy vegetable. The glucosinolate content of Chinese cabbage cultivars can show up to threefold difference between the cultivar with the lowest and the one with the highest content (Lewis and Fenwick, 1988). Even greater differences in glucosinolate content can be found when comparing types of Chinese leafy vegetables (He et al., 2003).

There is limited information on production practices and their effects on the bioactive contents in leafy vegetables. Studies on the effects of nitrate levels in hydroponic feeding systems have shown that reducing nitrogen feeding levels by half induces a greater than twofold increase in ascorbate levels of butterhead lettuce (Chiesa et al., 2006). Not only is the nitrogen rate in the nutrient medium important with regards to bioactive content in leafy vegetables, but also important is the ratio of ammonium to nitrate. Kim et al. (2006) found that as the per cent molar ratio of ammonium to nitrate increased to 100 in the nutrient media, the
glucosinolate content of rocket leaves declined. In a comparison between organic and conventional production systems, there was no difference in phenolic content for lettuce, but organically grown pak choi had higher phenolic content than conventionally grown pak choi (Young et al., 2005). However, the authors attributed the significant result in pak choi simply to the fact that the organically grown product suffered from flea beetle attack and therefore the leaves would have produced more phenolics in response to the wounding caused by the flea beetle feeding.

The growing environment can also influence the bioactives that accumulate in some leafy vegetables. Butterhead lettuce grown in the open field had a much higher content of flavonols and caffeic acid derivatives than those grown in polycarbon-covered greenhouses (Romani et al., 2002). In a study on production practices in spinach, it was found that shading decreased ascorbic acid content, while in contrast it increased carotenoid concentration (Bergquist, 2006). However, in that same study, shading did not have a consistent effect on flavonoid content of spinach. Some of these responses may be explained by the effect of UV-B light exposure. Caldwell and Britz (2006) found that supplemental UV-B light led to higher carotenoid content in green leaf lettuce cultivars, whereas it also led to decline in carotenoid content in red leaf cultivars.

The geographic location where the leafy vegetable is grown may also affect the bioactive content of the vegetable. A study comparing the concentrations of ascorbate, phenolics and flavonoids in drumstick tree leaves demonstrated that their contents varied depending on whether the tree was grown in Niger, India or Nicaragua (Siddhuraju and Becker, 2003). Ascorbate content did not parallel the total phenolic or flavonoid content among the regions. A wide range in ascorbate, total phenolics and flavonoids was also determined in drumstick tree leaves in different growing regions within one country, Pakistan (Iqbal and Bhanger, 2006). Similarly, folate content has been found to vary in different lettuce types grown in various European countries (Johansson et al., 2007).

The age of the leafy vegetable at harvest will have a significant effect on the functional component levels in the leaves. Bergquist (2006) found that ascorbate and flavonoid content in spinach declined with the chronological age of the leaves and so suggested harvest of young leaves to ensure the best bioactive levels. However, carotenoid content did not change between leaves of different maturities. In apparent contrast, Pandjaitan et al. (2005) concluded that mid-maturity leaves of spinach had higher levels of flavonoids than younger or older leaves. These apparent differences in conclusions may be related to the fact that Bergquist (2006) harvested leaves from plants of differing chronological age, whereas Pandjaitan et al. (2005) harvested leaves of differing chronological age on a single, more mature plant. In endive and Boston lettuce, the carotenoid concentrations of mature leaves were two- to fourfold greater than those of young leaves (de Azevedo-Meleiro and Rodriguez-Amaya, 2005). In contrast, the younger leaves of New Zealand spinach had slightly higher carotenoid levels than the mature leaves (de Azevedo-Meleiro and Rodriguez-Amaya, 2005).

Another aspect of production is the time in a growing season when the leafy vegetable is harvested (i.e. harvest date). There are several of reports showing that the harvest season is extremely important with regards to bioactive content in a particular leafy vegetable. For example, the carotenoid contents of minimally processed endive and New Zealand spinach grown in Brazil were significantly higher in the summer than in the winter growing season (de Azevedo-Meleiro and Rodriguez-Amaya, 2005; Rodriguez-Amaya et al., 2007). Howard et al. (2002) found that spring-planted spinach had almost twofold greater phenolic concentrations than autumn-planted spinach in Arkansas. They suggested that the reason spinach planted during the spring developed higher phenolic content and antioxidant capacity than autumn-grown spinach was due to the higher temperatures and greater light intensity typical of the earlier growing season. Similar patterns were seen in Sweden, where spring-sown spinach had higher phenolic content
than plants sown in August (Bergquist, 2006). Finally, phylloquinone content also appears to show the same pattern in spring-grown versus autumn-grown iceberg lettuce in Finland (Koivu et al., 1997).

10.4.2 Postharvest effects

Washing is ideally a process that most, if not all, fresh produce undergoes before use or consumption. There is some evidence to support the contention that washing protocols affect the functional value of leafy vegetables and their products. For example, Baur et al. (2004) showed that washing trimmed heads or shredded iceberg lettuce with chlorinated water (100–200 mg/l free chlorine) reduced phenylalanine ammonia lyase (PAL) activity significantly and led to concomitant rise in 3,5-di-O-caffeoylquinic acid (isochlorogenic acid) concentration in the tissues during storage, compared with washes using tap or ozonated water. However, there was little effect of washing treatment on O-cafeeyl tartaric (caftaric acid), di-O-cafeoyltartaric (chicoric acid), 5-O-cafeeyloyquinic (chlorogenic acid) and O-cafeoylmalic acid contents.

Esparza Rivera et al. (2006) found that immersion in hydro-cooling water containing 1% ascorbic acid would increase ascorbate content in green leaf lettuce threefold, whereas a hydro-cool spray with the same concentration would not have a significant effect on ascorbate residues in the tissues. They also determined that there was no impact of either immersion or spray on the phenolic or antioxidant contents in the lettuce. While the immersion-treated lettuce had significantly more ascorbate, there was a rapid decline in this compound in the tissue, so that the differences between treatments had disappeared by 21 days (Esparza Rivera et al., 2006), well past the expiry date of such a product.

Heat has been widely studied as a post-harvest or processing tool to reduce browning in lettuce (Hodges and Toivonen, 2007). The effect of heat on lettuce is to inhibit PAL activity, thus preventing the wound-induced accumulation of phenolics in fresh-cut product (Saltveit, 2000). While the original work was performed on lettuce rib tissue, subsequent work has also shown that there is a similar pattern of response in both midrib and lamella tissue of iceberg lettuce (Fukumoto et al., 2002). The greatest and most consistent response to heat treatment was the reduction in the accumulation of 3,5-di-O-cafeeyloyquinic acid (isochlorogenic acid). In addition, Moreira et al. (2006) demonstrated that heat treatments accelerated the loss in ascorbate in romaine lettuce. These examples suggest that the use of heat treatments to preserve visual quality in lettuce can lead to loss of bioactive content and functional quality.

Cutting, a common process during handling fresh leafy vegetables, is known to induce either increases or accelerated losses of bioactive components. Wound-induced increase in phenolics, a commonly known response to cutting in lettuce (Kang and Saltveit, 2002; Choi et al., 2005; Reyes et al., 2007), is achieved through upregulation of PAL activity (Campos-Vargas et al., 2005). In contrast, the cutting process leads to significant reduction in ascorbic acid content in iceberg lettuce, the actual level of loss being determined by cutting method and blade sharpness (Barry-Ryan and O’Beirne, 1999). In the latter study, the best retention of ascorbate was obtained by tearing, as opposed to cutting, the lettuce leaves.

Modified atmosphere packaging (MAP) is a widely used technology for whole and minimally processed leafy vegetables. Nitrogen-flushed MAP (called active MAP) retains ascorbate content of minimally processed iceberg lettuce better than either passive MAP (i.e. sealed without gas flushing the package) or non-sealed plastic wrapping of the processed lettuce (Barry-Ryan and O’Beirne, 1999). Schreiner et al. (2003) found that MAP in lettuce did not affect retention of carotenoids when compared with non-packaged lettuce. MAP has been shown to enhance flavonoid contents in Swiss chard stored at 6°C, while at the same time, ascorbate content loss was accelerated by MAP (Gil et al., 1998). Storage of Chinese leafy vegetables at 4 and 20°C for 6 days resulted in an increase of phenolic acid content, with the greatest response occurring at 20°C (Harbaum et al., 2008). The authors based their
explanation for this increase during storage on the belief that the storage environment imposed stress on the vegetables, which resulted in stress-induced upregulation of PAL activity, with a consequent rise in phenolics. However, other constituents may not be affected by MAP conditions. In contrast to these many studies, Serafini et al. (2002) found that MAP packaging of lettuce results in complete loss of bioavailability of phenolics. However, these authors did not provide accurate information on the MAP treatment, to allow interpretation as to whether the results reflected those found for commercially packaged products. Certainly, more work is required to understand better the impact of MAP packaging and storage on bioavailability of bioactives in leafy salad vegetables.

Recently, the effects of superatmospheric oxygen levels (i.e. levels about 21 kPa) have been reviewed and, while there are many reports of the effects of such apparently detrimental atmospheres on bioactive contents on many fruit, only one report deals with the effects on the bioactive content of leafy vegetables (Zheng and Wang, 2007). In that one study, Heimdal et al. (1995) reported that superatmospheric oxygen in MAP delays the degradation of ascorbic acid in shredded iceberg lettuce; however, it is unclear how superatmospheric oxygen atmospheres can be used practically and safely.

Storage temperature is also a well-documented factor regulating bioactive content in fresh leafy vegetables (Watada, 1987). Fresh-cut iceberg lettuce stored at 3°C retains 20% more ascorbic acid content than does the same product stored at 8°C (Barry-Ryan and O’Beirne, 1999). Total phenolic content increased in storage for minimally processed butterhead and romaine lettuces, but the increase was greater at 13°C storage temperature than at 5°C (Castañer et al., 1999). The increase was associated with an upregulation in PAL activity, as would be expected with de novo synthesis of phenolics (Degl’Innocenti et al., 2005). Flavonoid losses in sweet potato leaves were very much accelerated by storage at 25°C as compared with 4°C, with losses at day 1 for the higher temperature equating to the losses that occurred at day 9 if the leaves were stored at 4°C (Chu et al., 2000). In another example, carotenoid content declined by an average of 14% in minimally processed endive over 5 days’ storage at 7–9°C, while losses in New Zealand spinach were on average 29% under the same conditions (de Azevedo-Meleiro and Rodriguez-Amaya, 2005). Pandrangi and Laborde (2004) found that folate and carotenoids in commercially packaged spinach declined much more rapidly at 10 and 20°C than at 4°C, and that packaging had no effect on the decline. In that study, the commercial standard microperforated film was used to package the spinach.

Duration of fresh storage can be a significant factor in determining the content of some bioactive compounds at the consumer level. There were considerable losses in ascorbic acid during storage of spinach, whereas carotenoids and flavonoids were more stable and sometimes increased in concentration (Bergquist, 2006). Pandrangi and Laborde (2004) found declines in carotenoids in the range of 39% over 6 days at the same storage temperature as used by Bergquist (2006). The differences in the two studies may reflect differences in production systems, cultivar selection and/or the chronological age of the spinach used in the research. Declines in carotenoids similar to those shown by Pandrangi and Laborde (2004) have been shown, with time in storage at 7–9°C, for endive and New Zealand spinach (de Azevedo-Meleiro and Rodriguez-Amaya, 2005). Similarly, ascorbate decline increases with storage time in minimally processed, packaged iceberg lettuce (Barry-Ryan and O’Beirne, 1999). From these examples it becomes clear that ascorbate is the most labile of the bioactive compounds in leafy vegetables and that it may be the most limiting constituent when considering storage recommendations. This is a new concept, since research and commercial definitions of shelf life have been focused previously on the appearance of disorders or decay on the vegetable tissues. The nutritional value of fresh vegetables, whether minimally processed or whole, should be included in the consideration for determining the end of useful shelf life.

The use of irradiation to sanitize fresh leafy vegetables is increasing with the increased concern for food safety, particularly for packaged
salads that will not be cooked prior to consumption (Goularte et al., 2004). Although the impact of this treatment has not been studied to a large degree, there are some indications that doses effective in controlling microorganism growth on the cut leafy product also lead to increased phenolic content in lettuce (Fan et al., 2003) and arugula (Nunes et al., 2008). However, in contrast, irradiation treatment reduces ascorbate content significantly in iceberg and romaine lettuce (Fan and Sokorai, 2008). Irradiation can lead to quality defects in lettuce, but warm-water pretreatments have been found to improve quality retention and prevent losses of ascorbate (Fan et al., 2003). However, enhanced phenolic content in response to irradiation is prevented with warm-water treatment.

10.5 Future Research Needs

There are large gaps in understanding the factors that affect the content of most bioactive compounds in leafy vegetables. If the nutritional and functional values of these commodities are considered to be central to human health, then how healthfulness can be modulated through intentional preharvest and/or postharvest practices needs to be clearly understood. For instance, there is a need to develop an accepted threshold based on the minimum acceptable loss of ascorbic acid levels when modelling shelf life for whole and fresh-cut packaged salads.

Another emerging concern relates to the indigenous leafy vegetables that are central to the diet and health of those in the developing world. Can these indigenous leafy vegetables play a role in economic development concomitant with maintaining population health in developing countries? If such new products are introduced to the Western diet, then knowledge of the effects of transport, handling and storage on bioactive content will also require further work. Another aspect of indigenous vegetables is that a few, such as tree spinach, have been shown to have specific therapeutic benefits for managing some prevalent diseases in the Western world, such as diabetes (Kuti and Torres, 1996). It could be that inclusion of some of these exotic leafy vegetables may provide alternatives or adjuncts to medical interventions. However, such use must be tempered with clinical research to set appropriate consumption dosages and limits.

While there are measures of bioactive contents available for many of the leafy vegetables, the biological health-promoting significance of some of these compounds has yet to be confirmed. While health benefits attributed to ascorbate, folate, phylloquinones, carotenoids and tocopherols have been relatively well documented in the literature, there are questions as to the specific significance of phenolics as antioxidants and preventers of diseases such as cancer (Seifried et al., 2003). One reason for raising this question is that, while there are many reports showing generally good relationships between antioxidant capacity and phenolic content, there are other reports showing significant discrepancies. One study showed that there was a lack of a good correlation between ORAC and phenolic contents of beet greens and spinach, with the authors stating that the ORAC value was a measure of the quality of antioxidants and their interactions in the tissue matrix (Ninfali and Bacchiocca, 2003). Another study showed a poor relationship between the DPPH (2,2-diphenyl-1-picrylhydrazyl; another total antioxidant assay) assay and phenolic content of different types of lettuce (Liu et al., 2007). In a last example, researchers found that many antioxidant capacity assays (DPPH radical-scavenging activity, superoxide radical-scavenging activity in riboflavin/light/NBT system, hydroxyl radical-scavenging activity and inhibition of lipid peroxidation induced by FeSO₄ in egg yolk) did not exhibit a strong relationship with the phenolic content of leafy vegetables (Dasgupta and De, 2007). They found that Asteracantha longifolia Nees and L. reptans (Linn.) Poir. had high phenolic content (as measured by the Folin–Ciocalteu reaction), but the measured antioxidant capacity of the phenolic fraction was relatively low. In both cases, there was a much higher level of antioxidant capacity associated with ascorbate, which was known to be reactive in the Folin–Ciocalteu reaction (Huang et al., 2005). Therefore, the use of the Folin–Ciocalteu reaction to estimate total
phenolic content must be evaluated carefully, to determine if there are significant levels of interfering compounds in the tissue matrix. These discrepancies point to the need for future research to characterize the functionality of complete bioactive extracts, as well as identification and quantification of the bioactive molecules present in leafy vegetable extracts. It may be that there is significant interaction between bioactive constituents in a complete extract that modulates the functionality of the extract. However, research into the identification and quantification of bioactive constituents needs to be continued, to provide the necessary information to bolster the health-based argument for increased consumption of leafy vegetables (Goldman, 2003; van Dokkum et al., 2008).

Another area that needs further exploration is the bioavailability of the pure, isolated bioactive compounds themselves. More than one research report has shown that the food matrix is an important determinant of bioavailability of a bioactive molecule (e.g. β-carotene and lutein in spinach; Castenmiller et al., 1999). The relative bioavailability of specific molecules in differing leafy matrices is poorly understood. For example, the bioavailability of carotenoids is not uniform among different compounds – lutein is generally much more bioavailable than β-carotene in the leafy vegetable matrix (Erdman, 1999). Folate bioavailability in vegetable matrices is also poorly understood. While leafy vegetables are particularly rich in folates, the biological importance of these high levels in a specific leaf-tissue matrix is not truly understood with regards to human health, since the relative bioavailability varies according to the vegetable in question (Gregory, 2001). There is some evidence that microstructural and biochemical interactions occurring during digestion of leafy vegetable matrices are responsible for the consequent bioavailability of specific bioactive components (Parada and Aguilera, 2007). As a good example, recent work by Su and Arab (2006) has demonstrated that regular consumption of leafy green vegetables in a salad format, with dressing, led to above-median blood serum levels of folic acid, vitamins C and E, lycopene and α- and β-carotene in test subject populations. The authors found that the improved bioavailability of the bioactives was due to the addition of the salad dressing, which modified the matrix in the gut during digestion. Therefore, it appears that tissue content is only one determinant of the bioavailability of a bioactive molecule, which is also affected by many other factors, such as the tissue matrix itself and also the preparation format of the vegetable at the time of consumption.

Recently, the concept of high botanical diversity in human diets has emerged. There are clinical research findings suggesting that botanical diversity is important in expanding the bioactivity of diets high in fruit and vegetables, and that smaller amounts of many bioactive compounds have greater beneficial effects than larger amounts of relatively few bioactive compounds (Thompson et al., 2006). The authors of that work cite that this may be why the concept of antioxidant supplements for managing cancer has had mixed results, as concluded by Seifried et al. (2003). Leafy vegetables have a significant diversity in types of bioactive compounds (Table 10.2), and often within the same botanical family there are differences in the specific molecules that are found between leafy vegetable species. For example, among Brassicaceous leafy vegetables, the forms of flavonoids found in watercress are completely different from those found in rocket (Martínez-Sánchez et al., 2008). In another example, the relative quantities and content of specific glucosinolates vary significantly among different Asian leafy Brassicaceous vegetables (He et al., 2003). Consequently, leafy vegetables considered in this chapter could provide at least a partial source for a high botanical diversity diet if this becomes an approach that is supported in the long-term by further research results.

10.6 Conclusions

All leafy vegetables are generally rich sources of a wide range of bioactive health-promoting compounds, including carotenoids, tocopherols, phenolics, phylloquinones, folate and ascorbate. Some leafy vegetables are also rich
sources of glucosinolates, which also are considered to offer health benefits. The wide range of leafy vegetables is enhanced by the deployment of indigenous examples into mainstream consumption as populations migrate and cuisines follow. It is arguable to state that leafy vegetables have the most potential to provide health benefits, particularly in developing world economies.

While much is understood about the bioactive content of mainstream leafy vegetables, little is known about the health-promoting properties of most of the indigenous leafy vegetables that are consumed around the world. This is an area that requires more research, as the potential for inclusion of indigenous leafy vegetables into common diets increases concomitant with globalization.

In regards to increasing the consumption of mainstream leafy vegetables, there is some good preliminary information demonstrating the clinical benefits of such consumption, but still more work is required to underpin fully the recommendations for increased consumption. Also, bioavailability studies should focus on the availability and absorption of bioactives of leafy vegetables in the complete matrix in which they are consumed. For example, if the vegetable is eaten as part of a salad mix with salad dressing, then the analysis of benefits should be based on that complete product. One reason for this approach is that, in reference to fat-soluble bioactive compounds (e.g. carotenoids and tocopherols), the addition of a salad dressing containing oil can influence the bioavailability of those bioactives dramatically. Hence, future analysis must take into account the format for consumption.

It is difficult to conclude whether work should be conducted to create (via classical and/or molecular breeding) leafy vegetables that are extremely high in a particular bioactive. There are two reasons for this reticence: (i) some evidence from the literature indicates that it is not the quantity of a particular bioactive from a single vegetable that is important to health; rather, it is the diversity of bioactives in the diet that is important; and (ii) if many leafy vegetables are consumed in a diet, then there may be sufficient uptake of bioactives, rendering an enhancement in content irrelevant to further health improvement. Some have also raised the concern of potential toxicity and/or bitter taste if some classes of bioactives are increased excessively.

Bioactive content can be affected and/or manipulated by many factors. Future research should be conducted to optimize production (including cultivar selection) and postharvest management of leafy vegetables better, such that bioactive values are maintained at desirable levels. Some treatments/technologies potentially may enhance concentrations of some bioactive compounds.

Finally, leafy green vegetables are a significant source of most of the important bioactive compounds found in fruit and vegetables. The nature of the function of many compounds, particularly phylloquinones and folate, in the leaf provides a mechanistic explanation for the richness of these compounds in leaves. They potentially can provide a diversity of tastes and flavours that also enrich the pleasure of a healthy and healthful diet. In a world that will require options to ensure health in developing economies, the inclusion of indigenous leafy vegetables can provide a sustainable mechanism to deliver such a diet to less affluent consumers.

References


11 Pome Fruit

Chris B. Watkins and Rui Hai Liu

11.1 Introduction

A pome is an accessory fruit composed of five or more carpels in which the exocarp forms an inconspicuous layer. The mesocarp is usually fleshy and the endocarp forms a leathery case around the seed. Outside of the endocarp is the most edible part of this fruit, derived from the floral tube (torus) and other parts, which corresponds to what is commonly called the core. The best-known example of pome fruit is the apple, but other plants that produce fruit classified as a pome include cotoneaster, hawthorn, medlar, pear, pyracantha, toyon, quince, rowan and whitebeam. Of these species, a large literature exists about the apple and thus this chapter also has an unavoidable bias towards only one of the many pome fruit. However, examples of the health benefits of other pome fruit are included in this chapter wherever possible.

The health benefits of the apple fruit have long been recognized; in the Middle Ages the English said ‘To eat an apple before going to bed will make the doctor beg his bread’, which is commonly known in modern terms as the proverb ‘An apple a day keeps the doctor away’. Apples are one of the most popular and healthy fruit that are commonly enjoyed by people all over the world. In the East, the Oriental pear has long been associated with antitussive, anti-inflammatory and diuretic properties (Cui et al., 2005).

Apple nutrients are listed in Table 11.1. Apples are a natural source of fibre, minerals and vitamin C. Besides an excellent source of fibre, apples are a very significant source of phenolics in people’s diet. For example, of the top 25 fruit consumed in the USA, apples are the number one source of phenolics in the American diet and provide Americans with 33% of the phenolics they consume (Boyer and Liu, 2004; Wolfe et al., 2008). In contrast, the pear contributes only 3% (Wolfe et al., 2008).

11.2 Phytochemicals

11.2.1 Phenolics

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins and tannins (Liu, 2004; Fig. 11.1). They are the products of secondary metabolism in plants, providing essential functions in the reproduction and growth of the plant, and acting as defence mechanisms against pathogens, parasites and predators, as well as contributing to plant colour. In addition to
Table 11.1. Apple nutrient composition.a

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (100 g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (g)</td>
<td>85.6</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>52</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>218</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.26</td>
</tr>
<tr>
<td>Total lipid (fat) (g)</td>
<td>0.17</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>13.81</td>
</tr>
<tr>
<td>Total sugars (g)</td>
<td>10.39</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2.07</td>
</tr>
<tr>
<td>Glucose (dextrose)</td>
<td>2.43</td>
</tr>
<tr>
<td>Fructose</td>
<td>5.90</td>
</tr>
<tr>
<td>Total dietary fibre (g)</td>
<td>2.4</td>
</tr>
<tr>
<td>Pectin (g)</td>
<td>0.5</td>
</tr>
<tr>
<td>Ash, total minerals (g)</td>
<td>0.19</td>
</tr>
<tr>
<td>Potassium, K (mg)</td>
<td>107</td>
</tr>
<tr>
<td>Calcium, Ca (mg)</td>
<td>6</td>
</tr>
<tr>
<td>Magnesium, Mg (mg)</td>
<td>5</td>
</tr>
<tr>
<td>Phosphorus, P (mg)</td>
<td>11</td>
</tr>
<tr>
<td>Iron, Fe (mg)</td>
<td>0.12</td>
</tr>
<tr>
<td>Vitamin C, total ascorbic acid (mg)</td>
<td>4.6</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>54</td>
</tr>
<tr>
<td>Vitamin E (α-tocopherol) (mg)</td>
<td>0.18</td>
</tr>
<tr>
<td>β-Carotene (µg)</td>
<td>27</td>
</tr>
<tr>
<td>Lutein + zeaxanthin (µg)</td>
<td>29</td>
</tr>
</tbody>
</table>


Fig. 11.1. The generic structure of flavonoids.

![Flavonoid structure](image)

their roles in plants, phenolic compounds in diets may provide health benefits associated with reduced risk of chronic diseases.

Apples are a good source of phenolic compounds (Eberhardt et al., 2001; Sun et al., 2002; Boyer and Liu, 2004). The total extractable phenolic content has been investigated and ranges from 110 to 357 mg/100 g fresh apple (Podsedek et al., 2000; Liu et al., 2001). Phenolic concentrations in pear fruit are generally lower than those in apples, being in the range of 63–98 mg/100 g fresh weight (FW) (Vinson et al., 2001; Kevers et al., 2007; Wolfe et al., 2008). A 100 g serving of pear fruit (with peel) provides between 27.2 and 40.7 mg of phenolics, depending on the cultivar, compared with between 5.5 and 8.4 mg of L-ascorbic acid (L-AA) (Galvis-Sanchez et al., 2003).

11.2.2 Flavonoids and other phytochemicals

Flavonoids are a group of phenolic compounds with antioxidant and biological activity that have been identified in fruit such as apples, as well as in other fruit, vegetables and other plant foods. Flavonoids have been linked to reducing the risk of major chronic diseases. More than 4000 distinct flavonoids have been identified (Liu, 2004). They commonly have a generic structure consisting of two aromatic rings (A and B rings) linked by three carbons that are usually in an oxygenated heterocycle ring, or C ring (Fig. 11.1).
Differences in the generic structure of the heterocycle C ring classify them as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, or isoflavonoids (isoflavones) (Fig. 11.2). Flavonols (quercetin, kaempferol and myricetin), flavones (luteolin and apigenin), flavanols (catechin, epicatechin, epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)), flavanones (naringenin), anthocyanidins (cyanidin and malvidin), or isoflavonoids (genistein and daidzein) are common flavonoids in the diet. Flavonoids are found most frequently in nature as conjugates in glycosylated or esterified forms, but can occur as aglycones, especially as a result of the effects of food processing. Many different glycosides can be found in nature as more than 80 different sugars have been discovered bound to flavonoids.

Five major groups of phenolic compounds were detected in eight apple cultivars by HPLC: flavan-3-ols, flavonols, dihydrochalcones, anthocyanins and phenolic acids (hydroxybenzoic acid and hydroxycinnamic acids) (Tsao et al., 2003). The major group was the flavan-3-ols, or catechins, which accounted for 56% of polyphenols in the flesh (0–583.0 μg/g FW) and 60% of pholyphenols in the peel (151–1655 μg/g FW). The dominant catechins in apples were catechin and epicatechin, and the dimers, procyanidin B1 (epicatechin plus catechin) and procyanidin B2 (two epicatechin molecules) (Tsao et al., 2003). The second major group of polyphenols in apples was the flavonols, such as quercetin, kaempferol and myricetin. Quercetin and its glycosides are by far the most abundant flavonols, can contribute up to 18% of total phenolics and occur exclusively in the peel. Concentrations were found to be 220–350 μg/g FW (Tsao et al., 2003).

The other three minor polyphenolic groups found in these eight apple cultivars were dihydrochalcones, anthocyanins and phenolic acids (Tsao et al., 2003). The main dihydrochalcones were phloretin 2’-glycoside (phlorizin) and phloretin 2’-xyloglucoside, which occurred mostly in the peel at an average concentration of 124 μg/g FW. Anthocyanins, which occurred as cyanidin glycosides in red apples, ranged from 43 to 208 μg/g FW.

The two main groups of phenolic acids found in apples were hydroxybenzoic acids and hydroxycinnamic acids. Hydroxybenzoic acids accounted for less than 5% of phenolics in apples. In the peel, total benzoic acids

![Fig. 11.2. Structures of main classes of dietary flavonoids.](image-url)
Pome Fruit

11.2.3 Ascorbic acid

Vitamin C (L-AA) is an essential nutrient, required from the diet by humans because of the absence of the enzyme L-gulonolactone oxidase needed in its synthetic pathway. Vitamin C is widely distributed in foods, with the highest levels found in fruit and vegetables. Apples contain an average of 46 μg vitamin C/g FW of fruit. Once absorbed, it is present in the body in two forms in equilibrium: ascorbic acid and the reduced form, dehydroascorbic acid (DHA). Vitamin C is essential in the human body and participates in important physiological functions, including: as an enzyme cosubstrate in collagen synthesis, neurotransmitter metabolism and carnitine synthesis; as a redox agent responsible for maintaining enzyme-bound metals in the correct oxidation state for functionality; as a regulator of antihistamine reactions; and as a stimulator of immunoglobulins (Davey et al., 2000). In addition to the metabolic roles, vitamin C also functions as an antioxidant. Ascorbic acid can react with free radicals in the body to form a relatively unreactive ascorbyl radical, which then disproportionates to ascorbate and dehyroascorbate. Besides scavenging harmful free radicals, such as peroxyl radicals, hydroxyl radicals and superoxide, ascorbic acid may reduce the chromanoxyl radical to regenerate vitamin E (Davey et al., 2000).

11.2.4 Antioxidant activity

Although the L-AA content of raw cv. Red Delicious apples with skin was 5.7 mg/100 g, the antioxidant activities of 1 g of apple with 3-O-glicosides, rutinoside, galactorhamnoside, glucoside, malonyl galactoside and malonyl glucoside (Oleszek et al., 1994). In pear fruit, only chlorogenic acid was detected in the flesh, whereas higher concentrations of the acid as well as flavonols and arbutin were found in the peel (Galvis-Sanchez et al., 2003). The major phenolic compounds in Oriental pears are arbutin and chlorogenic acid (Cui et al., 2005).
skin and without skin were 83 and 46 μmol vitamin C equivalents/g, respectively (Eberhardt et al., 2001). The calculated antioxidant activity of L-AA in 1 g of cv. Red Delicious apple with skin was only 0.32 μmol vitamin C equivalents/g. The L-AA in apple with skin of this cultivar accounts for as little as 0.4% of total antioxidant activity (Eberhardt et al., 2001). Other research has shown a larger contribution, of 10% of the total antioxidant capacity (Lee et al., 2003; Vanzani et al., 2005), but nevertheless the majority of antioxidant activity of apples is not from L-AA but from other phytochemicals in the fruit. The combinations of different phytochemicals in apples may function additively or synergistically to be responsible for this potent antioxidant activity. This is likely to be true also for pears and hawthorn, in which L-AA is a small component of total antioxidant activity (Galvis-Sanchez et al., 2003; Guo et al., 2003; Kevers et al., 2007).

Pear fruit have lower total antioxidant capacity than apple fruit, as measured with a number of assay types (Wang et al., 1996), although the differences are small compared with fruit with relatively high capacity, e.g. berry fruit (Vinson et al., 2001; Leontowicz et al., 2002, 2003; Wolfe et al., 2008). Hawthorn fruit extracts contained chlorogenic acid, epicatechin, hyperoside, isoquercitrin, quercetin, rutin and protocatechuic acid, which were shown to inhibit oxidation of human low-density lipoprotein and α-tocopherol (Zhang et al., 2001) and linoleic acid (Sokol-Letowska, 2007). No comparisons of hawthorn fruit with either pear or apple appear to be available, although antioxidant capacity (FRAP assay) of hawthorn was 27 times that of duck pear (Guo et al., 2003). Antiradical activity of quince fruit is correlated with the concentrations of caffeoylquinic acid, phenolic, ascorbic acid and citric acid concentrations (Silva et al., 2004), and Chinese quince and quince phenolics gave greater inhibition of gastric ulcers in rats than did apples (Hamauzu et al., 2006). Compared with apple, quince and Chinese quince phenolic extracts were less effective in a linoleic acid peroxidation system, but phenolics from Chinese quince had the strongest anti-influenza vial activity in a haemagglutination inhibition test (Hamauzu et al., 2005).

### 11.2.5 Bioavailability of phytochemicals

Phytochemicals cannot exert any biological effects unless they are absorbed, metabolized and distributed in the body. The extent to which this happens depends on many factors, including the nature of the compound, the food matrix, the presence of microorganisms and subject conditions (age and gender).

Human intake of all flavonoids is estimated at a few hundred mg to 650 mg/day. The total average intake of flavonols (quercetin, myricetin and kaempferol) and flavones (luteolin and apigenin) was estimated as 23 mg/day, of which quercetin contributed ~70%, kaempferol 17%, myricetin 6%, luteolin 4% and apigenin 3%.

Several studies have been performed to investigate the bioavailability of quercetin. Quercetin administered orally in an ethanol solution was found to be 36.4–53.0% absorbed (Walle et al., 2001). To determine if different forms of quercetin can be absorbed, Hollman et al. (1995) fed nine ileostomy patients, who lacked colons, a single dose of quercetin in onions, which contained mostly quercetin 3-glucoside, pure quercetin 3-rutinoside (rutin), the predominant form of quercetin in tea, and pure quercetin aglycone. The average absorption of quercetin was 52% from onions, 17% for rutin and 24% for quercetin aglycone.

The same research group later fed quercetin to nine subjects as a single large dose using onions (see Chapter 2 of this volume), apples (which contained a variety of quercetin glycosides) and pure rutin, and monitored plasma quercetin levels over 36 h (Hollman et al., 1997). Quercetin from onions was absorbed the most rapidly and rutin the least rapidly, indicating that quercetin glucoside was likely absorbed from the stomach or small intestine, while quercetin from rutin was probably absorbed from the colon after microbial cleavage of the sugar. Peak plasma levels were 224 ng/ml after the onion meal, 92 ng/ml after the apples and 90 ng/ml after the rutin, showing quercetin from apples and rutin to have only 30% of the bioavailability of quercetin from onions. The half-life of quercetin in plasma was found to be about 24 h, suggesting that accumulation of the compound was possible.
A study involving examination of quercetin, quercetin 4'-glucoside and quercetin 3,4'-diglucoside transport through Caco-2 cells showed quercetin aglycone was absorbed more rapidly across the intestinal epithelial cells than either glucoside (Walgren et al., 1998). It also refuted the earlier theory of an active transport mechanism for quercetin. McAnlis et al. (1999) fed each of five healthy volunteers a single dose of onions, containing about 50 mg of quercetin, and measured plasma quercetin levels. Quercetin in the plasma increased from 28.4 ± 1.9 ng/ml at baseline to 248.4 ± 103.9 ng/ml after 2 h before returning to baseline in 24 h, an observation not noted by other experimenters. In another study, six subjects were fed a meal containing fried onions and fresh cherry tomatoes (Boyle et al., 2000). Plasma quercetin levels increased from 16.5 ± 2.7 ng/ml to 104.9 ± 10.42 ng/ml 4 h after ingestion and remained elevated at 8 h. After 24 h, plasma quercetin levels were still higher than baseline. Quercetin was shown to be absorbed from the stomach of rats, but the glycosides, rutin and isoquercitrin, were not, indicating that the aglycones might be partly absorbed in the stomach of humans (Crespy et al., 2002). It is obvious quercetin can be absorbed, but the glycosylation of quercetin largely determines to what extent.

11.2.6 Antimicrobial activity

Though little work is available, Fattouch et al. (2008) have found antimicrobial activity of apple, pear and quince extracts against a number of microorganisms associated with foodborne diseases and/or spoilage of contaminated product. Antimicrobial activity is not highly correlated with phenolic contents and further studies are warranted.

11.3 Health Benefits

11.3.1 Cancer

Consumption of apples has been linked to the prevention of chronic disease. Several epidemiological studies have linked apple consumption specifically with a reduced risk of cancer (Knekt et al., 1997; Feskanich et al., 2000; Le Marchand et al., 2000; Gallus et al., 2005).

In a study in Finland involving 10,000 men and women and a 24-year follow-up, a strong inverse association was seen between flavonoid intake and lung cancer development (Knekt et al., 1997). The mean flavonoid intake was 4.0 mg/day and 95% of the total flavonoid intake was quercetin. Apples and onions together provided 64% of all flavonoid intakes. The reduced risk of lung cancer associated with increased flavonoid consumption was especially strong in younger people and in non-smokers. Apples were the only specific foods that were related inversely to lung cancer risk in Finland. Men and women with the highest quartile of apple intake had a relative risk of 0.42 for lung cancer compared with those in the lowest quartile of apple intake (Knekt et al., 1997). Since apples were the main source of flavonoids in the Finnish population, it was concluded that the flavonoids from apples were most likely responsible for the decreased risk of lung cancer.

A Hawaiian case–control study found that apple and onion intake was associated with a reduced risk of lung cancer in both males and females (Le Marchand et al., 2000). Smoking history and food intake were assessed for 582 patients with lung cancer and 582 control subjects without lung cancer. The relative risk for lung cancer was decreased by 40% in individuals with high apple and onion intake when compared with those who consumed the lowest amount of these fruit and vegetables. No associations were seen with red wine, black tea or green tea. Both onions and apples are high in flavonoids, especially quercetin and quercetin conjugates. Le Marchand et al. (2000) found an inverse association between lung cancer and quercetin intake, although the trend was not statistically significant. Interestingly, the inverse association seen between apple and onion intake and lung cancer was stronger for squamous cell carcinomas than for adenocarcinomas.

In women from the Nurses’ Health Study, significantly lower risks of lung cancer were found with an increase of one serving/day of apples and pears (relative risk = 0.63)
In the Nurses’ Health Study and the Health Professionals’ Follow-up Study, involving over 77,000 women and 47,000 men, fruit and vegetable intake was associated with a 21% reduced risk in lung cancer in women, but this association was not seen in men (Feskanich et al., 2000). Very few of the individual fruit and vegetables examined had a significant effect on lung cancer risk in women; however, apples were one of the individual fruit associated with a decreased risk in lung cancer. Women who consumed at least one serving per day of apples and pears had a reduced risk of lung cancer. Of the men involved, there was no association seen between any individual fruit or vegetable and lung cancer risk.

Another epidemiological study including 2569 breast cancer patients showed that apple consumption was linked to a lower risk of breast cancer (Gallus et al., 2005). When compared with subjects consuming less than one apple/day, the multivariate odds ratio (OR) for at least one apple/day was 0.82 (95% CI 0.73–0.92) for breast cancer. After further allowance for consumption of vegetables and other fruit, the association with apples became even stronger for breast cancer (OR 0.76, CI 0.67–0.85) (Gallus et al., 2005).

The relationship between dietary catechins and epithelial cancer was examined in 728 men (aged 65–84), as part of the Zutphen Elderly Study (Arts et al., 2001). Tea, a naturally high source of catechins, contributed 87% of the total catechin intake in this study, while apples contributed 8.0% of catechin consumption. It was found that total catechin and tea consumption did not have an effect on lung cancer, but apple consumption was associated with decreased epithelial lung cancer incidence (Arts et al., 2001). This supported the findings of the previous studies discussed above, where apples were associated significantly inversely with lung cancer, and might suggest that catechins alone do not have an effect against lung cancers. Other data from the Zutphen Elderly study showed an inverse association between fruit and vegetable flavonoids and total cancer incidence and tumours of the alimentary and respiratory tract (Hertog et al., 1994). Again, tea flavonoids were not associated with a decrease in cancer risk.

Apples have been shown to have strong antioxidant activity (Wang et al., 1996; Sun et al., 2002). In previous studies, apple extracts have been shown to have potent antiproliferative activity against colon, liver and breast cancer cells in vitro, in a dose-dependent manner (Eberhardt et al., 2001; Sun et al., 2002; Wolfe et al., 2003). Apples with peel inhibit the growth of human liver cancer cells to a greater extent than those without peel (Eberhardt et al., 2001; Wolfe et al., 2003). Apple extracts inhibited 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary cancer in rats, in a dose-dependent manner, in doses equivalent to human consumption of one, three or six apples/day (Liu et al., 2005). The apple extracts downregulated proliferating cell nuclear antigen (PCNA), Cyclin D1 and Bcl-2, and upregulated Bax and nuclear fragments, reduced tumour size and tumour burden and delayed tumour onset in a clear dose-dependent manner (Liu et al., 2009). It was reported that apple extracts had activity inhibiting NFkB activation in human breast cancer MCF-7 cells (Yoon and Liu, 2007, 2008).

### 11.3.2 Cardiovascular diseases

Apple consumption has also been related to the reduced incidence of cardiovascular disease. Coronary mortality was reduced by 43% in Finnish women in the highest quartile of apple intake compared with the lowest quartile. Total flavonoid intake was found to be associated significantly and inversely with coronary mortality in women, but no significant reduction in risk of coronary mortality in men was found (Knekt et al., 1996).

The risk of thrombotic stroke was reduced in women in the highest quartile of apple intake when compared with those who consumed the lowest amounts of apples in a Finnish cohort study (Knekt et al., 2000). Onion intake and quercetin intake were not associated with thrombotic stroke or other cerebrovascular diseases.

The Women’s Health Study found apple consumption was associated with a reduced risk of cardiovascular disease, in a study examining the association of flavonoid intake
and cardiovascular disease (Sesso et al., 2003). Women ingesting the highest amounts of flavonoids had a 35% reduction in risk of cardiovascular events. Flavonoid intake was not associated with risk of stroke, myocardial infarction or cardiovascular disease death. Quercetin did not have any association with cardiovascular disease, cardiovascular events, myocardial infarction or stroke. However, both apple intake and broccoli intake were associated with reductions in the risk of both cardiovascular disease and cardiovascular events. Women ingesting apples had a 13–22% decrease in cardiovascular disease risk.

Apples and wine consumption was also associated inversely with death from coronary heart disease in postmenopausal women, in a study of nearly 35,000 women in Iowa (Arts et al., 2001). The intakes of catechin and epicatechin, both constituents of apples, were strongly inversely associated with coronary heart disease death. Although total catechin intake was inversely associated with coronary heart disease mortality, Arts et al. (2001) found that tea catechins were not associated with coronary heart disease mortality in postmenopausal women. Apple catechins may be more bioavailable than the catechin and epicatechin gallates commonly found in teas.

The Zutphen Elderly Study examined the relationship between flavonoid intake and risk of coronary heart disease and found that flavonoid intake was negatively correlated with mortality from heart disease in elderly men and also with myocardial infarction (Hertog et al., 1993). Tea was the main source of flavonoids in this study and was also negatively correlated with coronary heart disease. Apple intake contributed to approximately 10% of the total ingested flavonoids and was also associated with a reduced risk of death from coronary heart disease in men, although the relationship was not statistically significant (Hertog et al., 1993).

11.3.3 Pulmonary function and asthma

Apple consumption has been linked inversely with asthma and has also been associated positively with general pulmonary health. Shaheen et al. (2001) conducted a study about diet and lifestyle involving nearly 600 individuals with asthma and 900 individuals without asthma. Total fruit and vegetable intake was associated weakly with asthma, and apple intake showed a stronger inverse relationship with asthma. The association was very strong in subjects who consumed at least two apples/week. Onion, tea and red wine consumptions were not related to asthma incidence, suggesting the specific beneficial effect of apple flavonoids. Vitamin C and vitamin E were not correlated with asthma incidence, whereas carotene intake was associated weakly, but positively, with asthma.

In the Dutch MORGEN study of over 13,000 adults, it was found that apple and pear intake was positively associated with pulmonary function and negatively associated with chronic obstructive pulmonary disease (Tabak et al., 2001). Catechin intake was also positively associated with pulmonary function and negatively associated with chronic obstructive pulmonary disease, but there was no association between tea, the main source of catechins, and chronic obstructive pulmonary disease (Tabak et al., 2001).

In another study involving 1600 adults in Australia, apple and pear intake was associated with a decreased risk of asthma and a decrease in bronchial hypersensitivity, but total fruit and vegetable intake was not associated with asthma risk or severity (Woods et al., 2003). Specific antioxidants, such as vitamin E, vitamin C, retinol and β-carotene, were not associated with asthma or bronchial hypersensitivity.

11.4 Effect of Cultivar

Comparative studies on the health-promoting components of different apple and pear cultivars have tended to focus either on total and individual phenolic concentrations, or on L-AA and associated antioxidant systems. An extensive literature shows that cultivars can vary greatly in phenolic composition (Amiot et al., 1995; Lister et al., 1996a; van de Sluis et al., 2001; Imeh and Khokhar, 2002; Galvis-Sanchez et al., 2003; Lee et al., 2003; Leja et al., 2003; Wolfe et al., 2003; Napolitano et al., 2004;
Vrhovsek et al., 2004; Khanizadeh et al., 2007; Lata and Tomala, 2007). The concentration ranges for all individual phenolic compounds are available in Treutter (2001). L-AA concentrations also vary greatly among cultivars (Galvis-Sanchez et al., 2003; Davey and Keulemans, 2004; Vrhovsek et al., 2004; Lata et al., 2005; Davey et al., 2007). Also, it has been observed that older cultivars tend to have higher concentrations than newer cultivars (Planchnon et al., 2004), as do later harvested cultivars compared with early harvested cultivars (Davey et al., 2007).

Total phenolic concentrations of ten different apple cultivars, with and without skin, were reported by Liu et al. (2001). The cv. Fuji apples with skin had the highest total phenolic content (230.49 ± 4.4 mg/100 g apple), followed by cvs. Red Delicious, Gala, Liberty, Northern Spy, Golden Delicious, Fortune, Jonagold, Empire and NY674. The total phenolic content in apples without skin was highest for cv. Red Delicious (167.82 ± 1.7 mg/100 g apple) followed by cvs. Northern Spy, Fortune, Gala, Fuji, Liberty, Golden Delicious, NY674, Jonagold and Empire. Total phenolic content was highest in all cultivars for apples with skin when compared with apples without skin, with the exception of NY674 (Liu et al., 2001).

The distribution of phenolics among the flesh, flesh + peel and peel of four apple cultivars have been reported (Wolfe et al., 2003). In cvs. Idared, Rome Beauty, Golden Delicious and Cortland apples, total phenolic concentrations of the flesh were 75.7 ± 4.0, 93.0 ± 4.1, 97.7 ± 8.9 and 103.2 ± 12.3 mg/100 g fruit, respectively. In the same order, total phenolic concentrations of the flesh with the peel were 120.1 ± 15.0, 159.0 ± 15.1, 129.7 ± 9.7 and 119.0 ± 14.9 mg/100 g fruit. Total phenolic concentrations of the peel were 588.9 ± 83.2, 500.2 ± 13.7, 309.1 ± 32.1 and 388.5 ± 82.4 mg/100 g fruit. Most notable is the fact that the phenolics are highly concentrated in the peel. The total phenolic concentrations of the peels were higher than the flesh and flesh + peel values in all cultivars (P < 0.05) (Wolfe et al., 2003).

Total antioxidant capacity and antiproliferation activity also vary among cultivars. Total antioxidant capacity (TEAC assay) of five apple genotypes (whole fruit) varied by two- to threefold in the hydrophilic phase, but much less in the lipophilic phase (Scalzo et al., 2005). Wolfe et al. (2003) found that concentrations of total flavonoids, anthocyanins and antioxidant activity (TOSC assay) varied between cvs. Rome Beauty, Idared, Cortland and Golden Delicious, especially in the peel. Interestingly, antiproliferative activity of the flesh + peel of cv. Rome Beauty was greater than expected from the values for peel or flesh alone, suggesting synergistic effects between phytochemicals from the two tissue types. In addition, Wolfe et al. (2003) found that peels had the highest antioxidant capacity and antiproliferation activity against HepG2, human liver cancer cells. Total phenolic concentrations of 11 apple and six pear genotypes were associated closely with antioxidant capacities (FRAP assay (Khanizadeh et al., 2007) and DPPH assay (Galvis-Sanchez et al., 2003), respectively) although associations could be poor (Kakhkonen et al., 1999; Mareczek et al., 2000).

These studies, and others, also show that total phenolic concentrations, flavonoids and antioxidant activity (Burda et al., 1990; Ju et al., 1996; Escarpa and Gonzalez, 1998; Galvis-Sanchez et al., 2003; Wolfe et al., 2003; McGhie et al., 2005; Lata, 2007) and L-AA (Galvis-Sanchez et al., 2003; Davey et al., 2004; Li et al., 2008) are higher in the peel than in the flesh of apples and pears. However, the available studies differ greatly in the tissue type (peel, flesh or combined) and components that are analysed, and therefore comparisons between studies are often difficult. For both phenolics and L-AA, the variation between fruit of the same cultivar and between years can be very high (Planchnon et al., 2004; Lata et al., 2005). Geographical growing region may also affect the phenolic composition of apple cultivars (McGhie et al., 2005), although the absence of replication of orchards within regions and a study carried out in only 1 year makes this conclusion tentative. Seasonal variations of L-AA, flavonoids, anthocyanins and phenolics, as well as other bioactive compounds, were greater in peel than whole fruit (Lata and Tomala, 2007).
11.5 Effect of Maturation

Phenolic concentrations typically decrease during apple and pear fruit development, though sometimes after an initial increase (Harel et al., 1966; Mosel and Herrman, 1974; Coseteng and Lee, 1987; Burda et al., 1990; Awad et al., 2001b). After the decline, however, concentrations remain relatively unchanged during the maturation period. The main phenolic compounds, epicatechin and procyanidin B2, and phloretin glycosides in the peel and flesh remain constant during maturation (Burda et al., 1990). Decreased phenol concentrations during development are associated with lower antioxidant capacities of flesh extracts (Hamauzu et al., 1999). However, while concentrations of individual phenolic components decline during development, the total amount per apple increases (Awad et al., 2001a).

L-AAA concentrations in cv. Conference pears fluctuate in young fruit, remain stable during fruit maturation and start to decline 1 week before commercial harvest (Franck et al., 2003). Amiot et al. (1995) found that differences in phenolic concentrations and browning susceptibility of pear purees were associated more with cultivar than with maturity effects.

11.6 Effect of Preharvest and Postharvest Continuum

11.6.1 Orchard management

Modern orchard systems emphasize the high yields of large fruit and requirements of red coloration appropriate to the cultivar, whether it is bicoloured or full red. The major flavonoid, anthocyanin, is therefore the most visible phytochemical for red apple cultivars. Anthocyanin concentrations are affected by environmental and cultural practices (Saure, 1990; Lancaster, 1992), but generally increase markedly during ripening (Knee, 1972). Anthocyanin synthesis is reported to be associated closely with the activity of phenylalanine ammonia lyase (PAL) (Tan, 1979; Wang et al., 2000), which causes the non-oxidative deamination of L-phenylalanine to form trans-cinnamic acid and a free ammonium ion. However, associations between PAL activity and anthocyanin accumulation are not always apparent (Lister et al., 1996b); PAL activity is only the first step in the biosynthesis of a large range of phenylpropanoid-derived secondary products in plants, such as flavonoids and isoflavonoids, coumarins, lignins, wound-protective hydroxycinnamic acid esters and other phenolic compounds (Tomas-Barberan and Espin, 2001). PAL activity can be activated by ethylene in other storage and plant tissues (Ritenour et al., 1995; Tomas-Barberan and Espin, 2001) and its activity is associated with increasing ethylene concentrations in apple fruit (Faragher and Brohier, 1984; Blankenship and Unrath, 1988).

Concentrations and amounts of anthocyanin and quercetin glycosides were higher in the skin of cvs. Elstar and Jonagold apples from the outer canopy compared with the inner canopy, whereas concentrations of catechins, phloridzin and chlorogenic acid were independent of canopy position (Awad et al., 2001a,c). Exposure of fruit on the tree also has a major effect on ascorbic acid concentrations which are much greater on the red (sunny) side of individual fruit than on the green (shady) side and much greater in exposed fruit than in shaded fruit in the canopy (Davey et al., 2004; Planchon et al., 2004; Hagen et al., 2007).

Crop load did not affect chlorogenic acid or total and individual flavonoid concentrations in cvs. Jonagold and Red Elstar apples (Awad et al., 2001a), but lower crop loads resulted in higher concentrations of both total and individual phenolic compounds (Stopar et al., 2002). Nitrogen is applied to soils to increase productivity and fruit size, but may decrease red coloration (Johnson and Samuelson, 1990; Raese and Drake, 1997). Calcium is applied to trees to decrease the incidence of certain storage disorders, such as bitter pit and senescent breakdown. Awad and de Jager (2002b) found that chlorogenic acid concentrations were related negatively to the concentrations of N, K and Mg and the N/Ca ratio, but related positively to P and Ca in cv. Elstar flesh tissue. Anthocyanins, catechins and total flavonoids were
associated with lower red coloration in the skin of nitrogen-treated fruit. Calcium sprays were associated with higher anthocyanins, total flavonoids, epicatechin, chlorogenic and total phenols in apples (Sannomuru et al., 1998; Awad and de Jager, 2002b). Pre-harvest sprays of ethephon and phosphorus–calcium mixtures (Seniphos) stimulated red colour and concentrations of anthocyanins, proanthocyanidins and flavonols in the skin of cv. Fuji apples (Li et al., 2002).

The effects of photosynthesis on phenolics have been illustrated in a study of branch orientation in pear fruit (Colaric et al., 2006). Lower catechin, epicatechin, sinapic acid, syringic acid and total phenolic concentrations, as well as individual sugars, were found in fruit from branches bent down in summer compared with no branch manipulations. However, fruit from spring-bent branches had constituent concentrations closer to those of the control.

No studies that relate these preharvest management practices to total antioxidant capacity and antiproliferation activity appear to be available for pome fruit. However, the effects of organic versus non-organic production on phytochemical content of apples have been the focus of numerous studies. Many studies show no differences, between production systems, for phenolics, antioxidant bioavailability or protection against oxidative DNA damage in flesh samples, with or without peel (Tarozzi et al., 2004; Briviba et al., 2007; Roth et al., 2007). However, Weibel et al. (2000) found that organically grown cv. Golden Delicious (flesh and peel), and Hecke et al. (2006) that fruit from several organically grown cultivars (juice from pressed fruit), had higher total phenolic concentrations than fruit from integrated fruit production systems. Organically grown fruit sometimes had the highest sugar concentrations, although acid levels were not consistently different between the management systems (Reganold et al., 2001; DeEll and Prange, 1992; Hecke et al., 2006). In studies conducted in Washington State on cv. Gala apples, Peck et al. (2006) did not find any effects of cultivation system on soluble solids concentration (SSC), but they did find higher total antioxidant activity in organically grown fruit than in those from conventional or integrated fruit production systems. However, in similar studies carried out in New York on cv. Liberty apples, no difference for total phenolic concentrations or antioxidant capacity was found between organic and integrated fruit production systems (Peck et al., 2009). Cultivation system had little effect on ascorbic acid or thiobarbituric acid reactive substances in pears, but total phenolics and polyphenol oxidase (PPO) activity were higher in organic than in conventional systems (Carbonaro et al., 2002). With numerous differences between how and where the farming systems were implemented, results for apples and other fruit crops have been variable (Zhao et al., 2006), and no universal conclusions can be drawn. Long-term rigorous and comprehensive comparisons of these growing systems under a variety of climatic regions are needed to determine the consistency of fruit responses.

11.6.2 Effects of postharvest treatment

Apples and pears are horticultural products that are stored for long periods using a range of technologies such as refrigerated and controlled atmosphere (CA) and, more recently, the ethylene inhibitor, 1-methylcyclopropene (1-MCP) (Watkins, 2003, 2008). The majority of available research has focused on physical quality attributes, and an emphasis on characterization of the health-related components of apple after harvest has been relatively recent. Phenolics, for example, have been of interest as substrates for browning enzymes in both apples and pears (Ranadive and Haard, 1971; Coseteng and Lee, 1987; Richard-Forget et al., 1992). Browning of fruit during storage as a result of senescence and/or CA-induced disorders has been of increasing interest (Volz et al., 1998; Fernandez-Trujillo et al., 2001; de Castro et al., 2008). An important disorder of apples known as superficial scald, which results in browning of the apple skin, has also been the subject of considerable interest in terms of phenolics (Ju et al., 1996; Piretti et al., 1996; Golding et al., 2001). Phenolics may also be involved in scab resistance (Mayr et al., 1997), and changes in concentrations of benzoic acid, p-coumaryl-quinic acids
and chlorogenic acid may also be important in resistance of apple fruit against disease (Brown and Swinburn, 1973; Ndubizu, 1976; Noble and Drysdale, 1983; Lattanzio et al., 2001; Michalek et al., 2005). Finally, phenolics, in addition to L-AA, are important antioxidants involved in normal cellular function (Rice-Evans et al., 1996). As described earlier, however, total antioxidant capacity is associated more closely with total phenolic concentrations than those of L-AA (Eberhardt et al., 2001; Lee et al., 2003; Hagen et al., 2007). L-AA contributes as little as 0.4% (Sun et al., 2002) to 10% of the total antioxidant capacity of apples (Lee et al., 2003; Vanzani et al., 2005), but it may contribute to antioxidant activity of phenolics (Saucier and Waterhouse, 1999). Moreover, the contribution of individual antioxidant components is much smaller than total antioxidant capacity, as determined by assays such as DPPH (Chinnici et al., 2004) and peroxyl radical trapping efficiency (Wolfe et al., 2003; Vanzani et al., 2005). Polymeric anthocyanidins may be a factor (Vanzani et al., 2005), but the available data do not exclude the possibility that synergistic interactions among antioxidant components explain the discrepancy (Eberhardt et al., 2001; Chinnici et al., 2004).

Little information is available for pear fruit. L-AA concentrations have been investigated in relation to core breakdown of pears stored in CA. Development of this disorder is associated with decreasing L-AA concentrations in the core and may occur only when concentrations fall below a threshold value (Veltman et al., 1999, 2000; Zerbini et al., 2002).

Postharvest changes of phenolics in apples appear modest compared with cultivar and preharvest effects such as exposure to light. Although phenolics undergo constant turnover and degradation (Barz and Hoesel, 1979; Stafford, 1990), little is known about the biochemical processes underlying these changes. The major exception is PAL activity, described earlier.

Although total antioxidant capacity of apples is tested routinely and antiproliferation activity of apples has been well described (Liu et al., 2001; Wolfe et al., 2003), there is an absence of information about antiproliferation activity against cancer cell systems in any postharvest system.

Air storage

Early research with nine apple cultivars found that phenolic concentrations remained relatively constant over 4 months of cold storage (Coseteng and Lee, 1987). Individual phenolic compounds in peel and flesh tissues of cvs. Rhode Island Greening, Empire and Golden Delicious also changed little over 6 months of storage, although epicatechin concentrations appeared to increase in peel and flesh tissues of cv. Rhode Island Greening after harvest (Burda et al., 1990). However, no statistical information for changes over time was provided by the authors. In cvs. Red Delicious and Ralls, chlorogenic acid, flavonoid and anthocyanin concentrations in the peel remained stable for 4–5 months of storage (Ju et al., 1996). Golding et al. (2001) found that concentrations of total phenolics, total benzoic acid derivatives, total procyanidins, phloridzin, total cinnamic derivatives and chlorogenic acid in peels differed greatly among cvs. Lady Williams, Crofton and Granny Smith. These concentrations increased in the early stages of storage, but then generally declined over a 9-month storage period. Piretti et al. (1994) also found that epicatechin, quercetin glycosides and procyanidins in peel of cv. Granny Smith apples decreased from day 100 to day 205 of storage. Lattanzio et al. (2001) found an increase of chlorogenic acid, phlorodzin and phloretin glycoside concentrations in the peel after 60 days of air storage, which then either levelled off or declined. In a detailed study of harvest date effects, MacLean et al. (2006) found that chlorogenic acid concentrations in cv. Red Delicious peel tissues increased during air storage, while those of anthocyanins decreased. Total antioxidant activity of peel tissues increased during storage of cv. Empire, but decreased in cv. Red Delicious (MacLean et al., 2003). Total phenolic concentrations increased in the skin of cv. Jonagold but not cv. S’ampion, while anthocyanin concentrations decreased in both cultivars (Leja et al., 2003). However, total antioxidant activity (inhibition of linoleic acid peroxidation and
DPPH assays) increased during storage of both cultivars (Leja et al., 2003). Total quercetin glycoside, phloridzin, cyanidin galactoside and chlorogenic acid concentrations were unaffected by storage in whole fruit of four cultivars, whereas catechin decreased over storage in each cultivar, depending on harvest year (van de Sluis et al., 2001). Napolitano et al. (2004) found that epicatechin concentrations increased in flesh tissues of cvs. Golden Delicious, Red Delicious and Empire, while a major increase in chlorogenic acid and catechin was found in cv. Annurca, a cultivar that was harvested at an immature stage and then treated to exposure to sunlight for a month before storage. The total antioxidant activity (ABTS) increased during storage of all cultivars, but especially cv. Annurca; activity was correlated with catechin and phloridzin concentrations and not that of chlorogenic acid (Napolitano et al., 2004). Air storage did not affect antioxidant activity (inhibition of lipid peroxidation) in whole fruit of four cultivars (van de Sluis et al., 2001). However, Tarozzi et al. (2004) found that antioxidant bioactivity in vitro, measured in terms of intracellular antioxidant, cryoprotective and antiproliferation activity in human colon carcinoma (Caco-2) cells, decreased over time.

During the shelf-life period at warmer temperatures after cold storage, phenolics may be stable (Awad and de Jager, 2000), but decreases in various phenolic components, including chlorogenic acid, flavonoids and anthocyanins, in cvs. Granny Smith, Red Delicious and Ralls, have been reported (Piretti et al., 1994; Ju et al., 1996). Phenolic compounds in the peel, but not the flesh, increased during a shelf-life period of 21 days (Perez-Izarbe et al., 1997).

Overall, the literature, while contradictory, suggests that phenolics are relatively stable and therefore that the health benefits of phenolics are likely to be maintained during storage. Where increases in total phenolics and individual components have been reported, these occur in the early stages of storage. It is possible that these changes during early storage are associated with ethylene stimulation of PAL activity. What is unclear, however, is the bioactivity of these phenolics, with poor understanding of the interactions among individual compounds and ascorbic acid, or even the effects of changing acid and sugar levels in the fruit.

In contrast, total L-AA concentrations generally decrease during air storage (Vilaplana et al., 2006; de Castro et al., 2008; Fawbush et al., 2009), although not always (Tarozzi et al., 2004). Cultivars vary in the rates of L-AA loss over time (Davey and Keulemans, 2004). Davey et al. (2004) reported that ascorbic acid (L-AA) could not be synthesized de novo by fruit. Although Li et al. (2008) found that both peel and flesh tissues were able to synthesize ascorbic acid, and L-AA concentrations increased in fruit exposed to visible and UV-B light after harvest (Hagen et al., 2007). Losses of L-AA might be associated with declining cellular function and might be especially important during defence against physiological disorders.

**Controlled atmosphere (CA) storage**

Compared with air storage, relatively few studies on the effects of CA on antioxidative compounds of apple are available. CA storage conditions had little effect on total quercetin glycoside, phloridzin and cyanidin galactoside concentrations in whole fruit of four cultivars (van de Sluis et al., 2001). Chlorogenic acid and catechin concentrations decreased in cv. Jonagold, whereas only catechin decreased in cv. Golden Delicious, and storage did not affect antioxidant activity (inhibition of lipid peroxidation) in any cultivar (van de Sluis et al., 2001). Catechin, epicatechin and quercetin glycoside concentrations in the peel decreased in CA storage of cvs. Jonagold, Sampion and Elstar apples in a similar fashion to that in air storage (Piretti et al., 1994; Awad and de Jager, 2000, 2003). In contrast, total phenolic concentrations and total antioxidant activity in the peel of cvs. Jonagold and Sampion increased, while anthocyanin concentrations during CA storage were maintained at levels similar to those at harvest (Leja et al., 2003). Total phenolics in peel of cv. Golden Reiders, but not cv. Gala Must, increased during 7 months of CA storage (Mareczek et al., 2000); these concentrations decreased only in cv. Gala Must during a subsequent shelf life. In pear fruit, hydroxycinnamic
derivatives and flavonols were stable in air and CA (0, 0.5 and 5% kPa CO₂ in 2% kPa O₂) for 4 months, but flava-3-ol concentrations decreased (Galvis-Sanchez et al., 2006). Accumulation of phenolic compounds that was observed in air-stored cv. Williams pears was inhibited by CA storage (Amiot et al., 1995).

L-AA levels generally decline in apples during CA storage (de Castro et al., 2008; Fawbush et al., 2009), although Lata (2008) found increased L-AA concentrations (in addition to thiols and phenolic compounds) in apple peel during CA storage. Loss of L-AA in elevated CO₂ concentrations was reduced with lower O₂ concentrations and might be associated with susceptibility of fruit to flesh browning (de Castro et al., 2008).

In pears, L-AA concentrations decreased more rapidly in CA than in air storage for 22 days (Larrigaudiere et al., 2001a) but regenerated during subsequent storage, depending on gas concentration (Larrigaudiere et al., 2001b). L-AA concentrations also increased when the CO₂ concentration in the storage atmosphere was increased from 0 to 10% kPa (Veltman et al., 2000). However, Galvis-Sanchez et al. (2006) found that L-AA concentrations decreased, while those of DHA decreased during storage in air and CA. Franck et al. (2003) found that losses of L-AA from pears was similar in fruit cooled slowly or quickly in conjunction with CA storage, but specific CA regimes had significant differences.

1-Methylcyclopropene (1-MCP)

An inhibitor of ethylene perception, 1-MCP, has been registered recently for use on food crops around the world, and it is used extensively to maintain quality of apple fruit during air and CA storage (Watkins, 2006, 2008). To date, studies on the effects of 1-MCP on antioxidant components are limited. MacLean et al. (2006) found that 1-MCP treatment inhibited an increase in chlorogenic acid in peel tissues of cv. Red Delicious apples during storage, and resulted in higher flavonoid concentrations. The effects of 1-MCP on chlorogenic acid decreased as fruit harvest date and internal ethylene increased; this effect was consistent with a role of ethylene in modulating PAL activity, as later harvest date presumably would have a smaller inhibition of ethylene production and thus less effect on PAL activity. MacLean et al. (2003) also showed that total antioxidant activity (TOSC) was higher in peels of 1-MCP-treated cvs. Empire and Red Delicious apples than in untreated fruit. The total antioxidant activity (DPPH) of cv. Golden Smoothee flesh was unaffected by 1-MCP treatment, but total L-AA concentrations were slightly lower after 30 and 90 days of air storage (Vilaplana et al., 2006).

In pears, 1-MCP treatment inhibited an increase of chlorogenic acid observed in untreated fruit, and overall the results suggested that 1-MCP inhibited the transcription of the key flavonoid biosynthetic enzymes, PAL and chalcone synthase (MacLean et al., 2007).

Postharvest chemical treatments

Postharvest treatment of fruit with diphenylamine (DPA), an antioxidant used to prevent superficial scald development, resulted in higher total phenolics during air storage, but the overall effects were minor (Golding et al., 2001). In contrast, Duvenage and DeSward (1973) concluded that DPA inhibited both the synthesis and oxidation of flavonols during storage. DPA did not affect the loss of L-AA in fruit during the first 2 months of storage, but concentrations were higher, in both air- and CA-stored fruit, after 4 months (de Castro et al., 2008).

Calcium is often applied to apple fruit as a postharvest drench or dip at the same time as DPA treatment. Little is known about the effects of such applications on nutritional components, but Bangerth (1976) found that L-AA concentrations in fruit were increased by calcium dips or infiltration.

UV irradiation

Postharvest irradiation of fruit, usually as combined visible and UV-B radiation, induces anthocyanin accumulation in both non-red and red apples (Arakawa et al., 1985; Reay, 1999; Lancaster et al., 2000; Reay and Lancaster, 2001; Ubi et al., 2006). Hagen et al.
(2007) extended these studies to show that exposure of fruit from the inner (shaded) and outer (exposed) part of the tree to visible light and UV-B radiation could be used to increase health-related components; radiation caused higher accumulations of anthocyanin, quercetin glycosides, chlorogenic acid, ascorbic acid, total phenols and antioxidant (ORAC) capacity in the peel, but not flesh, compared with untreated fruit. Total antioxidant capacity was correlated more strongly with the sum of phenols than with ascorbic acid concentrations (Hagen et al., 2007). Methyl jasmonate (MJ) interacts synergistically with ethylene in UV/white light-treated apple fruit to influence peel pigment synthesis pathways, the effects being specific to key pigment components (Rudell et al., 2002; Rudell and Mattheis, 2008).

11.7 Conclusion

The apple has been well studied compared with other pome fruit. The evidence from epidemiological studies strongly suggests that consumption of apples is associated with a decreased risk of developing chronic diseases such as cardiovascular disease, cancer and asthma. In vitro studies have demonstrated that apples have high antioxidant activity and antiproliferative activity against colon, liver and breast cancer cells, and can decrease lipid oxidation and lower cholesterol. Apple extracts inhibit NFkB activation, induce G1 arrest and decrease expression of Cyclin D1 and Cdk4 in human breast cancer MCF-7 cells. Most recently, several studies have found that apple extracts prevent DMBA-induced mammary cancer in rats, downregulate PCNA, Cyclin D1 and Bcl-2, upregulate Bax and induce apoptosis in vivo. The health benefits of apples, and probably other pome fruit, are attributed to the complex mixture of phytochemicals, plant bioactive compounds with a variety of functions. The interaction of the phytochemicals, especially the additive and synergistic effects, warrants more study to explain further the mechanism behind the ability of apple and other fruit to reduce risk of chronic disease. More work is needed to understand better the bioavailability of pome fruit phytochemicals. While more research is warranted with apple fruit, this review illustrates clearly the paucity of information for pear and other pome fruit.

Understanding of the processes involved in intake and bioavailability of phytochemicals in pome fruit may lead to much better knowledge of exactly how important pre- and postharvest management and handling practices are in maintaining nutritional quality. Selection processes by breeders inevitably are focused on appearance and yield, resistance to disease and physiological disorders. These factors may or may not be associated with nutritional quality.

References


Pome Fruit


12 Potato and Other Root Crops

Anne Pihlanto

12.1 Introduction

Diet is known to play an important role in many major diseases of our society, such as cardiovascular diseases, cancer, obesity and diabetes. Certain bioactive compounds in food plants have been known for a long time for their beneficial effects, whereas others have been recognized more recently. Diets rich in polyphenolics and carotenoids have been associated with a lower incidence of atherosclerotic heart disease and certain cancers. Reduction of atherosclerotic heart disease in association with antioxidant-rich diets is hypothesized to be related to a reduction in the oxidative polymerization of low-density lipoproteins and consequent lesion formation and plaque build-up in key coronary arteries. Cancer reduction is further hypothesized to be due to protection of DNA from destruction by reactive oxidative species. Consumption of diets high in fruit and vegetables increases the antioxidant levels in blood serum in human subjects. In recent years, some studies have focused on the role of polyphenols and potato proteins in the prevention and treatment of obesity.

Potatoes (*Solanum tuberosum* L.) and other root crops are perhaps the most important vegetables consumed in the Western diet. This chapter reviews the current knowledge about the major beneficial compounds derived from potatoes and carrots, as well as sweet potato, red beet, red radish, cassava and yam. Special emphasis is given to their potential health effects (Table 12.1). Suggestions for future research are also mentioned.

12.2 Identity and Role of Bioactivities

12.2.1 Potato

The potato is one of the world’s most widely grown crops. Potato production is about 320 million tonnes (Mt) globally, of which about 66% is used as food, 12% as feed and 10% as seed (FAO, 2010). The rest is used mainly in the starch industry, since increasing amounts of starch are now extracted from potato tubers and modified for further uses in processed foods and in non-foods, including the paper industry. Potatoes represent a staple source of nutrients and energy in many different countries. They are a proven source of proteins, carbohydrates and minerals such as calcium, potassium and phosphorus, and their value in the human diet is often underestimated or overlooked. Several small molecular weight compounds, many of which have reported beneficial effects on health, have been found in potatoes (Friedman, 1997). These compounds include secondary metabolites,
Table 12.1. Bioactive compounds and potential health effects *(in vitro, cell, animal and human studies)*.

<table>
<thead>
<tr>
<th>Food</th>
<th>Component</th>
<th>Potential benefit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proven effect on animal and human studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>Tuber protease</td>
<td>Prevents experimental protease-induced dermatitis <em>(in vivo)</em> (humans)</td>
<td>Ruseler-van Embden et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Protease</td>
<td>Prevents protease-induced perianal dermatitis (faeces of subjects suffering perianal dermatitis): Suppresses proteolytic activity in faeces</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peel extract</td>
<td>Protection of liver injury <em>(in rats)</em></td>
<td>Singh et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Protein hydrolysate</td>
<td>Protective effect against ethanol-induced mucosal damage in rats</td>
<td>Kudoh et al. (2003)</td>
</tr>
<tr>
<td>Carrot</td>
<td>Protein hydrolysate</td>
<td>Beneficial effects on cholesterol metabolism <em>(mice and rats)</em>: Increases antioxidant status</td>
<td>Nicolle et al. (2003, 2004)</td>
</tr>
<tr>
<td></td>
<td>Insoluble fibre</td>
<td>Lowers serum triglyceride, total cholesterol and liver lipids <em>(in hamster)</em></td>
<td>Chou et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>and cellulose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Falcarinol</td>
<td>Chemopreventive impact on lymphoblastic leukaemia cell line CEM-C7H2. Reduces development of tumours in rats.</td>
<td>Christensen and Brandt (2006)</td>
</tr>
<tr>
<td><strong>In vitro evidence on positive effect on cardiovascular health and prevention of certain cancer cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>Patatin</td>
<td>Radical, hydroxyl radical scavenging activity, prevention of LDL peroxidation</td>
<td>Liu et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Protein hydrolysate</td>
<td>Antihypertensive and antioxidative activity <em>(in vitro)</em></td>
<td>Pihlanto et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Early potato extracts</td>
<td>Free radical, oxyradical scavenging activity Inhibition of breast cancer MCF-7 cell proliferation</td>
<td>Leo et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Methanol extract</td>
<td>Free radical, oxyradical, hydroxyl radical scavenging activity, inhibition of lipid peroxidation</td>
<td>Chu et al. (2000)</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>Glycoalkaloids</td>
<td>Inhibition of cholinesterases, complexes with cholesterol and other phytosterols</td>
<td>Friedman (2006)</td>
</tr>
<tr>
<td></td>
<td>Phenolic</td>
<td>Antioxidant activity as Trolox equivalent, ranging from 1.3 to 4.6 mg/g DW</td>
<td>Padda and Picha (2008)</td>
</tr>
<tr>
<td></td>
<td>Trypsin inhibitor</td>
<td>Induced NB4 promyelocytic leukaemia cell apoptosis through the inhibition of cell growth</td>
<td>Huang et al. (2007)</td>
</tr>
<tr>
<td><strong>Satiety in vitro and in vivo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>Protein hydrolysate</td>
<td>Induce CCK release from enteroendocrine cells</td>
<td>Foltz et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Proteinase inhibitor II</td>
<td>Reduced energy intake in lean subjects</td>
<td>Hill et al. (1990)</td>
</tr>
</tbody>
</table>
such as polyphenols, protease inhibitors and glycoalkaloids. Storage and heat treatments, during commercial processing or domestic cooking, may affect the total content of these secondary metabolites, which has to be taken into account when evaluating the intake of these compounds. Outside the centre of origin of cultivated potato in the Andes of South America, it is rare to find varieties with anthocyanidin pigments conferring red or purple flesh. However, much of the world’s production is occupied by the yellow-flesh potatoes, which have higher total carotenoid than the white-fleshed varieties of North America and Great Britain.

**Polyphenolic compounds**

Potato tubers are one of the richest sources of polyphenols. Content of polyphenols is affected mainly by variety, year of cultivation, stress factors (mechanical damage of tubers, attack of pathogens, action of light on tubers or irrigation) and by storage and cooking treatment. To a lesser extent, the effect of geographical location, soil type, potassium fertilization, storage temperature, γ-irradiation and other factors could be involved, but there is only a little demonstrable empirical evidence for this in the literature (Friedman, 1997).

Phenolic acids are distributed mostly between the cortex and skin (peel) tissues of the potato (Fig. 12.1). About 50% of the phenolic compounds are located in the potato peel and adjoining tissues, which are often wasted, while the remainder decrease in concentration from outside toward the centre of the potato tubers (Friedman, 1997). Lewis et al. (1998a) found that cultivated potato tuber skin contained 2–5 mg/g fresh weight (FW) phenolic acid and 0.2–0.3 mg flavonoids. In wild *Solanum* species, phenolic acids ranged from 6 to 27 μg/g FW in skin and from 1 to 6 μg/g FW in the flesh (Lewis et al., 1998b). Purple- and red-skinned tubers contained twice the concentration of phenolic acids as white-skinned tubers. Tuber flesh contained lower concentrations, ranging from 0.1 to 0.6 mg of phenolic acids and from 0 to 0.03 mg/g FW of flavonoids. Furthermore, purple- and red-fleshed cultivars had twice the flavonoid concentration of white-fleshed cultivars and three to four times the concentration of phenolic acids. Mattila and Hellström (2007) found the highest content of phenolic acids in raw and boiled potato peels, varying from 0.23 to 0.45 mg/g FW. When peeled potatoes were boiled for 18–25 min with or without NaCl, the phenolic acid content was clearly lower.

Studies on the phenolic composition of potatoes have reported that the predominant phenolic acid is chlorogenic acid, which constitutes about 80% of the total phenolic acids. Chlorogenic acid is formed between caffeic and quinic acid and is hydrolysed rapidly to caffeic acid under alkaline conditions. Traces of other phenolic acids, such as protocatechic, ferulic, vanillic and p-coumaric acids, can be found. Chlorogenic acid content varied from 0.1 to 0.2 mg/g in different potato varieties (Friedman, 1997). In skin extracts of wild *Solanum* species, chlorogenic acid accounted for 40–50% of the total phenolic acid content, with caffeic acid also present at high levels (10–30%). Flesh phenolic acids comprised 30–40% protocatechic acid, 20–30%.
chlorogenic acid and 20–30% p-coumaric acid (Lewis et al., 1998b). In early potatoes, the cultivars had high concentrations of free chlorogenic acid (0.47–0.92 mg/g dry weight (DW)) and moderate amounts of caffeic acid (0.05–0.12 mg/g DW), lower concentrations of ferulic acid (0.006–0.039 mg/g DW) and traces of p-coumaric acid and some unidentified compounds. Nearly all the phenolic acids in potatoes are in a soluble form (Mattiila and Hellström, 2007). In the wild species (white or light purple tubers), concentrations of total flavonoids ranged between 20 and 170 µg/g FW in skin and 0 and 25 µg/g FW in flesh. The major flavonoids were catechin, epicatechin, eriodictyol and naringenin (Lewis et al., 1998b). Chu et al. (2000) reported that in potatoes purchased from local markets, the total amount of flavonols and flavones was 0.13 mg/kg and the major compound was kaempferol. The major flavonoid in early potatoes was catechin (0.43–1.57 mg/g DW) and this was the major compound present in a bound form (Leo et al., 2008).

Anthocyanins are the major group of pigments in potatoes and tubers which exhibit a range of colours, with heterogeneous flushes of red, and have been studied by several workers (Fig. 12.2). The pigments have been determined to be various types of acylated anthocyanidin glucosides. Rodriguez-Saona et al. (1998) reported anthocyanin contents of partially and solidly red-fleshed potatoes ranging from 0.03 to 0.40 mg/g FW. The major pigments were identified to be acylated glucosides of pelargonidin. Lewis et al. (1998a) found much higher concentration of anthocyanins in certain cultivars, extending up to 3.68 mg/g FW in the purple-fleshed cv. Urenika and up to 0.22 mg/g FW in red-fleshed types. Concentrations were considerably higher in skin, approaching 9 mg/g FW in purple-fleshed and 5 mg/g FW (of the skin alone) in red-fleshed types. p-Coumaryl conjugates of anthocyanins were present in peel (up to 7 mg/g) and flesh (up to 2 mg/g) (Lewis et al., 1998a). Pelargonidin and peonidin were in nearly equal amounts in the red flesh, while petunidin and malvidin were predominant in the purple flesh. Wild species had no anthocyanins in the flesh but up to 0.27 mg/g (FW) in the skin (Lewis et al., 1998b). Jansen and Flamme (2006) found, on average, higher amounts of anthocyanins in skin (0.65 mg/g FW) in 31 analysed potato cultivars/breeding clones. The corresponding values of whole tubers (0.31 mg/g FW) and flesh (0.22 mg/g FW) were significantly lower. The cv. Peru Purple was shown to have the highest anthocyanin content in the skin: 2.96 mg/g FW. Fossen and Andersen (2000) determined the anthocyanins of the purple-fleshed cv. Congo to consist of ferulyl glucose- and rhamno-pyranosides of malvidin and petudin. Fossen et al. (2003) further reported the finding of acylation with caffeic acid in extracts from an unnamed, purple-fleshed, Norwegianderived cultivar.

Several results support the theory that one of the reasons why potatoes produce phenolic acids and flavonoids is to aid in plant defence. In diseased tubers, increased output via the cinnamic acid, benzoic acid and flavonoid, especially flavonol, pathways has been found. Also supporting this hypothesis is the observation that much higher concentrations of phenolic acids and flavonoids (plus anthocyanins) have been found in tuber skin than in the flesh, and this may be related to the skin being the first line of defence against pathogens and pests (Friedman, 1997; Brown, 2005).

There are only a few publications describing the effect of cooking on the polyphenolic compounds in potatoes, a situation that is bizarre considering that potatoes are not eaten raw. Phenolic acids seemed to remain quite stable in potatoes during boiling. Mattila and Hellström (2007) found that 71–92% and 65–100% of soluble and total phenolic acids on a dry matter basis, respectively, were left in potato peels after boiling (18–25 min), as compared with unboiled peels. Boiling destroyed 65%, microwave baking 45% and oven baking 100% of the original amount of chlorogenic acid. Anthocyanins in potatoes survived to a large degree during various cooking methods, including frying in oil (Brown, 2005).

Potato antioxidant compounds are associated closely with a strong antioxidant capacity and their antioxidant effect is due mainly to their redox properties and is the result of various possible mechanisms: free
radical scavenging activity, transition metal chelating activity and/or singlet-oxygen-quenching capacity. Phenolic content and antioxidative activity of potato cultivars have been shown to be genotype-dependent and not related to flesh colour. Polyphenolic compounds in potatoes show antioxidative activity in several food systems. For example, freeze-dried extracts from peels of potato varieties prevented soybean or sunflower oil oxidation. These results suggest the possible value of potato peel in the prevention of oxidative rancidity of food oils (Brown, 2005).

**Glycoalkaloids**

Glycoalkaloids (GAs) are secondary metabolites that are produced following the general
sterol biosynthesis pathway, starting from acetyl-coenzyme A and followed by the intermediates, mevalonic acid, squalene, cycloartenol and cholesterol. α-Chaconine and α-solanine are the main GAs of the cultivated potato (Fig. 12.3), whereas many other GAs are known in the wild potato species. GAs may be toxic to bacteria, fungi, viruses, insects, animals, and even humans. Their natural function is probably to serve as stress metabolites for the protection of potato when attacked by insects, fungi, etc. The potential human toxicity of GAs has led to the establishment of strict guidelines limiting the GA content of new cultivars before they can be released for commercial use. In Finland, as in many countries, the official recommended upper limit for total GA concentration in cultivated S. tuberosum tubers is 0.20 mg/g FW.

At least 90 structurally different steroidal alkaloids have been isolated and characterized in over 300 Solanum species. The GAs identified to date are composed of C-27 steroidal alkaloid along with various sugar

---

**Fig. 12.3.** Intermediates in the hydrolysis of the trisaccharide side chains of α-chaconine and α-solanine to the aglycon solanidine (Friedman, 2006).
moieties, usually composed of di-, tri- or tetrasaccharides. In commercially cultivated potato, the major GAs, α-solanine and α-solatin, are triglycosylated derivatives of the same aglycone, solanidine, but differ in their carbohydrate moiety. In α-solanine, the carbohydrate moiety is composed of galactose, glucose and rhamnose (β-solatriose), while in α-solatin glucose, rhamnose and rhamnose β-chacotriose are present. The trisaccharide chains can be cleaved by acid or enzymatic hydrolysis to form aglycon solanidine. Other GAs that are present, but in much lower concentration, are α- and β-solamins and chaconines, α- and β-solamarines, aglycones demissidine and 5-α-solanidan-3-β-ol. In wild potatoes such as S. chacoense, numerous other GAs and aglycones have been identified. Two other structural classes of potato GAs, the leptines and leptidines, are present in the leaves of S. chacoense but not in the leaves of S. tuberosum, and are not present in potato tubers (Friedman, 2006; Nema et al., 2008).

GAs are found throughout the potato plant, with levels varying considerably between different tissues and between the same parts in different plants and varieties. The highest GA concentration is found in the outer layer of the tuber, especially next to eyes, wounds and in the sprout, yet they decrease towards the centre of the tuber. The total GA concentration in the peels was reported to vary from 0.25 to 0.95 mg/g FW. For peeled potatoes, the average content can vary from 1 μg/g to 0.20 mg/g FW in the whole tuber. When potato tubers are exposed to light, the solanine content in the peel may increase by as much as tenfold. The ratio of α-solane to α-chaconine concentration differs, depending on the part of the potato plant and the variety. Most studies report the value of this ratio to be between 1:2 and 1:7. Both rates and patterns of accumulation, as well as the ratio of α-chaconine to α-solane during tuber growth and development, are influenced strongly by genotype (Friedman, 2006; Nema et al., 2008).

**Proteins and peptides**

Potato tuber proteins are classified into three groups: patatins, protease inhibitors and other proteins. The patatin and protease inhibitor classes, which form the bulk of the potato tuber protein, are considered to be mostly storage proteins. The majority of the isoforms have defined enzymatic and inhibitory activities that might be of physiological relevance.

Patatin, a glycoprotein of molecular weight c.45 kDa, accounted for about 40% of the total soluble protein in potato. Patatin exists in a number of charge forms, which differ between potato cultivars. A high degree of homology between the isoforms of patatin was also indicated by NH₂-terminal amino acid sequence analysis (Shewry, 2003). Pots et al. (1999) separated the patatins of cv. Bintje into four pools with different chromatographic and electrophoretic characteristics, but similar biophysical properties. Little information on other proteins present in the tuber is available and, until recently, no systematic gene discovery or protein sequencing had been undertaken. Jorgensen et al. (2006) determined 43 different potato tuber proteins from the starchy potato cv. Kuras. Potato tuber protease inhibitors are a diverse group of proteins that inhibit a variety of proteases and some other enzymes, for example invertase. They differ in their amino acid sequence, chain length and subunit composition (monomer to pentamer).

The role of patatin in potato tubers remains unclear. It has been speculated that, besides being the main storage protein of potato tubers, patatin might also be involved in the resistance reaction induced by pathogen attack. The lipid acyl hydrolase activity of patatin could be important for the rapid degradation of cell membranes, and thus rapid degradation of certain metabolites. In addition to enzymatic and inhibitory activities, patatin was shown to have antioxidant or antiradical activity. Liu et al. (2003) found that purified patatin showed antioxidant or antiradical activity in a series of in vitro tests, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assays, anti-human low-density lipoprotein peroxidation tests, and protections against hydroxyl radical-mediated DNA damages and peroxynitrite-mediated dihydrorhodamine 123 oxidations. Gaillard and Matthew (2006) measured two lipid-degrading
enzymes, namely a hydrolytic enzyme (lipolytic acyl hydrolase) and an oxidizing enzyme (lipoxygenase), in tubers from 23 varieties of potato at harvest. All varieties contained high levels of lipoxygenase activity, and 22 varieties had very high levels of the hydrolytic enzyme, ranging from 5 to 50 μmol of substrate hydrolysed/min/g of FW of tuber. One variety, Desiree, contained a much lower level of this enzyme (0.06–0.2 units/g FW). From these results it may be concluded that the major soluble protein may be beneficial when it is consumed, owing to its antioxidant activity. In addition, recent findings indicate that potato isolates and by-products from the potato industry could be useful sources of bioactive compounds (Pihlanto et al., 2008).

Peptides with specific biological activity may be located in the amino acid sequence of a given protein. Enzymatic degradation of foodstuffs in the gut and in food processing releases short-chain peptide sequences from intact proteins, glycoproteins and lipoproteins. In some cases, peptides may act as regulatory compounds with a hormone-like activity, based on their amino acid composition and sequence. The beneficial health effects may be attributed to numerous known peptide sequences exhibiting, for example, antimicrobial, antioxidative, antithrombotic, antihypertensive and immunomodulatory activities. Such activities are based on the peptides’ amino acid composition and sequence. Bioactive peptides usually contain between three and 20 amino acid residues per molecule. In order to elicit a biological response, peptides must both cross the intestinal epithelium and enter the blood circulation, or bind directly to specific epithelial cell-surface receptor sites (Pihlanto and Korhonen, 2003).

There are a few reports on the bioactivities of potato hydrolysates; however, no peptides have been identified so far. Based on the amino acid sequences of potato proteins, there are several potential precursor proteins, from which peptides with various activities could be released. Recently, it has been found that hydrolysis of protein isolates from potato tubers results in enhanced inhibition of angiotensin I converting enzyme (ACE) (Pihlanto et al., 2008), which plays a major role in the control of blood pressure. Further studies showed that autolysis of protein isolates from vascular bundle and inner tuber tissues of potato enhanced the inhibition of the ACE. In addition, the physiological age of the tuber affected the strength of ACE inhibition, the rate of its increase during autolysis and the tuber tissue where ACE inhibition was most pronounced. Many tuber proteins, including two aspartic protease inhibitors, were observed to degrade during autolysis (Mäkinen et al., 2008). Foltz et al. (2008) found that potato protein hydrolysate stimulated cholesterylokinin (CCK) release from a murine cell line of enteronecocrine origin (ST-1 cell line) and directly stimulated CCK-R-expressing cells. As CCK-R is expressed in the gastrointestinal tract, direct interaction of CCK-R with dietary peptides may contribute to their satiety effects.

**Other bioactivities**

White- and yellow-fleshed potatoes are very familiar to people around the world. Iwanzik et al. (1983), in one of the most complete studies, compared potatoes with various degrees of yellow intensity, finding a range of total carotenoids from 0.27 to 3.29 μg/g FW. They listed lutein, neoxanthin, violaxanthin and lutein-5,6-epoxide as components and found a strong correlation between carotenoid concentration and colorimetric measurements of yellowness. The summer and spring potatoes studied were found to contain 0.13 and 0.60 μg/g FW, respectively, of xanthophyll identified as lutein. Furthermore, a range of 0.97–5.36 μg/g FW in a series of cultivars and breeding lines has been obtained, although, in this study, carotenoid content did not appear to be related to colour of flesh (Brown, 2005).

The potato pulp resulting from industrial starch processing is a potential source of dietary fibre. Potato pulp has a high content of soluble fibre. Meyer et al. (2009) reported that in potato pulp about 22% by weight of the non-starch polysaccharide material was made up of cellulosic, leaving about 38–40% by weight of the dry matter as pectin and hemicellulose polymers containing different monosaccharides, with a remarkably high proportion of galactose and relatively high uronic acid levels – the latter indicating galacturonic acids and hence presence of pectin.
12.2.2 Carrots

Carrot (Daucus carota) is one of the major vegetables consumed in the Western world, whatever the season. Its consumption has been increasing in Western countries. Carrot is one of the richest vegetables in fibres and carotenoids. It also contains other antioxidants such as vitamin E (0.52 mg/g), vitamin C (0.07 mg/g) and polyphenols such as p-coumaric, chlorogenic and caffeic acids.

Carotenoids

Carotenoids are a group of natural pigments responsible for the yellow, orange or red colour of many foods. Carotenoids are defined by their chemical structure. The majority of carotenoids are derived from a 40-carbon polyene chain, which could be considered the backbone of the molecule. This chain may be terminated by cyclic end-groups and may be complemented with oxygen-containing functional groups. Besides the well-known provitamin A activity of some of these compounds, they have also been associated with lowered risk of developing degenerative diseases such as cancer, cardiovascular diseases, cataracts and macular degeneration.

Carrots are especially rich in carotenoids. α-Carotene and β-carotene are the principal carotenoids in carrots, while lutein is a minor component. Total carotenoid content and distribution of carotenoids at harvest are influenced by genetic and environmental factors. The data for α-carotene in carrot tissue ranged from 5.3 to 51.6 μg/g, for β-carotene they ranged from 33.0 to 130 μg/g and for minor component, lutein, they ranged from 3.6 to 5.6 μg/g (Müller, 1997; Niizu and Rodriguez-Amaya, 2005).

Thermal treatments reduce the total carotenoid content in carrots. During home processing, 14.4–39.9% of carotenoids are lost. Water cooking, without pressure, carried out under controlled time and temperature (6 min, 96°C), was the best method, as the losses in the amounts of α- and β-carotenoids were approximately 24% in relation to total (Pinheiro-Santana et al., 1998). Nguyen et al. (2007) found that pressure-assisted thermal processing (temperatures up to 105°C) resulted in higher carotenoid content than thermal processing alone.

Polyacetylenes

Carrot storage roots normally contain three major polyacetylenes, namely (Z)-heptadeca-1,9-diene-4,6-diyn-3-ol (falcarinol), (Z)-heptadeca-1,9-diene-4,6-diyn-3,8-diol (falcarindiol) and (Z)-3-acetoxyheptadeca-1,9-diene-4,6-diyn-8-ol (falcarindiol 3-acetate); however, most investigations have concentrated on falcarinol and falcarindiol. Polyacetylenes have been identified as the dominant cause of the commonly experienced bitter taste in carrot. The correlation between their concentration and individual bitter detection threshold clearly indicated falcarindiol as the main compound responsible for the bitter off-taste of fresh and stored carrots. Falcarindiol is present mainly in the peel and outer layer of the carrot root. In peeled carrots, the content varied significantly between 0.006 and 0.030 mg/g FW for falcarindiol, between 0.010 and 0.022 mg/g FW for falcarindiol 3-acetate and between 0.035 and 0.067 mg/g FW for falcarinol. Carrot peel contained over a tenfold higher concentration of falcarindiol than the corresponding peeled carrots.

Polyacetylenes are potent antifungal and antibacterial compounds. They are also known to be inhibitors of a number of enzymes such as diacylglycerol acyltransferase, inducible nitric oxide synthase and cholesteryl ester transfer protein, as well as microsomal and mitochondrial enzymes. In vitro experiments indicate that some polyacetylenes might exhibit antiallergenic and anti-inflammatory activities. In addition, polyacetylenes have proven to be cytotoxic against a number of solid and leukaemic cancer cell lines and to potentiate cytotoxicity of other anticancer drugs (Christensen and Brandt, 2006).

Flavonoids and other bioactivities

Gebczyński (2006) found that fresh carrot (cv. Koral) contained polyphenols at 20.9 mg/100 g, with a corresponding antioxidative activity of 19.4% (expressed as radical
scavenging activity). After 12-months storage, carrot retained 73–78% of polyphenols, while its antioxidative activity was reduced to 30–39% of the initial level. Mattila and Hellström (2007) reported that carrots contained 0.081–0.17 mg/g FW chlorogenic acid, 0.086–0.20 mg/g FW unknown phenolic compounds, and traces of other phenolic acids. The major anthocyanins in black carrot (D. carota ssp. sativus var. Atrorubens Alef) plant tissues and cell cultures possess cyanidin as an aglycon. In addition, traces of anthocyanins possessing a peonidin- or pelargonidin-type aglycon have been described (Kammerer et al., 2003; Schwarz et al., 2004). Also, carrot peels are a good source of antioxidant dietary fibre (Chantaro et al., 2008).

12.2.3 Other root crops

Yang et al. (2008) analysed the flavonoid contents as aglycones (for quercetin, kaempferol, isorhamnetin, luteolin and apigenin) for 115 edible plants (91 species), among them sweet potato (Ipomoea batatas). The latter’s total flavonoid content varied from 0 to 0.446 µg/g DW. The purple sweet potato variety contained the highest amount of flavonoids and was especially rich in quercetin. Padda and Picha (2008) found significant differences in total phenolics, in different genotypes of sweet potato, ranging from 1.4 to 4.7 µg/g DW. The highest total phenolic content and antioxidant activity was observed in a purple-fleshed genotype. Chlorogenic acid and 3,5-dicaffeoylquinic acid were the predominant phenolic acids, while caffeic acid was the least abundant in most genotypes. The highest content of chlorogenic acid (422.4 µg/g DW) was present in a white-fleshed cv. Quarter Million imported from Jamaica. However, a purple-fleshed genotype had the highest total phenolic content and antioxidant activity was observed in a purple-fleshed genotype. Chlorogenic acid and 3,5-dicaffeoylquinic acid were the predominant phenolic acids, while caffeic acid was the least abundant in most genotypes.

Several studies have shown that red beet/beetroot (Beta vulgaris L.) is a good source of natural antioxidants. The red beet colour results from the betalains, which are used extensively as natural food colorants. In vitro studies have demonstrated that the betalains from red beets possess high antiradical and antioxidant activity. Consequently, it has become a popular belief that betalains provide protection against oxidative stress-related disorders by acting as antioxidants in vivo (Stintzing and Carle, 2004). If this belief is correct, consumers may benefit from regular consumption of products rich in betalains (e.g. red beet juice). Besides these hydrophilic pigments, the presence of further health-promoting constituents of red beets, such as phenolics (phenolic acids, phenolic acid esters and flavonoids) and folic acid, has been reported.

Schreiner et al. (2002) found bioactive indolyl glucosinolates in red radish (Raphanus sativus L. var. sativus) and production process effects on the content of these compounds. Furthermore, there are several other root and tuber crops that are grown worldwide but usually have low commercial value for direct consumption.

The yams (Dioscorea villosa) are some of the most important tuber crops in West Africa. Yams have a complex phytochemical profile; the most predominant compounds are dioscorine alkaloid and diosgenin saponin. These compounds traditionally are considered toxic, but such toxicity can be removed by washing, boiling and cooking. Okwu and Ndu (2006) reported that different varieties of yam expressed different levels of alkaloids and flavonoids. The highest (0.195 mg/g) saponin content was found in the D. rotundata hybrid and the lowest (0.03 mg/g) in the D. alata hybrid (TDa 117). Alkaloids apart from saponins were found in concentrations from 0.01 to 0.02 mg/g. Good quantities of flavonoids were found in tubers (0.06–1.0 mg/g), whereas tannins and phenolic compounds were in smaller quantities (Okwu and Ndu, 2006). Apart from food, yams are used for medicinal purposes; the sapogenins, aglycons of yam saponins, are medically important, mainly because of their steroid structure.

Cassava (Manihot esculenta) is an important tropical root crop, ranking fourth on the list of major food crops in developing countries. An item of concern for public health authorities, plant breeders, producers, processors and, principally, the consumers is its...
toxic potential. The potential toxicity is due to the presence of two cyanogenic glycosides, linamarin and lotaustralin, in a ratio of approx 20:1, which, after hydrolysis into cyanohydrins, yield the toxic compound, hydrogen cyanide. Cassava root contains linamarin, equivalent to 0.002–1.0 mg HCN/g of cassava flesh. Traditional methods of processing cassava, such as sun drying, soaking, boiling and fermentation, eliminate most of the cyanide. The cyanogenic potential of insufficiently processed cassava has been identified as a factor in health problems like acute toxic effects, iodine deficiency disorders, tropical ataxic neuropathy and the paralytic disease, konzo (Essers, 1995; Egan et al., 1998).

12.3 Potential Health Benefits

12.3.1 Bioavailability

Any suggested health-promoting agent in foods would, after consumption, probably be required to appear in the blood to a significant extent to have an effect on target sites other than those of the gastrointestinal tract. The notion of bioavailability integrates several variables, such as intestinal absorption, metabolism by the microflora, intestinal and hepatic metabolism, plasma kinetics, the nature of circulating metabolites, cellular uptake and intracellular metabolism. The difficulty lies in integrating all the information and relating the variables to health effects at the organ level.

The health effects of polyphenols depend on the amount consumed and on their bioavailability. The structural diversity of polyphenols makes the estimation of their content in food difficult. Furthermore, they are not evenly distributed and processing may result in a loss or enrichment of some phenolic compounds. In potato, phenolic acids and flavones are found in the peel. It should be pointed out that peel polyphenols have little dietary significance if the polyphenols are destroyed during processing (baking, cooking, frying) or they are discarded during cooking preparation (e.g., peeling). Peels of baked or fried potatoes are the principal source of potato polyphenols in the human diet.

Direct evidence on the bioavailability of a few polyphenolic compounds has been obtained by measuring their concentrations in plasma and urine after the ingestion of either pure compounds or foodstuffs with known content of the compounds. Although very abundant in our diet, proanthocyanidins and anthocyanins are absorbed either very poorly or not at all, or their metabolites have not yet been identified. Thus, their action is restricted to the intestine. Intakes of monomeric flavonols, flavones and flavanols are relatively low, and plasma concentrations rarely exceed 1 µmol/l because of limited absorption and rapid elimination. Flavanones and isoﬂavones are the ﬂavonoids with the best bioavailability proﬁles, and plasma concentrations may reach 5 µmol/l. However, the distribution of these substances is restricted to citrus fruit and soybean. Finally, hydroxycinnamic acids are found in a wide variety of foods, often at high concentrations, but esteriﬁcation decreases their intestinal absorption. As a general rule, the metabolites of polyphenols are eliminated rapidly from plasma, which indicates that consumption of plant products on a daily basis is necessary to maintain high concentrations of metabolites in the blood (Scalbert and Williamson, 2000; Manach et al., 2004).

The consumption of carotenoid-rich foods such as carrots has been associated with a decrease in the risk of developing certain types of degenerative and chronic diseases. Processing of food and the interaction of carotenoids with lipophilic food components or ingredients may modify the amount of the pigments released from the food matrix, and therefore potentially increase or decrease their bioavailability. Carotenoids derived from food have been detected in human and rat plasma; however, carotenoids in foods are much less available than are purified sources of β-carotene. The relative bioavailability of β-carotene from vegetables compared with purified β-carotene ranged between 19 and 34% for carrots (Micozzi et al., 1992; Nicolle et al., 2003). Hornero-Méndez and Mínguez-Mosquera (2007) found that food processing and mainly lipid content improved carotenoid bioaccessibility from carrots significantly.
and therefore might increase bioavailability in humans. The amount of dietary fat required to ensure carotenoid absorption seems to be low (3–5 g/meal), although it depends on the physicochemical characteristics of the carotenoids ingested (Van Hof et al., 2000). The presence of dietary fibre in vegetables and fruit may explain in part the lower bioavailability of carotenoids from plant foods. It has been suggested that fibre interferes with micelle formation by partitioning bile salts and fat in the gel phase of dietary fibre. Some studies have shown that carotenes from cooked carrots are absorbed more efficiently than are those from raw carrots, because the plant cells are disrupted during preparation and because the mechanical homogenization of carrots allows greater efficiency of absorption (Van Hof et al., 2000).

Although the potency of the exogenous peptides is lower than that of endogenous peptides or peptide-based drugs, they may well have physiological effects, because food proteins are usually ingested in fairly large amounts. To exert physiological effects in vivo, bioactive peptides must be released during intestinal digestion or/and be resistant to digestive enzymes, and then reach their target sites at the luminal side of the intestinal tract or, after absorption, in the peripheral organs. The gastrointestinal tract of humans contains a number of enzymes involved in the hydrolysis of proteins and peptides and they are located in a number of sites. It is important to recognize that peptidase enzymes never occur alone. Throughout the gastrointestinal tract there is always a mixture of peptidases working synergistically. The main event in the intraluminal digestion of proteins consists of cleavage of polypeptides by pancreatic proteases, such as trypsin, chymotrypsin, elastase and carboxypeptidase. Furthermore, the microorganisms of the colon produce large numbers of peptidase enzymes, in considerable quantities, that participate in protein digestion. The small intestine is the principal site of absorption. Di- and tripeptides, such as immunopeptides and several ACE inhibitors, may pass across the intestine, in quantitatively significant amounts, to reach peripheral target sites. After absorption in the intestinal tract, serum peptidases can hydrolyse the peptide bonds further. Resistance to peptidase degradation may, in fact, be a prerequisite for a physiological effect following oral ingestion and/or the intravenous infusion of biologically active peptides/hydrolysates (Pihlanto and Korhonen, 2003).

### 12.3.2 Cancer studies

Carrots are one of the plant products that have shown the strongest protective anticancer effects. Carrots contain β-carotene, dietary fibres and polyphenols, which have been shown in experimental studies to be potentially anticarcinogenic. Antioxidant properties of carotenoids are thought to be at least partly responsible for protecting against colon cancer. There are large amounts of in vitro data supporting this hypothesis but there is little known about the antioxidant effects of carotenoid-rich food in vivo, particularly in the gastrointestinal tract. Briviba et al. (2004) found that consumption of carotenoid-rich juices for 2 weeks increased the carotenoid level in plasma and faeces; the antioxidant capacity of low-density lipoprotein tended to be increased by only approximately 4.5%, and lipid peroxidation in men’s plasma and faeces was not affected. Thus, processes other than lipid peroxidation could be responsible for the preventive effects of tomatoes and carrots against colon cancer. A human intervention study with carotenoid-rich juices led to only minor changes in luminal biomarkers relevant to colon carcinogenesis (Schnäbele et al., 2008). The results by Nyberg et al. (1998) supported evidence linking a diet rich in vegetables and non-citrus fruit with decreased lung cancer risk and suggested that, among vegetables, carrot consumption was the most important component or marker for this effect in Sweden.

A recent in vitro study aiming to screen for potential health-promoting compounds from vegetables showed that falcarniol, but not β-carotene, could stimulate differentiation of primary mammalian cells in concentrations between 0.004 and 0.4 μM falcarniol. Therefore, falcarniol appears to be one of the bioactive components in carrots and related...
vegetables that could explain their health-promoting properties, rather than carotenoids or other types of primary and/or secondary metabolites. This hypothesis is further supported by recent studies on the bioavailability of falcarinol in humans. The carrot and falcarinol treatments showed a significant tendency to reduce numbers of (pre)cancerous lesions with increasing size of lesion in rat models (Christensen and Brandt, 2006). Traditionally, polyacetylenes in food are generally considered undesirable toxins but this belief may need to be revised, and perhaps these compounds may instead be regarded as important nutraceuticals. The major challenge with regard to bioactive polyacetylenes is to test their health-promoting effects in vivo in clinical as well as in further preclinical studies.

12.3.3 Cardiovascular diseases

The biological activities of polyphenols have often been evaluated in vitro on pure enzymes, cultured cells or isolated tissues, by using polyphenol aglycones or some glycosides that are present in food. Very little is known about the biological properties of the conjugated derivatives present in plasma or tissues because of the lack of precise identification and commercial standards. Polyphenols exhibit strong in vitro antioxidant activity with heart disease-related lipoprotein. Since in vivo oxidation of low density lipoproteins (LDL) appears to be a major cause of heart disease, it is possible that chlorogenic acid and other polyphenols may also lessen such disease. Lazarov and Werman (1996) found that the consumption of potato peel induced a lowering of cholesterol in rats. They ascribed this result to the fibre content in the peel. However, it is likely that the polyphenols and other antioxidants as well as the glycoalkaloids in the peel contributed to the observed hypcholesterolaemia. Han et al. (2006) investigated the antioxidant effects of purple anthocyanin-rich potato flakes in vivo and found that the flakes enhanced the serum Trolox equivalent antioxidant capacity values and suppressed hepatic thiobarbituric acid reactive substances (TBARS) levels in rats. Shindo et al. (2007) showed that administering anthocyanin-rich colours from purple sweet potato and red radish decreased the blood pressure of spontaneously hypertensive rats (SHR). Robert et al. (2006, 2008) suggested that consumption of cooked potatoes (consumed with skin) might enhance antioxidant defence and improve lipid metabolism. These effects limit oxidative stress and reduce the risk of developing the associated degenerative diseases, including cardiovascular disease, and could have potential in cardiovascular disease prevention.

The effect of carrot consumption on cholesterol metabolism has been studied. Robertson et al. (1979) found that 200 g of raw carrot eaten each day for 3 weeks reduced serum cholesterol significantly by 11%, increased faecal bile acid and fat excretion by 50% and increased stool weight modestly by 25%. The latter effect was attributed to carrot fibre. Nicolle et al. (2003, 2004) found that carrot consumption modified cholesterol absorption and bile acid excretion and increased antioxidant status. It is likely that these effects could be due to the synergistic effect of fibre and associated antioxidants.

12.3.4 Other beneficial effects

Gastrointestinal hormones such as cholecystokinin (CCK) are important in the regulation of food intake and in maintaining energy homeostasis. The role of CCK as a major endocrine determinant in food intake regulation is well documented. Studies in animals and humans using different protein sources suggest that, in order to stimulate CCK secretion efficiently, the protein needs to be hydrolysed to short-chain peptides and amino acids (Schwartz et al., 2000). Discovering either specific protein hydrolysates or other food-grade bioactive compounds with optimized CCK-releasing properties is an interesting target in the development of functional food products for weight management purposes. Foltz et al. (2008) found that potato hydrolysates induced CCK-releasing properties and stimulated CCKR expression in enteroendocrine cells.
Potato tubers are an extraordinarily rich source of protease inhibitors, which represent 25–30% of potato juice protein. As well as containing serine, cysteine and aspartage proteinases, they also contain a metal-containing carboxypeptidase and pancreatic proteases. In 11 lean subjects, the addition of proteinase inhibitors extracted from potatoes was studied. Reduced energy intake and increased CCK release was found, suggesting proteinase inhibition might have therapeutic potential for reducing food intake (Hill et al., 1990). Potato protein fractions were capable of inhibiting a large part of the high proteolytic activity in faeces from patients with gastrointestinal resections and infants (in vitro) and prevented experimental protease-induced dermatitis (in vivo) (Ruseler-van Embden et al., 2004).

Free radicals and reactive oxygen species play a central role in liver disease pathology and progression. Accordingly, dietary antioxidants have been proposed as therapeutic agents to counteract liver damage. Studies by Singh et al. (2008) indicated that potato peel extract treatment had a potent protective effect against oxidative stress and acute liver damage induced by CCl₄ in rats, as revealed by the remarkable decrease in hepatic malondialdehyde (MDA) content accompanied with enhanced reduced glutathione (GSH) and superoxide dismutase (SOD) activities.

### 12.4 Preharvest and Postharvest Continuum

Climatic conditions, especially temperature and light intensity, have a strong effect on nutritional quality. Soil type, irrigation, fertilization and other cultural practices influence the water and nutrient supply to the plant, which affect the composition and quality attributes (appearance, texture, taste and aroma) of the harvested plant parts. Maturity at harvest, harvesting method and extent of physical injuries have an influence on the content of several components in the tubers. In addition, many biochemical and physical changes, including variations in the concentration of bioactive compounds, occur during storage. A better understanding of how changes in the health-promoting compounds vary with preharvest factors and postharvest storage conditions will allow optimization of the bioactive components in these products at the time of their ingestion.

#### 12.4.1 Potato

Preharvest considerations must include cultivar selection, as individual cultivars have significantly different proportions of bioactive components. The differences in specific antioxidant activity observed in several studies suggest that potato genotype and harvest location influence the accumulation of polyphenols in different quantities and/or types of polyphenols. Pigmented potato cultivars are an especially rich source of anthocyanins. Jansen and Flamme (2006) analysed 27 potato cultivars and four breeding clones grown in field plots for tuber production. The analysis revealed considerable differences in the amounts of anthocyanins between the cultivars/breeding clones. Interestingly, two different rates of nitrogen fertilization, at 100 and 200 kg/ha, had no significant effect on the pigment content of the potatoes. In dry matter, starch and protein contents, the coloured potato cultivars/breeding clones were comparable with traditional cultivars. Reyes et al. (2005) found that the anthocyanin content of potatoes ranged from 0.11 to 1.74 mg cyanidin-3-glucoside/g FW in purple-fleshed tubers and from 0.21 to 0.55 mg cyanidin-3-glucoside/g FW in red-fleshed tubers, and showed large variation between genotypes and locations. The antioxidant capacity of purple- and red-fleshed potatoes ranged from 0.513 to 1.426 mg Trolox equivalents/g FW, and phenolic compounds, especially anthocyanins, were responsible for the antioxidant capacity. Lachman et al. (2009) analysed total anthocyanins and individual anthocyanidins in 15 red- and purple-fleshed potato cultivars produced in five different locations in the Czech Republic. A significantly different abundance of anthocyanins was found in the individual cultivars. Different site conditions affected polyphenol content in tubers significantly. Increased height above sea level, higher annual sum of precipitation and lower annual temperatures caused a
higher level of antioxidant activity and total anthocyanins (Hamouz et al., 2007; Lachman et al., 2009).

Content of polyphenols is dissimilar at different stages of tuber maturity. Lewis et al. (1999) observed that, as tubers reached maturity, the concentration of anthocyanins at the stem end was always higher than at the bud end. In addition, the observed differences were more marked in smaller (50–70 g) tubers. The polyphenol content decreased with tuber growth and maturity (Reyes et al., 2004).

Effective control of sprouting is a fundamental requirement of potato storage. This can be accomplished by very low temperature storage and the use of sprout suppressants (namely, maleic hydrazide (MH) and chlorpropham (CIPC)), which include the natural plant-growth regulator, ethylene. Potatoes are low producers of ethylene (< 0.1 μl/kg/h at 20°C) but, since the 2003 publication of a commodity approval, exogenous application of ethylene has been approved for use as a postharvest sprout suppressant (Terry, 2008). It is apparent that ethylene treatment can extend the storage life of potatoes; however, the biological mechanism(s) by which this occurs is currently unknown. To date, there has been a scarcity of research carried out that has profiled the detailed biochemical and rheological changes induced by ethylene treatment during storage and compared these against physiological effects such as sprout inhibition. Storage of potato tubers at different temperatures (2, 10 and 20°C) and fluctuating temperature (from 2 to 20°C) did not affect the total accumulation of polyphenols. Similarly, ethylene treatments of air-stored samples led to no significant differences in either anthocyanin or phenolic content. Only tubers with low initial anthocyanin levels treated with methyl jasmonate showed 60% anthocyanin accumulation, and wounding induced the accumulation of phenolic compounds (Reyes and Cisneros-Zevallos, 2003). Jansen and Flamme (2006) found that anthocyanins were preserved in stored tubers without the risk of any degradation over a longer period of time.

Total amounts of GAs are influenced by environmental factors such as soil and climate. The relative concentration of GA falls with increasing tuber size and maturation. Immature tubers contain 28–38 mg α-solanine and 66–84 mg α-chaconine per kg, whereas mature tubers contain 4–10 mg α-solanine and 11–30 mg α-chaconine per kg. Storage conditions, especially light and temperature, are responsible mainly for increases in solanine during marketing. Although the GA content can increase in the dark, the rate of formation is only about 20% of that in the light (Friedman, 2006; Nema et al., 2008).

Studies have shown consistently that the developmental stage and physiological age of potato tubers are associated with significant alterations in the protein composition of tubers. Lehesranta et al. (2006) observed significant changes in the potato tuber proteome during tuber development and physiological aging, but different tissues or fractions of the tuber were not compared. Qualitative and quantitative changes have been detected in protein content during the development of tubers (Borgmann et al., 1994) and dormancy (Espen et al., 1999) and after dormancy (Desires et al., 1995a,b). Differences in enzymatic activities were confined to different parts of the potato tuber at different physiological stages. These changes were also associated with differences in the production of ACE-inhibitory activity (Mäkinen et al., 2008).

12.4.2 Carrots

β-Carotene content in carrots is affected by various environmental factors such as growing conditions, processing and storage. Purple and orange carrot have been found to contain the highest amount of total carotenoids; only trace amounts of these compounds were detected in yellow carrot and none was found in white carrot (Alasalvar et al., 2001; Surles et al., 2004). Lee (1986) found that the provitamin A content increased during maturation and then decreased. Moreover, during storage at 2°C and 90% relative humidity, the total carotene content increased during the first 100-day storage but decreased thereafter. Sanitation procedures affect carotenoid content during storage. Washing
carrots with acidified sodium chlorite retained higher carotenoid content than seen in carrots washed with water or other sanitizers (Ruiz-Gruz et al., 2007).

During handling and distribution of fresh carrots from field to consumer, a less desirable taste can often develop. Bitterness and harshness are two usual sensory characteristics mentioned in connection with less desirable taste and flavour of carrots. Part of the reduction in taste quality could be due to exposure of carrots to ethylene during transport or storage. The production of the bitter compound, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (6-methoxymellein), after ethylene exposure is well documented. Different kinds of mechanical stress (e.g. dropping on hard surfaces, harsh handling during harvest, packing or slicing) are known to enhance ethylene production as well as respiration, and cause splitting of the carrot roots.

**12.5 Future Research Needed**

The occurrence of many natural biomolecules in plants, such as those found in potatoes and carrots, has been shown in several studies during the last decades. Several bioactive food components are now well established; their structure and concentration, as well as in vitro activities are known. However, the primary interest of bioactive components is their physiological functions in the human body after consumption. For example, the total antioxidant activity of food, including the bioactive components, could provide a potentially useful measure of the physiological function of that food. The most important future research needs related to bioactive components are summarized hereunder:

- Screening for potential bioactivity among proteins in potato.
- To use by-products from starch manufacture as a starting material to produce novel products, including health-promoting ingredients such as fibre, polyphenols, proteins and peptides.
- Study of the effects of conventional and novel processing technologies on bioactivities.
- Study of the interactions of polyphenols, peptides and fibres with other food components during processing and the effects of these interactions on bioactivity.
- Basic research on allergenicity and toxicity of the compounds.
- Evaluation of the efficacy of polyphenols, proteins and peptides in animal model and human clinical studies per se and in food systems.

**12.6 Conclusions**

An increasing number of studies have revealed that potatoes and other root crops such as carrots and sweet potatoes are good sources of various bioactive components. These components are mainly the secondary metabolite products, such as polyphenols and glycoalkaloids in potato and carotenoids, polyacetylenes and polyphenols in carrot. The influence of genetic and environmental factors on the concentration of polyphenols, glycoalkaloids and carotenoids needs greater attention. The activity of these compounds has been shown with various in vitro methods. Potatoes are also a good source of bioactive proteins, since they have shown antioxidant as well as enzymatic and inhibitory activities. Furthermore, the digestion of potato proteins produces hydrolysates with satiety and antihypertensive activities in vitro. Potato and carrot are promising sources of components that have beneficial effects on cardiovascular health. The data available suggest that carrot polyacetylenes may inhibit the growth of cancer cells, and that potato proteins and hydrolysates can have satiety-promoting effects. Further studies are needed to establish the in vivo efficacy of these components, as well as their long-term effects when administered orally on a regular basis. Based on present knowledge, potato could and perhaps should be included as one of the major important sources of bioactive compounds. Furthermore, potato and carrot components provide a highly interesting base to be used as active ingredients for the formulation of functional health-promoting foods.
References


Potato and Other Root Crops


13  

Prunus

Ariel R. Vicente, George A. Manganaris, Luis Cisneros-Zevallos and Carlos H. Crisosto

13.1 Introduction

The genus *Prunus* includes about 430 species of deciduous or evergreen trees and shrubs naturally widespread throughout temperate regions. It belongs to the subfamily Prunoideae, within the Rosaceae family. While some species do not yield edible fruit and are used for decoration, others are grown commercially for fruit and 'nut' production. Most of these species are originally from Asia or Southern Europe, such as peach (*Prunus persica*), nectarine (*P. persica* var. nectarina), European plum (*P. domestica*), Japanese plum (*P. salicina*), apricot (*P. armeniaca*), mume or Japanese apricot (*P. mume*), sweet cherry (*P. avium*), sour cherry (*P. cerasus*) and almond (*P. amygdalus*).

The fruit of these species is defined botanically as a 'drupe' (Brady, 1993). This name is derived from the Latin word *druppa*, which means overripe olive. Drupes mostly develop from flowers with superior ovaries having a single carpel. The fruit usually have a clear ventral suture, do not retain floral residues next to the pedicel and are characterized by a membranous exocarp, with an outer fleshy mesocarp consisting mainly of parenchyma cells (Romani and Jennings, 1971). The mesocarp surrounds a shell (the pit or stone) of hardened endocarp with a seed inside and, due to this, *Prunus* species are also referred to as 'stone fruit'. Unlike almonds, in which the consumed portion is the seed within the pit, the edible part in most stone fruits includes the mesocarp, and eventually the exocarp. Growth of stone fruit usually shows a double sigmoid pattern, with an initial stage after fertilization characterized by active cell division, followed by a phase in which all the parts of the ovary besides the embryo and endosperm grow. Later on, whole fruit growth is decelerated, while seed development and endocarp lignification occur and, lastly, mesocarp expansion resumes (Romani and Jennings, 1971).

*Prunus* species cultivation is common in temperate regions throughout the globe. The most popular fruit within this group are by far peaches/nectarines and plums, with annual productions of around 17.5 and 9.7 million tonnes (Mt), respectively, in 2007 (Table 13.1). Apricot production is around 3 Mt/year, while almonds and cherries account for 2 Mt annually (Table 13.1). The main world producing countries of stone fruit include China, the USA, Italy, Spain and Turkey. Serbia and Syria are also important producers of plums and almonds, respectively.

Probably in association with the differences in the economical importance of *Prunus* species, much more research has been devoted historically to peaches and nectarines (the biochemistry and physiology of...
peaches and nectarines are similar, with the main difference between them being the appearance of the skin, with more variation within each of the groups). Cherries and plums have been studied to a lesser extent, and some other species, such as apricot and sour cherry, have received little attention. The biochemical and physiological changes associated with ripening of these fruits have been reviewed (Romani and Jennings, 1971; Brady, 1993). Some areas in which there have been significant advances include: (i) evaluation of the influence of orchard practices on fruit yield and quality (La Rue and Johnson, 1989; Crisosto et al., 1997); (ii) development of proper harvest indices and definition of optimal harvest operations (Crisosto et al., 1995; Crisosto and Mitchell, 2002); (iii) determination of optimal cooling strategies, temperatures for storage and transportation and modified atmospheres (Crisosto et al., 2009); and (iv) the characterization of the physiological basis of some disorders, such as internal breakdown, mealiness and flesh reddening (Brummell et al., 2004a,b; Lurie and Crisosto, 2005; Manganaris et al., 2008).

Stone fruit are highly appreciated due to their unique aesthetic and organoleptic characteristics. Traditionally, attributes evaluated in relation to fruit quality include mostly appearance, sugars and acids. However, the fruit also contain a myriad of phytochemicals that, though present in relatively low concentrations, have a key role in overall quality. Some of these chemicals might be the main determinants of colour and flavour. In addition, many of these compounds have been found to play protective roles against some human diseases (Ames et al., 1993; Rice-Evans and Miller, 1996; Olsson et al., 2004). When ingested regularly and in significant amounts as part of the diet, these metabolites may have noticeable long-term physiological effects (Espín et al., 2007). Increasing the consumption of fruit and vegetables results in a significant increase of plasma antioxidants (Cao et al., 1998). The metabolites that have received more attention include ascorbic acid, tocopherols, tocotrienols, carotenoids and phenolics (Robards, 2003). It is currently recommended to have five to ten servings of fruit and vegetables daily (http://www.mypyramid.gov), and in the past years there have been efforts to develop educational and promotional programmes related to fruit and vegetable consumption (http://www.5aday.nhs.uk/topTips/default.html).

Studying plant phytochemicals is not an easy endeavour, especially for carotenoids and phenolics, which include thousands of different compounds, commonly present at relatively low concentrations. In the past 20 years, the improvement in analytical techniques (based mostly on HPLC coupled with mass spectrometry detection) has allowed rapid progress in the identification, quantification and overall study of fruit phytochemicals, with special emphasis on those showing bioactivity.

Fresh Prunus species are significant contributors of bioactive compounds to the diet during spring and summer, although the increase in year-round supply in the developed world has lessened these seasonal eating habits. Most Prunus fruit and seeds are commonly used for processing, including jam production, canning, drying or roasting, and regularly are consumed year-round. Black plum varieties are among fruit with the highest antioxidant capacity and are very rich.

### Table 13.1. Production of Prunus species in 2007 (FAOSTAT, 2009).

<table>
<thead>
<tr>
<th></th>
<th>Production (t)</th>
<th>Main producing countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
<td>2,065,489</td>
<td>USA, Spain, Italy, Syria</td>
</tr>
<tr>
<td>Apricot</td>
<td>3,067,952</td>
<td>Turkey, Iran, Italy</td>
</tr>
<tr>
<td>Cherry</td>
<td>1,995,751</td>
<td>Turkey, Iran, USA</td>
</tr>
<tr>
<td>Peach and nectarine</td>
<td>17,502,245</td>
<td>China, Italy, Spain, USA</td>
</tr>
<tr>
<td>Plum</td>
<td>9,719,451</td>
<td>China, Serbia, USA</td>
</tr>
<tr>
<td>Total</td>
<td>34,350,888</td>
<td></td>
</tr>
</tbody>
</table>

Prunus
in phenolic compounds, while apricots are high in carotenoids. Cherries and almonds are also rich in phenolics and have an oxygen radical absorbing capacity (ORAC) similar to that found in strawberry or raspberry (which are commonly recognized as good sources of antioxidants; see Chapters 14 and 15 of this volume). Although peaches and nectarines do not rank top among fruit in terms of antioxidant capacity or content of bioactive compounds, they still do have moderate levels of carotenoids and ascorbic acid. Due to their popularity, either in fresh or processed form, they may contribute significantly to global intake of bioactives. Vinson et al. (2001) estimated that peach contribution of phenolics to the diet, despite the modest level of phenolic compounds relative to plums and cherries, is highest among stone fruit. Despite these general features, both the qualitative and quantitative profiles of these compounds in stone fruit vary markedly depending on the variety (Tomás Barberán et al., 2001; Dalla Valle et al., 2007; Vizzotto et al., 2007; Díaz-Mula et al., 2008; Ruiz et al., 2008). Orchard practices, growing location, season, environmental conditions, postharvest management and processing operations may also determine great changes in the levels of phytochemicals. In this chapter, we describe the antioxidants and bioactive compounds in Prunus species and we analyse the main factors affecting their levels.

### 13.2 Antioxidants

The ORAC assay has been applied routinely to determine antioxidant capacity and currently there are databases generated for different foods (Wu et al., 2004a; USDA, 2007a). The ORAC values of some common fruit are shown in Fig. 13.1. Blackcurrants and cranberries rank at the top, with ORAC values of around 10,000 μmol Tropax equivalents (TE)/100 g. Blueberries, which have been recognized repeatedly for their high content of antioxidants, have ORAC comparable to that observed in plums (Fig 13.1). Cherries also show relatively high ORAC, not far from that found in strawberries.

An original limitation of the ORAC method was the inability to determine both hydrophilic and lipophilic antioxidants. A modified ORAC method was developed for that purpose. Hydrophilic and lipophilic antioxidants were extracted in polar solvent and hexane, respectively. The use of randomly methylated β-cyclodextrin as a solubility enhancer allowed the use of the same peroxyl-free radical source (Wu et al., 2004b). This method has the advantage that similar assay conditions and standards are used for both the hydrophilic and lipophilic assays and could be used to determine a total ORAC value for a given sample.

Table 13.2 shows the hydrophilic and lipophilic ORAC values for the main cultivated Prunus species. As mentioned, there is a broad range of antioxidant capacities among stone fruit. Plums are by far the stone fruit that rank at the top, with ORAC values of 6239 μmol TE/100 g. Black plum varieties have even higher ORAC than several fruit commonly recognized as antioxidant-rich fruit (e.g. blueberries). The values in Table 13.2 have to be interpreted cautiously since broad variation for a given fruit has been reported, depending on the variety evaluated. In a survey of eight plum cultivars, Blackamber showed highest antioxidant capacity, followed relatively closely by cvs. Golden Globe and Larry Ann and, with much lower levels, by cvs. TC Sun, Sonogold, Angeleno, Golden Japan and Black Diamond (Díaz-Mula et al., 2008). Almonds and cherries also have high ORAC (3361 and 4454 μmol TE/100 g, respectively). The ORAC reported for peach, nectarine and apricot are usually lower than those found in other Prunus species (Wu et al., 2004a).

In all cases, it is seen clearly that the lipophilic contribution to total ORAC is low. This suggests that the relative relevance of phenolic compounds, ascorbic acid and possibly xanthophylls in the antioxidant capacity of stone fruit is higher than that of carotenes and tocopherol (however, whether or not the conditions for measurement of lipophilic antioxidant are distinct from those occurring in vivo, and that the real antioxidant contribution of these metabolites is underestimated, is not known). Stone fruit total antioxidant capacity
Fig. 13.1. Total oxygen radical absorbance capacity (ORAC) of different fruit. The black columns highlight Prunus species (USDA, 2007a).
Table 13.2. Hydrophilic, lipophilic and total oxygen radical absorbance capacity (ORAC) of different Prunus species (adapted from USDA, 2007a, and Wu et al., 2004a).

<table>
<thead>
<tr>
<th></th>
<th>Hydrophilic ORAC (μmol TE/100 g)</th>
<th>Lipophilic ORAC (μmol TE/100 g)</th>
<th>Total ORAC (μmol TE/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
<td>4282</td>
<td>172</td>
<td>4454</td>
</tr>
<tr>
<td>Apricot</td>
<td>1309</td>
<td>32</td>
<td>1341</td>
</tr>
<tr>
<td>Cherry, sweet</td>
<td>3344</td>
<td>17</td>
<td>3361</td>
</tr>
<tr>
<td>Nectarine</td>
<td>720</td>
<td>29</td>
<td>749</td>
</tr>
<tr>
<td>Peach</td>
<td>1813</td>
<td>50</td>
<td>1863</td>
</tr>
<tr>
<td>Plum</td>
<td>6222</td>
<td>17</td>
<td>6239</td>
</tr>
<tr>
<td>Plum, black</td>
<td>7301</td>
<td>38</td>
<td>7339</td>
</tr>
</tbody>
</table>

Note: TE = Trolox equivalents.

(TAC) have shown high positive correlations with total phenolics and antioxidants, suggesting that this group of compounds is the predominant contributor to TAC (Serrano et al., 2005; Drogoudi et al., 2008).

The distribution of antioxidants also varies within the fruit. In plum and cherry, TAC is always higher in the skin than in the flesh (Serrano et al., 2005; Díaz-Mula et al., 2008). In peach, removal of the peel also results in a significant loss of total antioxidant capacity (Remorini et al., 2008).

13.3 Main Bioactive Compounds in *Prunus* Species

13.3.1 Carotenoids

Carotenoids comprise a group of over 600 secondary plant metabolites with several functions in plants (Gross, 1991). They are essential structural components of the photosynthetic apparatus and contribute to light absorption in regions of the spectrum where chlorophyll absorption is low (Cuttriss and Pogson, 2004). They also provide protection against photo-oxidation. Carotenoids are responsible for the distinctive yellow and orange colours of the pulp and peel of most *Prunus* species. They are liposoluble terpenoids due to their relatively long (40 C) hydrocarbon structure (Sandmann, 2001). Carotenoids are synthesized *de novo* from geranyl-geranyl diphosphate by all photosynthetic organisms and accumulated in plastids (Rodríguez-Amaya, 2001). Then the enzyme phytoene synthase catalyses the condensation of two molecules of geranylgeranyl pyrophosphate to form phytoene, a colourless carotenoid (Gross, 1991). After that, double bonds are added sequentially to generate compounds with higher levels of conjugated double bonds (3 in phytoene, 5 in phytofluene, 7 in ζ-carotene, 9 in neurosporene and 11 in lycopene). Interestingly, the addition of double bonds is related to carotenoid spectral properties. Terpenoid polyenes absorb light in the UV and visible regions. Most show three absorbance maxima and the position of these peaks usually is related to the number of conjugated double bonds (as they increase, carotenoid colour shifts from yellow to orange and red) (Gross, 1991). Carotenoids can be subdivided into two groups, carotenes containing only carbon and hydrogen such as α-carotene, β-carotene and lycopene, and the oxygenated derivatives or xanthophylls such as lutein, cryptoxanthin, zeaxanthin and violaxanthin (Rodríguez-Amaya, 2001).

One of the main important features of carotenoids is being precursors of vitamin A (Sandmann, 2001). Humans depend on dietary carotenoids for making their retinoids, which are crucial for normal vision (Kopsell and Kopsell, 2006). Structurally, vitamin A (retinol) is basically a half molecule of β-carotene with an extra molecule of water, and retinoids are generated on cleavage by dioxygenases. Other carotenoids with at least one unsubstituted β-ionone ring (such as γ-carotene, α-carotene and cryptoxanthin) are
precursors of vitamin A. In contrast, those carotenoids that are acyclic, or with β rings with hydroxy, epoxy and carbonyl substituents, do not have provitamin A activity (Rodríguez-Amaya, 2001). Retinol equivalents (µg RE) from fruit carotenoids are calculated as µg of β-carotene/12 or as µg of other provitamin A carotenoids/24, to account for differences in the absorption and biotransformation of carotenoids into retinol.

Besides being precursors of vitamin A, some benefits of carotenoids have been associated with their antioxidant properties (Bendich, 1993). The conjugated double bonds are not only responsible for the colour of carotenoids but also are main determinants of their antioxidant properties. Carotenoids have been shown to react with singlet oxygen and peroxyl radicals (Stahl and Sies, 1997). The different group members can vary on their antioxidant capacity in humans. Larger conjugated systems, such as astaxanthin, are known to have a higher antioxidant activity (Miki, 1991). Lycopene also has high antioxidant capacity, surpassing that of β-carotene (Di Mascio et al., 1989). The fate of most carotenoids on consumption, besides lycopene and β-carotene, is not completely known (Rao and Rao, 2007). The bioavailability of carotenoids from plant foods is variable and depends on the type of compounds present in the food, release from the food matrix, absorption in the intestinal tract, and the nutritional status of the ingesting host, as well as the presence of other compounds in the food matrix (Rao and Rao, 2007). Recent studies have shown that absorption of carotenoids increases when they are ingested with dietary lipids. Processing activities usually increase bioavailability through increased release of bound carotenoids from the food matrix; after that, carotenoids are assimilated and oriented into lipid micelles, incorporated into chylomicrons, and eventually delivered to the liver (Kopsell and Kopsell, 2006).

From the whole group of more than 600 known carotenoids only 40 are present in a typical human diet, and just five of them (β-carotene, α-carotene, lutein, cryptoxanthin and lycopene) represent close to 90% (Gerster, 1997). The bicyclic β-carotene is the most widespread of all carotenoids in foods (Rodríguez-Amaya, 2001). In Prunus species, β-carotene is also by far the most abundant carotenoid; α-carotene is also found in apricots but at lower concentration. The most abundant xanthophylls present in most Prunus fruit is lutein, but β-cryptoxanthin is also present in orange-fleshed commodities such as apricot, peach and nectarine, and to a lower extent in plum (Tourjee et al., 1998; Rodríguez-Amaya, 2001; USDA, 2009). In this group, apricots show the highest levels of carotenoids and provitamin A, followed by cherry (Table 13.3). Peaches, nectarines and plums have lower concentrations of carotenoids. However, the variation among varieties is large. For instance, when studying eight plum varieties, Díaz-Mula et al. (2008) found fourfold variation in total carotenoid content. In apricot, Ruiz et al. (2005) found that total carotenoid content ranged from 1500 to 16,500 µg/100 g, depending on the variety being considered. Carotenoids were not distributed evenly in the food itself and various investigators found that they were usually more concentrated in the peel than in the pulp. In plums and peaches, the ratio of carotenoids in the peel to the pulp was around five (Díaz Mula et al., 2008; Remorini et al., 2008).

Though it has been suggested that breeding programmes might consider the selection of lines based on the content of bioactive compounds, using traditional analytical techniques for screening might be impractical. Yellow-flesh peaches have a higher concentration of carotenoids than light-coloured ones. Ruiz et al. (2005) reported that in apricot, carotenoid content showed high and positive correlation with the colour (hue) of both flesh and pulp. In addition, the authors developed a model to predict carotenoid content from single non-destructive colour measurements (Ruiz et al., 2008).

### 13.3.2 Ascorbic acid

Vitamin C is an essential nutrient for humans and a small number of other mammalian species (Hancock and Viola, 2005). Ascorbic acid plays an important role in hydroxylation reactions (e.g. in the synthesis of collagen,
Table 13.3. Carotenoid content in *Prunus* species (USDA, 2009).

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>β-CAR (µg RE/100 g)</th>
<th>α-CAR (µg/100 g)</th>
<th>LUT-ZEA (µg/100 g)</th>
<th>β-CRY (µg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Apricot</td>
<td>96</td>
<td>1094</td>
<td>19</td>
<td>89</td>
</tr>
<tr>
<td>Cherry, sweet</td>
<td>64</td>
<td>770</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>Nectarine</td>
<td>17</td>
<td>150</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td>Peach</td>
<td>16</td>
<td>162</td>
<td>0</td>
<td>91</td>
</tr>
<tr>
<td>Plum</td>
<td>17</td>
<td>190</td>
<td>0</td>
<td>73</td>
</tr>
</tbody>
</table>

Notes: β-CAR = β-carotene; α-CAR = α-carotene; LUT-ZEA = lutein + zeaxanthin; β-CRY = β-cryptoxanthin; RE = retinol equivalents 1 µg RE = 12 µg β-carotene or 24 µg of other carotenoids having provitamin A activity (in this case, α-car and β-cry).

Table 13.4. Ascorbic acid content in *Prunus* species (USDA, 2009).

<table>
<thead>
<tr>
<th>Ascorbic acid (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
</tr>
<tr>
<td>Apricot</td>
</tr>
<tr>
<td>Cherry, sweet</td>
</tr>
<tr>
<td>Nectarine</td>
</tr>
<tr>
<td>Peach</td>
</tr>
<tr>
<td>Plum</td>
</tr>
</tbody>
</table>

13.3.3 Vitamin E

Vitamin E is liposoluble and exists in eight different forms (four tocopherols and four tocotrienols). All the isomers have aromatic rings and are named alpha (α), beta (β), gamma (γ) and delta (δ). The designation of the isomers is related to the number and position of methyl groups in the molecule ring. From all the possible forms, α-tocopherol is the most active. The main function of these compounds seems to be as antioxidants. α-Tocopherol has also been implicated in other biological processes, such as inhibition of some protein kinases, prevention of platelet aggregation, enhancement of vasodilation and modulation of the activities of enzymes associated with the immune system (Food and Nutrition Board, Institute of Medicine, 2000; Traber, 2001). Foods rich in vitamin E include vegetable oils, nuts and avocado. Broccoli and leafy vegetables have lower tocopherol content than fat-rich products, but they are good sources compared with other fruit and vegetables.
α-Tocopherol content in *Prunus* fruit varies from 0.07 to 26 mg/100 g (Table 13.5). Almonds are extremely rich in vitamin E, with 60 g covering the daily recommended intake (22 IU or 15 mg α-tocopherol).

### 13.3.4 Phenolic compounds

Plants produce thousands of phenolic compounds as secondary metabolites. They are synthesized via the shikimic acid pathway. This is the biosynthetic route to the aromatic amino acids and is restricted to microorganisms and plants (Robards, 2003). Phenolics contribute greatly to the sensory qualities (colour, flavour, taste) of fresh fruit and their products (Kim et al., 2003a,b). Astringency generally is recognized as a loss of lubrication, a feeling of extreme dryness in the palate believed to be associated with the interaction of polyphenols with praline-rich proteins present in the saliva (Haslam, 2007). Phenolic compounds have been associated with ecological functions. Some benefits associated with phenolic compound consumption have been related to their antioxidant properties (Tall et al., 2004). Phenolic compounds may exert their antioxidant activity by different mechanisms. They may act by direct-scavenging free radicals (Kris-Etherton et al., 2002). Some phenolics can prevent the formation of free radicals by chelating copper and iron. Finally, they can regenerate other antioxidants such as tocopherol (McAnlis et al., 1999). Fruit, vegetables and beverages are the major sources of phenolic compounds in the human diet (Balasundram et al., 2006). Some *Prunus* fruit, such as red and black plum varieties, cherries and almonds, are very good sources of phenolic compounds (Vinson et al., 2001; Wu et al., 2004a) (Table 13.6). Sour cherries were reported to have a higher level of total phenolics than sweet cherries (Kim et al., 2005). Peach, nectarine and apricot have lower total phenolics, as measured with the Folin–Ciocalteu reagent. However, wide intervarietal differences exist (Tomás-Barberán et al., 2001). Pinelo et al. (2004) suggested that relatively high levels of phenolic antioxidants might be recovered from almond hulls for further use. The peel of *Prunus* fruit has also been found to contain higher amounts of phenolics than other fleshy parts (Balasundram et al., 2006).

Various studies have shown that total antioxidant capacity shows better correlation with total phenolic compound content than with ascorbic acid, tocopherol or carotenoids (Serrano et al., 2005; Drogoudi et al., 2008). However, it is currently unknown which would be an appropriate polyphenol daily uptake that would exert a beneficial effect (Espin et al., 2007). Regarding phenolic compound metabolism, the bowel microflora can hydrolyse phenolic compound glycosides, releasing aglycon, which could be transformed further to produce derivatives of acetic and phenyl propionic acids (Formica and Regelson, 1995).

Phenolic compounds might be divided in groups represented differently, depending on the species considered. Here, we summarize the main characteristics of phenolic acids,

| Table 13.5. Vitamin E content in *Prunus* species (USDA, 2009). |
|-------------------|-------------------|
| Fruit            | Vitamin E (mg α-TOC/100 g) |
| Almond           | 26.22             |
| Apricot          | 0.89              |
| Cherry, sweet    | 0.07              |
| Nectarine        | 0.77              |
| Peach            | 0.73              |
| Plum             | 0.26              |

*Note: α-TOC = α-tocopherol.*

| Table 13.6. Total phenolics (TP) in *Prunus* species (adapted from USDA, 2007a, and Wu et al., 2004a). |
|-------------------|-------------------|
| Fruit            | TP (mg GAE/100 g) |
| Almond           | 418              |
| Apricot          | 79               |
| Cherry, sweet    | 339              |
| Nectarine        | 107              |
| Peach            | 148              |
| Plum             | 367              |
| Plum, black      | 478              |

*Note: GAE = gallic acid equivalents.*
flavonoids, proanthocyanidins and tannins, describing the different metabolites found within these groups in *Prunus* species.

**Phenolic acids**

Phenolic acids occur in two classes: derivatives of benzoic acid or cinnamic acid. Hydroxycinnamic acids exhibit higher antioxidant activity than the corresponding hydroxybenzoic acids. These phenolic acid derivatives in turn combine with sugars to become glycosylated. Caffeic acid is generally the most abundant phenolic acid and accounts for between 75 and 100% of the total hydroxycinnamic acid content of most fruit, with the highest concentrations typically in the outer parts of ripe fruit. Caffeic and quinic acids may combine to form chlorogenic acid (5-O-caffeoylquinic acid, 5-CQA; Manach *et al.*, 2004). Apples and pears contain mainly 5-CQA (Mölle and Herrmann, 1983). In cherries, another isomeric form is the most abundant (3-CQA) (Herrmann, 1989). Relatively high amounts of 4-CQA are characteristic of prunes. Interestingly, the concentrations of chlorogenic and caffeic acid, two major phenolic acids in the epidermis and subtending cell layers of peach, are especially high in peach genotypes with a high level of resistance to the brown rot fungus, *Monilinia fructicola* (Bostock *et al.*, 1999).

**Flavonoids**

Flavonoids are a large group of structurally related compounds with a chromane-type skeleton, and a phenyl substituent in the C2 or C3 position. They are present in all terrestrial vascular plants and have been used historically in chemotaxonomy. To date, more than 6000 flavonoids have been identified. They are classified into at least ten chemical groups. Among them, anthocyanins, flavones, flavonols, flavanones, flavan-3-ols and isoflavones are particularly common in the diet. Members of the first five groups can be found in *Prunus* species.

**ANTHOCYANINS.** The name anthocyanin derives from *anthos*, which means flower, and *kyanos*, which means blue (Kong *et al.*, 2003). Anthocyanins are the most widespread of the flavonoid pigments. As they confer red, blue and purple colours to plant tissues, they contribute largely to the visual quality of fruit (Mazza, 1995). Anthocyanin is actually a name used to designate glycosides of flavonoid molecules known as anthocyanidins. Six anthocyanidins occur most frequently in plants: pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. Anthocyanins are either 3- or 3,5-glycosylated, with the most prevalent sugars being glucose, rhamnose, xylose, galactose, arabinose and fructose. Different patterns of hydroxylation and glycosylation in anthocyanins appear to modulate their antioxidant properties. Comparing the antioxidant capacity of different anthocyanins, cyanidin-3-glucoside (the most common anthocyanin found in *Prunus* species) had the highest ORAC activity, which was 3.5 times stronger than Trolox, while pelargonidin had the lowest antioxidant activity, but was still as potent as Trolox (Wang *et al.*, 1997). Cherries are by far the stone fruit with the highest content of cyanidin (75.18 mg/100 g), followed by Black Diamond® plums (40 mg/100 g) and red-flesh plums (13.7). Peonidin is found in cherry and plum in lower but still significant proportions (Table 13.7). Anthocyanin content in other *Prunus* species is low. In peach, anthocyanins are associated mainly with the peel. In some varieties, anthocyanin accumulation produces endocarp staining, a trait that can give an attractive appearance to fresh fruit, but is detrimental in the canning process, as the redness becomes brown and unsightly.

FLAVONES, FLAVONOLS, FLAVANONES AND FLAVAN-3-OLS. The main flavones in the diet are apigenin and luteolin. Among *Prunus* species, they are found at low concentrations in red plum varieties. Among flavonols, quercetin is the compound found most commonly in *Prunus* species. It is present in relatively high concentration, especially in cherry and red plum, but at levels lower than those found in flavonol-rich products such as onion. Flavonols are located almost exclusively in the peel.
Table 13.7. Flavonoids in *Prunus* species (USDA, 2007b).

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Group</th>
<th>Compound</th>
<th>mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
<td>Anthocyanidins</td>
<td>Cyanidin</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>Flavan-3-ols</td>
<td>(-)-Epicatechin</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-Epigallocatechin</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)-Catechin</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>Flavanones</td>
<td>Eriodictyol</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Naringerin</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isorhamnetin</td>
<td>7.05</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
<td>Kaempferol</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quercetin</td>
<td>0.36</td>
</tr>
<tr>
<td>Apricot</td>
<td>Flavan-3-ols</td>
<td>(-)-Epicatechin</td>
<td>5.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-Epigallocatechin-3-galate</td>
<td>4.79</td>
</tr>
<tr>
<td>Cherry, sweet</td>
<td>Anthocyanidins</td>
<td>Cyanidin</td>
<td>75.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pelargonidin</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peonidin</td>
<td>4.47</td>
</tr>
<tr>
<td></td>
<td>Flavan-3-ols</td>
<td>(-)-Epicatechin</td>
<td>6.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-Epigallocatechin-3-galate</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)-Epicatechin</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)-Catechin</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
<td>Quercetin</td>
<td>2.64</td>
</tr>
<tr>
<td>Nectarine</td>
<td>Anthocyanidins</td>
<td>Cyanidin</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>Flavan-3-ols</td>
<td>(-)-Epicatechin</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)-Catechin</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
<td>Quercetin</td>
<td>0.69</td>
</tr>
<tr>
<td>Peach</td>
<td>Anthocyanidins</td>
<td>Cyanidin</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>Flavan-3-ols</td>
<td>(-)-Epicatechin</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-Epigallocatechin</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-Epigallocatechin-3-galate</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)-Catechin</td>
<td>4.92</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
<td>Quercetin</td>
<td>0.68</td>
</tr>
<tr>
<td>Plum, red</td>
<td>Anthocyanidins</td>
<td>Cyanidin</td>
<td>4.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delphinidin</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pelargonidin</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peonidin</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>Flavones</td>
<td>Apigenin</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Luteolin</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
<td>Kaempferol</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myricetin</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quercetin</td>
<td>1.85</td>
</tr>
</tbody>
</table>

(Tomás-Barberán et al., 2001). Flavanones are typical of citrus, but eriodictyol and naringenin are also present in almond nuts. Finally, flavan-3-ols, including (+)-catechin and (-)-epicatechin and their gallic acid esters, show six times higher levels in black plums than in most other *Prunus* fruit that have similar total contents.

Proanthocyanidins (PAs) and tannins

Proanthocyanidins are the second most abundant natural phenolics after lignin (Espín et al., 2007). Proanthocyanidins, also known as condensed tannins, are oligomers. Despite their role as potent antioxidants, they have been associated with whole plant ecological
functions (Rawat et al., 1998). Proanthocyanidins produced by P. armeniaca roots were suggested to limit the germination and growth of surrounding species (Rawat et al., 1999). Polymers of catechin and epicatechin are the most common PAs in food. Colonic flora could metabolize these compounds, producing phenolic acids (Deprez et al., 2000). PAs have received increasing attention due to evidence associating their action with some health benefits (Lazarus et al., 1999). Plum and almonds are rich in PAs, with values even higher than those found in grape (USDA, 2004). PAs with a higher degree of polymerization are normal in these fruit as opposed to PAs from other Prunus species which show lower mean molecular mass (Table 13.8).

Ellagitannins (ETs) are a subgroup of tannins (Hümmer and Schreier, 2008). Structurally, they contain at least two galloyl units C–C coupled to each other, but lack glycosidically linked catechin units (Khanbabaee and van Ree, 2001). With more than 500 natural products characterized so far, they are the largest group of tannins. They are found in pomegranates, black raspberries, raspberries, strawberries, walnuts and almonds.

### 13.4 Phytonutrients

Nutraceutical compounds are defined as extracts of foods that exert a medicinal effect on human health by preventing or limiting the progression of chronic diseases. Extracts from fruit and vegetables contain mixtures of phytochemicals, and recent studies on nutraceutical properties have been conducted isolating chemical compounds, defining fractions and using mixtures to determine the specific bioactive compounds present. Many of the phytochemical categories present in Prunus species, including carotenoids, vitamins, phenolics and others, may play a role as nutraceuticals through the prevention of chronic diseases such as cancer, cardiovascular disease, Alzheimer’s, metabolic syndrome and others (Sun et al., 2002; Kottová et al., 2004; Heo and Lee, 2005; Chen et al., 2007; Chen and Blumberg, 2008). The following is a short summary of nutraceutical properties of selected Prunus species.

Almond extracts have been characterized for their effects against cancer and cardiovascular disease. For example, whole almonds and almond fractions have been shown to reduce aberrant crypt foci in a rat model of colon carcinogenesis (Davis and Iwahashi, 2001). Clinical studies have shown that inclusion of almonds in the daily diet may elevate the blood levels of high-density lipoproteins (HDL) while lowering levels of low-density lipoproteins (LDL), and these lipid-altering effects have been associated with the interactive or additive effects of the numerous bioactive constituents found in almonds (Spiller et al., 1998). A dose–response study on hyperlipidaemic subjects confirmed these results, showing that 73 g of almonds in the daily diet reduced LDL by ~ 9.4%, increased HDL ~ 4.6% and reduced the LDL/HDL ratio ~ 12% (Jenkins et al., 2002). Several constituents of almond have been associated with anti-inflammatory properties, antihepatotoxicity effects and immunity boosting properties (Puri, 2003).

Studies with peach and plum have been conducted to determine their effects against cancer, cognitive deficits and hepatotoxicity. On the other hand, nectarines and apricots still await characterization for their nutraceutical properties.

Peach and plum phenolic compounds have been shown to inhibit growth and induction of differentiation in colon cancer cells (Lea et al., 2008). Furthermore, plum extracts (dried plums) have been shown to alter several risk factors related to colon carcinogenesis in rats, including a reduction of faecal total and secondary bile acid concentrations, decrease of colonic β-glucoronidase and 7α-dehydroxylase activities and an increase in antioxidant activity. The effect was associated with the extracts’ phytochemical content (Yang and Gallaher, 2005). More recently, chlorogenic acid and neochlorogenic acid in plum and peach fruit have been identified as bioactive compounds with potential chemopreventive properties against an oestrogen-independent breast cancer cell line, while having little effect on normal cells (Noratto et al., 2009). On the other hand, plum (P. domestica L.) juice was effective in mitigating cognitive deficits in aged rats, and this effect was associated with the amount of
<table>
<thead>
<tr>
<th>Fruit</th>
<th>Proanthocyanidin</th>
<th>mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond</td>
<td>Monomers</td>
<td>7.77</td>
</tr>
<tr>
<td></td>
<td>Dimers</td>
<td>9.52</td>
</tr>
<tr>
<td></td>
<td>Trimers</td>
<td>8.82</td>
</tr>
<tr>
<td></td>
<td>4-6 mers</td>
<td>39.97</td>
</tr>
<tr>
<td></td>
<td>7-10 mers</td>
<td>37.68</td>
</tr>
<tr>
<td></td>
<td>Polymers</td>
<td>80.26</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>184.02</td>
</tr>
<tr>
<td>Apricot</td>
<td>Monomers</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>Dimers</td>
<td>11.33</td>
</tr>
<tr>
<td></td>
<td>Trimers</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>4-6 mers</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td>7-10 mers</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>Polymers</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21.05</td>
</tr>
<tr>
<td>Cherry, sweet</td>
<td>Monomers</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td>Dimers</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>Trimers</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>4-6 mers</td>
<td>6.51</td>
</tr>
<tr>
<td></td>
<td>7-10 mers</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>Polymers</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>19.13</td>
</tr>
<tr>
<td>Nectarine</td>
<td>Monomers</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>Dimers</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>Trimers</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>4-6 mers</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td>7-10 mers</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>Polymers</td>
<td>7.31</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29.18</td>
</tr>
<tr>
<td>Peach</td>
<td>Monomers</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td>Dimers</td>
<td>12.24</td>
</tr>
<tr>
<td></td>
<td>Trimers</td>
<td>4.41</td>
</tr>
<tr>
<td></td>
<td>4-6 mers</td>
<td>17.66</td>
</tr>
<tr>
<td></td>
<td>7-10 mers</td>
<td>10.94</td>
</tr>
<tr>
<td></td>
<td>Polymers</td>
<td>22.02</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>71.75</td>
</tr>
<tr>
<td>Plum, red</td>
<td>Monomers</td>
<td>10.88</td>
</tr>
<tr>
<td></td>
<td>Dimers</td>
<td>38.54</td>
</tr>
<tr>
<td></td>
<td>Trimers</td>
<td>22.25</td>
</tr>
<tr>
<td></td>
<td>4-6 mers</td>
<td>58.04</td>
</tr>
<tr>
<td></td>
<td>7-10 mers</td>
<td>33.79</td>
</tr>
<tr>
<td></td>
<td>Polymers</td>
<td>57.28</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>220.78</td>
</tr>
</tbody>
</table>

Phenolics supplemented (Shukitt-Hale et al., 2009). In addition, *P. persica* pericarp extracts were tested against cisplatin-induced acute toxicity in mice. Results showed a significant protection against the acute nephrotoxicity and hepatotoxicity, probably the result of a reduction in cisplatin-induced oxidative stress (Lee et al., 2008). Similar results were observed with *P. persica* flesh (Lee et al., 2007). Likewise, studies with methanolic extracts of immature plum fruit (*P. salicina* L. cv. Soldam, 20–40 days before final harvest) showed inhibitory effects against benzo(α)pyrene (B(α)P) induced toxicity in mice, by inhibiting the induction of
CYP1A1 expression, CYP1A1 being the primary cytochrome P450 involved in the metabolism and bioactivation of B(a)P (Kim et al., 2008). Recent studies with peach, nectarine and apricot extracts have shown in vitro binding of a mixture of bile acids (secreted in human bile at a duodenal physiological pH of ~6.3). Using cholestyramine (a bile acid-binding, cholesterol-lowering drug) as a reference indicated that binding potential followed the order peach > apricot > nectarine. The effects have been associated with the mixture of phytochemicals present in the extracts (Kahlon and Smith, 2007).

Research with tart and sweet cherry fruit extracts and fractions has been reported in the areas of metabolic syndrome, cancer, inflammation and effects on neuronal cells. For example, studies have shown that cherry-enriched diets reduce metabolic syndrome and oxidative stress in rats (Seymour et al., 2008), while regular tart cherry intake by obesity-prone rats fed with a high-fat diet altered the abdominal adiposity and affected adipose gene transcription and inflammation (Seymour et al., 2009). Furthermore, intake of tart cherry in diets reduced several phenotypic factors that were associated with risk for metabolic syndrome and Type 2 diabetes in rats (Seymour et al., 2008). In clinical studies, it was shown that consumption of cherries lowered plasma urate in healthy women (Jacob et al., 2003). This has important implications, since high levels of plasma urate have been associated with cardiovascular disease, diabetes and the metabolic syndrome.

Studies with anthocyanin-rich tart cherry extracts have shown inhibition of intestinal tumorigenesis in mice (Bobe et al., 2006) and antiproliferation activity against human colon cancer cells (Kang et al., 2003). Tart, sweet and sour cherries have shown cyclooxygenase inhibitory activities (Wang et al., 1999; Seeram et al., 2001; Mulabagal et al., 2009), and suppressed inflammation pain behaviour has been associated with tart cherry anthocyanin intake in rats (Tall et al., 2004). In fact, clinical trials have confirmed that consumption of cherries (Bing sweet cherries) lowers circulating concentrations of inflammation markers in healthy men and women (Kelley et al., 2006). Finally, studies using sweet and sour cherry phenolics have shown a protective effect on neuronal cells (Kim et al., 2005).

There is a need to continue further research on the nutraceutical properties of Prunus species, as well as the different genotypes involved in each type of fruit, since the phytochemical make-up may vary, as mentioned earlier in this chapter. In addition, novel approaches may be used as tools for bioactive compound discovery and elucidating the mechanisms of action, such as exploring the human gene expression associated with chronic diseases. If a pattern of gene expression linked to a desired bioactive compound effect in the targeted cell is established, then post-treatment gene expression may be used to screen bioactive compounds from different species for their ability to induce the target phenotype (Evans and Guy, 2004).

13.5 Factors Affecting Bioactive Compounds in Prunus Species

The concentration of bioactive compounds can be affected by genetic factors, cultural practices, environmental conditions and storage processing treatments.

13.5.1 Genetic factors

As previously shown, large differences in total antioxidants, carotenoids and phenolics exist between Prunus species (Tables 13.2, 13.3 and 13.6). In addition, for any given species, the concentration of bioactives among varieties can also show dramatic differences. Analysis of 37 apricot varieties showed tenfold difference in the accumulation of carotenoids (Ruiz et al., 2005). In another study, also in apricot, total phenolics varied from 30.3 to 742 mg of gallic acid equivalents per 100 g (Drogoudi et al., 2008). In plum, varieties with dark purple-coloured skin showed 200% higher total phenolics than others (Rupasinghe et al., 2006). The plum cvs. Black Beauty and Angeleno were especially rich in phenolics (Tomás-Barberán et al., 2001). In peach,
fruit showing more intense endocarp staining presented higher antioxidant capacity.

The effects induced by the rootstocks on controlling plant development and production are well known (Caruso et al., 1996, 1997). In contrast, the influence on fruit bioactive compounds is quite variable. In some cases, no pronounced differences were observed (Drogoudi and Tsipouridis, 2007). On the other hand, some works reported that the levels of phytochemicals might be influenced significantly by rootstock (Giorgi et al., 2005; Remorini et al., 2008).

13.5.2 Cultural practices and environmental conditions

The accumulation of bioactive compounds is affected greatly by the fruit-ripening process. In most Prunus species, the concentration of ascorbic acid increases at advanced ripening stages (Table 13.9). In sweet cherry (cv. 4-70), high content of ascorbic acid was detected at early phases of development. As ripening proceeded, ascorbic acid levels dropped and then increased progressively to reach maximum content at full maturity (Serrano et al., 2005).

Carotenoid concentration also increases steadily during ripening in most stone fruit (Katayama et al., 2006; Díaz-Mula et al., 2008). Besides its contribution to full colour development, enzymatic carotenoid degradation of nectarines was shown to play a role in C-13 norisoprenoid aroma compound formation (Balderman et al., 2005). The modifications in total phenolics showed no clear trend during ripening of peach, nectarine and plum cultivars (Tomás-Barberán et al., 2001). In cherry, total phenolic compounds decrease during the early stages of development, but later on skin colour development is associated with the accumulation of anthocyanins (Díaz-Mula et al., 2008; Usenik et al., 2008b). Plum colour development is also related directly to increased anthocyanidin content (Díaz-Mula et al., 2008; Usenik et al., 2008a), and delaying harvesting might lead to significant increases in these compounds and total antioxidant activity (by 10–20%) (Díaz-Mula et al., 2008).

Sunlight has been shown to be associated with increased content of several bioactive compounds, including ascorbic acid (Lee and Kader, 2000) and anthocyanins. Shading of plum fruit resulted in poor colour development (Murray et al., 2005). UV radiation is known to be associated with anthocyanin accumulation (Arakawa, 1993). Phenylalanine ammonia lyase, chalcone synthase and dihydroflavonol reductase (enzymes involved in phenylpropanoid metabolism) are induced by UV radiation (Tomás-Barberán and Espín, 2001). The differences between day and night temperatures have been shown to affect anthocyanin accumulation in plums (Tsuji et al., 1983). Regulated water deficit resulted in reduced content of vitamin C and carotenoids in the fruit peel, while increasing anthocyanins and procyanidins (Buendía et al., 2008).

Results comparing the effect of conventional or organic production on bioactive compounds in fruit and vegetables are variable. Organic peaches showed higher content of phenolic and ascorbic acid than conventionally produced fruit (Carbonaro and Mattera, 2001; Carbonaro et al., 2002). Ascorbic acid, vitamin E and β-carotene were higher in organic plums grown on soil covered with natural meadow, while total phenolic content was higher in conventional plums (Lombardi-Boccia et al., 2004). Bourn and Prescott (2002) concluded that further studies are required to understand fully the effect of

<table>
<thead>
<tr>
<th>Fruit (cultivar)</th>
<th>Green</th>
<th>Half-ripe</th>
<th>Ripe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricot (Tilton)</td>
<td>11.7</td>
<td>12.9</td>
<td>14.3</td>
</tr>
<tr>
<td>Peach (Elberta)</td>
<td>7.8</td>
<td>10.2</td>
<td>12.2</td>
</tr>
</tbody>
</table>
inorganic and organic fertilizers on the nutritional value of crops. Winter and Davis (2006) affirmed that it was not possible to ensure that organically grown products were nutritionally superior to those obtained by conventional agricultural techniques.

### 13.5.3 Storage and processing

In general, ascorbic acid is the compound showing the highest losses during storage and processing. In apricots a loss of around 26% ascorbic acid was found after 4 days at 20°C. However, the contribution of this compound to total antioxidant capacity in stone fruit is minimal compared with that of phenolic compounds. Several reports have shown that the content of total phenolics does not change or even increases during postharvest storage. Asami et al. (2002) reported no significant loss of total phenolics in clingstone peaches during cold storage. In dark purple and yellow plum varieties, increases in total phenolics were found even during storage (Diaz-Mula et al., 2008). Carotenoid content also increased in apricots during storage (Egea et al., 2007).

Although it seems clear that phenolic compound synthesis can proceed after harvest, the changes in phenolic compounds depend on the balance of de novo synthesis and degradation. Peaches are an excellent example of fruit susceptible to enzymatic browning, and this is a major problem encountered during handling and processing (Brandelli and Lopes, 2005). Improper storage conditions can lead in many cases to tissue browning due to phenolic compound degradation by the action of several enzymes, such as polyphenol oxidases and peroxidases. Refrigeration can reduce the activity of phenol oxidizing enzymes (Tomás-Barberán and Espín, 2001). In addition, fast cooling and low temperature storage reduce losses of other antioxidants such as ascorbic acid. Finally, gamma irradiation of almond skins increased the yield of total phenolic content, as well as enhanced antioxidant activity. Ultraviolet treatments have been shown to increase the accumulation of phenolic antioxidants.

A substantial amount of research literature has been published reporting the effects of processing on the nutritional quality of fruit and vegetables (Rickman et al., 2007). Various processing operations have profound effects on the level of bioactive compounds. Since bioactive compounds are generally present at higher concentration at the fruit surface, peel removal during processing results in significant losses. In addition, it increases leaching of soluble metabolites. Slicing favours ascorbic acid oxidation, the enzymatic degradation of phenolic compounds and tissue browning (McCarthy and Mattheus, 1994). Vitamin E is highly susceptible to oxidation during storage and processing. Blanching and other heat treatments might be useful to inactivate enzymes involved in phenolic compound oxidation, but might decrease the content of heat-sensitive compounds such as ascorbic acid. Carotenoids are quite heat stable (Nicoli et al., 1999) and their absorption could even be improved by heat treatments. Heating can lead to the formation of cis isomers of carotenoids (Lessin et al., 1997), which have lower relative provitamin A activity than the corresponding trans forms (Minguez-Mosquera et al., 2002).

Clingstone peaches are commonly preserved by thermal processing methods such as canning (Hong et al., 2004). Since simple phenolic compounds are water soluble, they are susceptible to leaching. Peach canning resulted in a 21% loss in total phenolics. The reduction was associated with migration of procyanidins into the canning syrup (Hong et al., 2004). The effects of freezing on bioactive compounds are less marked than those of other processing operations (Rickman et al., 2007).

Preservation methods usually are believed to reduce the level of antioxidants in food. While this might be true in some cases, the level of bioactive compounds in processed foods might still be higher than that found in many other food groups. In some cases, processing can even increase total antioxidant capacity. For instance, industrial drying (oven drying) of almond and roasting almond skins increased (twofold) the contents of phenolic compounds. In prunes, although drying results in marked losses of ascorbic acid and anthocyanins, total antioxidant capacity may be increased by formation of new antioxidants (Ryley and Kayda, 1993).
13.6 Future Prospects and Directions of Research and Development

Several years of active research have led to great advances in the area of fruit bioactive compounds, and the exploitation of these metabolites is increasing rapidly. Accumulating evidence suggests that having five to ten servings of fruit daily may play an important role in preventing some health disorders. However, it is important to interpret results cautiously, to avoid making premature and unjustified claims on health benefits when direct data are lacking.

Fresh Prunus species are significant contributors of bioactive compounds to the diet during the spring and summer. All the main groups of bioactive compounds (ascorbic acid, vitamin E, carotenoids, phenolic compounds) are represented to different degrees in most Prunus species.

In all cases, total antioxidant capacity seems to correlate best with the level of phenolics, suggesting that this is the predominant group. Plums rank top among Prunus species in antioxidant capacity, and black varieties have ORAC values close to those observed in fruit richest in antioxidants, such as blackcurrants and cranberries (Fig. 13.1). Almonds and cherries are rich in total phenolics and also show high antioxidant capacity. Peaches and nectarines show moderate levels of carotenoids and phenolics, but due to their high consumption, either fresh or processed, ultimately contribute significantly to dietary intake of bioactive compounds. Despite the general features of the main Prunus species, several works have shown that there is a wide variation among varieties for any given species. The accumulation of antioxidants is also variable within a single fruit, with the peel usually showing two- to 40-fold higher content of these substances than the other tissues. This may have implications where the peel is removed before consumption, a common practice in some parts of the Mediterranean.

Many Prunus species are also commonly used for processing. This might result in some losses of some antioxidants, such as ascorbic acid due to oxidation or leakage during heat treatments or long-term storage, or anthocyanins during drying. However, the contribution of bioactive compounds in frozen, canned or dried products still supersedes that found in many other food groups. In some cases (plum drying, almond roasting), processed foods could have more antioxidant capacity than fresh commodities due to the formation of new metabolites and a concentration effect. Consequently, intake of all forms of fruit and vegetables should be encouraged, as long as added ingredients, such as sugar, fat and salt, are not significant (Rickman et al., 2007).

Complete understanding of many aspects of the bioactive compounds in fruit is lacking, and multiple aspects are to be learned. While the identification of these compounds and the study of differences among species have flourished, further combined research efforts (in chemical, biochemical, medical and agronomic areas) are required in order to:

- characterize the mechanisms that account for specific bioactive compound protection.
- study bioactive compound bioavailability, metabolism, dose–response and toxicity.
- start evaluating the interactions among bioactive compounds, since they are consumed almost exclusively in combinations.
- generate guidelines and protocols to bring some order related to the analytical determination of antioxidant capacity on food (Huang et al., 2005).
- identify the molecular determinants of the distinct accumulation of bioactive compounds in external and internal fruit tissues or in different varieties.
- incorporate into breeding programmes traits related to higher production of bioactive compounds.
- determine more completely the influence of orchard variables on fruit bioactive compounds in order to design practices oriented to maximize accumulation of desirable metabolites.

In all, the positive attributes associated with bioactive compounds in fruit in general, and in Prunus species in particular, provide an opportunity that might be capitalized on.
References


Prunus


14  **Ribes and Rubus**  
[Blackberry, Currants and Raspberry, etc.]

Jordi Giné Bordonaba and Leon A. Terry

### 14.1 Introduction

A great deal of berry fruit which are commercially available in fresh or processed form belong to the *Ribes* and *Rubus* genera, which encompass several species including blackberry (*Rubus* spp.), black raspberry (*Ru. occidentalis* L.; *Ru. leucodermis* Torr. & A. Grey), red raspberry (*Ru. idaeus* L.), black currant (*Ribes nigrum* L.), red currant (*Ru. rubrum* L.), white currant (*Ru. glandulosum* Grauer), arctic bramble (*Ru. arcticus* L.), boysenberries (*Ru. ursinus × idaeus*), cloudberries (*Ru. chamaemorus* L.), gooseberry (*Ru. uva-crispa* L.), loganberry (*Ru. loganobaccus* L.H. Bailey), etc. Plants from both *Ribes* and *Rubus* are generally shrubs of small to medium size and usually are characterized by giving attractive small fruit rich in potential health-related compounds. As with many other fruit and vegetables, they represent an important source of micro- and macronutrients, including fibre, sugars, vitamins, minerals, etc.; however, most of their health-promoting properties have been associated largely with their high levels of bioactive compounds (namely, ascorbic acid, phenolic acids and flavonoids including anthocyanins) with known antioxidant capacity (Table 14.1). Nowadays, scientific evidence suggests that increased production or ineffective scavenging of reactive oxygen species (ROS) may play a crucial role in the development of certain pathologic conditions, especially cancer or chronic diseases (Wolfe *et al.*, 2008). Consumption of fruit and vegetables is likely to be responsible for decreasing the severity or incidence of these diseases, by reducing oxidative stress and modulating signal transduction pathways involved in cell proliferation and survival (Wolfe *et al.*, 2008). In this context, several studies to date have shown higher antioxidant activity, in cell-free systems, within *Ribes* and *Rubus* fruit than in many other food sources. Accordingly, numerous health-promoting properties have been attributed to fruit from either the *Ribes* or *Rubus* genera during the past few decades. Rather than health-related benefits due to single compounds, it is believed that most of their benefits come from the additive or synergistic effect from several bioactives (Seeram, 2008) present in these berries.

The present chapter describes the different key bioactive compounds of fruit from *Ribes* and *Rubus* species and discusses the latest scientific evidence on the health-promoting properties of these fruit.

#### 14.1.1 Ribes

The genus *Ribes* embraces the shrubs of both currants and gooseberries and belongs to the family *Grossulariaceae*. It includes more than 150 described species of bushes that are
Table 14.1. Nutrient and mineral composition of main Ribes and Rubus berries.

<table>
<thead>
<tr>
<th></th>
<th>Blackcurrant</th>
<th>Blackberries</th>
<th>Raspberries</th>
<th>Redcurrants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>20</td>
<td>12</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>15.4</td>
<td>9.6</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Organic acids</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Proteins (g)</td>
<td>1.4</td>
<td>1.4</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>–</td>
<td>5</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>85–500</td>
<td>11–28</td>
<td>25</td>
<td>22–53</td>
</tr>
<tr>
<td>Anthocyanins (mg)</td>
<td>152–400</td>
<td>160</td>
<td>–</td>
<td>1.1–136</td>
</tr>
<tr>
<td>Antioxidant activity (AU)</td>
<td>–</td>
<td>56.6–71.8</td>
<td>–</td>
<td>40–63</td>
</tr>
</tbody>
</table>

native throughout Northern Europe, North America and Asia, and in mountainous areas of north–west Africa and South America (Brennan, 2005). Five main Ribes subgenera are grown for their fruit and these include blackcurrants, redcurrants, white currants, gooseberries and jostaberries (Brennan, 2005). Historically, blackcurrants, for instance, were imported from Holland to England in 1611 by Tradescant (Brennan, 2005). Later, during the 18th century, blackcurrants were domesticated in Eastern Europe and spread over Russia, and in the particular case of the UK, blackcurrant cultivation was especially encouraged by the British government during the Second World War, due, in part, to the fruit’s suitability to the UK weather and the berries’ high content of vitamin C, as no other sources of this vitamin were really available. In those days, the major part of the production was made into blackcurrant syrup. Nowadays blackcurrants are the leading Ribes crop worldwide and are still mainly processed rather than used fresh, due to their strong flavour (Brennan et al., 1997; Barney and Hummer, 2005). Redcurrants are grown to be eaten fresh or to be processed into juice and conserves, while white currants provide the greatest yields and are freshly consumed or used for baby food processing (Barney and Hummer, 2005). Similarly, gooseberries are cultivated mainly for the fresh market and for inclusion in jams and pies (Brennan, 2005).

Ribes fruit have been appreciated for centuries as a nutritious food. Berries, including blackcurrants, redcurrants, etc., may be considered as an ancient food in northern Europe. The use of blackcurrant fruit as a herbal medicine emerged in the Middle Ages. In the 16th century, European herbalists started to recommend Ribes berries or their syrups for the treatment of several illnesses, including bladder stones and liver disorders, coughs and lung ailments. However, it was not until the 18th century that the use of Ribes fruit became widespread among European herbalists and physicians. Several berry-derived products were employed for treatment of numerous intestinal conditions, typhoid fever, gout and rheumatism, and for infections of the mouth, skin and urinary tract.

Economically, currant and gooseberry production around the world is based mainly in Asia, Europe and Oceania. In Asia, the Russian Federation represents more than 99% of the total production, while Poland and Germany together produce more than 70% of the European crop (Table 14.2). New Zealand represents almost the totality of the crop harvested in Oceania; 6110 t out of the 7110 t harvested during 2005. Currently, North American acreage for currants and gooseberries is increasing and this is due in part to both the lifting of the legislation that prohibited blackcurrant cultivation in several states and the release of new resistant varieties. This said, the crop still has not reached the popularity it currently has in Europe, and such is reflected by the paucity of research undertaken on these berries by the USA as compared with that on other berry fruits.
Table 14.2. Production (1000 t) of currants and gooseberries for the main producing countries within the European Union.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Poland</td>
<td>165.3</td>
<td>196.9</td>
<td>175.3</td>
<td>203.5</td>
</tr>
<tr>
<td>Germany</td>
<td>230.7</td>
<td>252.3</td>
<td>246.5</td>
<td>186</td>
</tr>
<tr>
<td>UK</td>
<td>18.9</td>
<td>21.9</td>
<td>13.8</td>
<td>22.4</td>
</tr>
<tr>
<td>Austria</td>
<td>25.8</td>
<td>19.7</td>
<td>24.7</td>
<td>21.1</td>
</tr>
<tr>
<td>Czech Republic</td>
<td></td>
<td>32.5</td>
<td>24.9</td>
<td>18.6</td>
</tr>
<tr>
<td>Hungary</td>
<td>23.4</td>
<td>16</td>
<td>16.5</td>
<td>13.4</td>
</tr>
<tr>
<td>France</td>
<td>7.5</td>
<td>11.3</td>
<td>8.4</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>471.6</td>
<td>550.6</td>
<td>510.1</td>
<td>478</td>
</tr>
</tbody>
</table>


14.1.2 Rubus

*Rubus* is a broad genus of flowering plants in the family *Rosaceae*, subfamily *Rosoideae*. It is found worldwide, except in desert areas, but is present mainly in the northern hemisphere. The important cultivated species from the genus include the European red raspberry (*Ru. idaeus* ssp. *vulgatus*), the North American red raspberry (*Ru. strigosus* (Michx.) Maxim.), the eastern North American blackberry (*Ru. occidentalis* L.) and the Andean blackberry hybrid (*Ru. glaucus* Benth., *Ru. adenotrichus* Schlech.) (Mertz *et al.*, 2007). Blackberry fruit, for instance, tend to be first green and red to brown-red and hard when immature but turn into black-coloured and juicy fruit as the berry ripens. Most of the commercial blackberry production occurs in the USA, but with appreciable amounts also grown in the UK and New Zealand (Dai *et al.*, 2007) (Fig. 14.1).

In the USA, the Pacific Coast region produces c.80% of the total national production. Raspberries are the most important species in the genus *Rubus*, although considered one of the most perishable fruit, with the risk of decay, colour darkening and changes in flavour occurring rapidly after harvest (Krüger *et al.*, 2003). Historically, raspberries and other *Rubus* species can be tracked to ancient times. However, the first written mention of raspberries can only be found in an English book on herbal medicine dated 1548. Juices and extracts from *Rubus* fruit were used extensively in the 16th century for the treatment of several conditions, but mainly for the treatment of infections (Dai *et al.*, 2007).

14.2 Identity and Role of Bioactives

*Ribes* and *Rubus* species are characterized by their high anthocyanin and phenolic contents, as well as other bioactives (e.g. ascorbic acid). Berries from these species have some of the highest antioxidant capacities of any common fruit. Generally, it is accepted that total phenolic content (TP) and total flavonoids (TF) are well correlated with antioxidant capacity, as determined by any of the standard assays such as FRAP or ORAC (see Chapters 18 and 19 of this volume for further information). For instance, TP and TF of four different raspberry cultivars were found to be strongly correlated with antioxidant capacity ($R^2 = 0.988$ and $R^2 = 0.996$, respectively) in a study conducted by Liu *et al.* (2002).

14.2.1 Polyphenolic compounds

Phenolic compounds are widely distributed in both *Ribes* and *Rubus* species, ranging from simple moieties with an individual hydroxylated aromatic ring to complex polymeric molecules (Harborne and Williams, 1995). Phenolics are plant secondary metabolites that are synthesized and accumulate in the plant via processes that are controlled endogenously or regulated by exogenous factors.
such as environmental conditions (namely, temperature and light) (Dixon and Paiva, 1995). Indeed, the diverse range of phenolics found in berries from Ribes or Rubus species are responsible for the fruit’s astringency, bitterness, colour and flavour, and also for the oxidative stability of their derived products. Due in part to the high concentrations of phenolic compounds in Ribes and Rubus fruit, a great deal of research has investigated the different polyphenolic fractions of these berries. However, it is important to notice that most of this information is focused on blackberries, raspberries and blackcurrants (Zadernowski et al., 2005; Mertz et al., 2007; Gine Bordonaba and Terry, 2008), whereas little is known about other minor Ribes and Rubus berries.

Generally, polyphenol content may be estimated by adaptations of the standard Folin–Ciocalteu method. Briefly, this method is based on the reduction of a phosphowolframate-phosphomolibdate complex by phenolic compounds, resulting in blue reaction products (see Chapter 18 of this volume for further information), which are then measured spectrophotometrically. By using this method with any of its reported modifications, many papers refer to the high total phenolic content, expressed as gallic acid equivalents (GAE), of different Ribes and Rubus fruit, often in comparison with other fruit and vegetables (Fig. 14.2). That said, the values for TP content found in the literature are often controversial, since the reported variation in the content of total phenolics between berry types is due mainly to differences in cultivar, agroclimatic and growing conditions and, finally, to differences in the methods used in each study (Gine Bordonaba and Terry, 2008).

**Flavonoids**

Flavonoids are a group of polyphenolic compounds that can be divided into different subclasses such as flavanols, flavonols, flavones, flavanones, isoflavones and anthocyanins (Pinent et al., 2008). Most berries from the Ribes and Rubus genera are rich sources of these compounds, with blackcurrants, for instance, containing c.tenfold greater flavonol concentrations than other berries (Häkkinen et al., 1999). Some of these flavonols (i.e.
quercetin) are found ubiquitously in most *Ribes* berries, accounting for 46.3, 39.6, 29.8, 14.3 and 10.1% of the total phenolic and flavonol fraction in green gooseberry, redcurrant, blackcurrant, green currant and white currant, respectively (Hakkinen et al., 1999). In contrast, *Rubus* species, including red raspberry, arctic bramble and cloudberry, had no more than 2.5% of quercetin (Fig. 14.4). In the same study, relative concentrations of myricetin and kaempferol ranged from 0 to 9.4% for all the above-mentioned *Ribes* and *Rubus* species (Häkkinen et al., 1999).

Anthocyanins are considered one of the main plant pigments visible to the human eye. They belong to the flavonoid class and they usually conjugate to form glycosides of polyhydroxy and polymetoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. The differences between various anthocyanins relate to the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of these sugars, usually C3 and less frequently at C5 or C7, and the nature and number of aliphatic or aromatic acids attached to the sugars (Fig. 14.3b). In fruit, anthocyanins are found generally in the external layers of the skin (hypodermis), and within the skin these compounds are encountered in vacuoles of different sizes. Anthocyanins in berries including raspberry, blackberry and blackcurrant have been studied extensively during the past years, not only for their interest as natural colorants but also for their health-promoting properties. Indeed, blackcurrant extracts have hitherto acted as an important model for understanding anthocyanin absorption in both humans and animals (Netzel et al., 2001; Nielsen et al., 2003; Wu et al., 2005; Matsumoto et al., 2006). A simple survey of the literature, using any of the available search engines, reveals that the number of published articles referring to blackcurrant anthocyanins has increased exponentially during the last 15 years (from one in 1991 up to 15 articles in 2008), and similar results can be obtained if searching for other *Ribes* or *Rubus* species. All studies so far have concluded that four major anthocyanins (Fig. 14.3b) (namely, cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and delphinidin-3-rutinoside) constitute almost 90% of the total anthocyanin content of blackcurrants (Häkkinen et al., 1999; Anttonen and Karjalainen, 2006; Manhita et al., 2006; Rubinskiene et al., 2006; Jordheim et al., 2007; Giné Bordonaba and Terry, 2008). Other anthocyanins, including peonidin-3-rutinoside and...
Ribes and Rubus anthocyanins have been reported in blackcurrants, but in lesser amounts (Froytlog et al., 1998; Slimestad and Solheim, 2002; Fig. 14.3b). In raspberries, cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-glucorutinoside, pelargonidin-3-sophoroside and pelargonidin-3-glucoside have all been identified (De Ancos et al., 2000; Fan-Chiang and Wrolstad, 2005). Marionberry anthocyanins include cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-glucoside and acylated cyanidin-based anthocyanins (Wu et al., 2004). Anthocyanin concentration in raspberries, as determined by the pH differential method (see Chapters 18 and 19 of this volume), ranged from 1.7 to 576 µg/g FW, depending on the cultivar (Liu et al., 2002). Blackberries contain cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, pelargonidin-3-glucoside, cyanidin-3-xylidoside and malvidin-3-glucoside, cyanidin-3-glucoside being the dominant anthocyanin (Goiffon et al., 1991; Fan-Chiang and Wrolstad, 2005). Indeed, anthocyanin distribution in Ribes and Rubus is species dependent. Certain European gooseberry cultivars...
<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Berry</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delphinidin-3-glucoside</td>
<td>OH</td>
<td>OH</td>
<td>α-Glucose</td>
<td>Blackcurrant, gooseberry</td>
<td>Freytag et al. (1998); Wu et al. (2004); Jordheim et al. (2007)</td>
</tr>
<tr>
<td>Delphinidin-3-rutinoside</td>
<td>OH</td>
<td>OH</td>
<td>α-Glucose-L-rhamnose</td>
<td>Blackcurrant, gooseberry</td>
<td>Freytag et al. (1998); Wu et al. (2004); Jordheim et al. (2007)</td>
</tr>
<tr>
<td>Cyanidin-3-glucoside</td>
<td>OH</td>
<td>H</td>
<td>α-Glucose</td>
<td>Blackcurrant, raspberry, blackberry, boysenberry, marionberry, gooseberry, redcurrant</td>
<td>Goiffon et al. (1991); Freytag et al. (1998); Cooney et al. (2004); Wu et al. (2004); Jordheim et al. (2007); Mertz et al. (2007); Giné Bordonaba and Terry (2008)</td>
</tr>
<tr>
<td>Cyanidin-3-rutinoside</td>
<td>OH</td>
<td>H</td>
<td>α-Glucose-L-rhamnose</td>
<td>Blackcurrant, raspberry, blackberry, boysenberry, marionberry, gooseberry, redcurrant</td>
<td>Goiffon et al. (1991); Freytag et al. (1998); Cooney et al. (2004); Wu et al. (2004); Jordheim et al. (2007); Mertz et al. (2007); Giné Bordonaba and Terry (2008)</td>
</tr>
<tr>
<td>Cyanidin-3-arabinoside</td>
<td>OH</td>
<td>H</td>
<td>α-Arabinose</td>
<td>Blackberry</td>
<td>Goiffon et al. (1991); De Ancos et al. (2000); Protegente et al. (2002); Wu et al. (2004); Fan-Chiang and Wrolstad (2005)</td>
</tr>
<tr>
<td>Pelargonidin-3-glucoside</td>
<td>H</td>
<td>H</td>
<td>α-Glucose</td>
<td>Marionberry, blackberry</td>
<td>Goiffon et al. (1991); De Ancos et al. (2000); Protegente et al. (2002); Wu et al. (2004); Fan-Chiang and Wrolstad (2005)</td>
</tr>
<tr>
<td>Peonidin-3-rutinoside</td>
<td>OCH₃</td>
<td>H</td>
<td>α-Glucose-L-rhamnose</td>
<td>Blackcurrant, gooseberry</td>
<td>Freytag et al. (1998); Slimestad and Solheim, (2002); Wu et al. (2004); Jordheim et al. (2007)</td>
</tr>
<tr>
<td>Peonidin-3-glucoside</td>
<td>OCH₃</td>
<td>H</td>
<td>α-Glucose</td>
<td>Blackcurrant, gooseberry</td>
<td>Freytag et al. (1998); Slimestad and Solheim, (2002); Wu et al. (2004); Jordheim et al. (2007)</td>
</tr>
<tr>
<td>Malvidin-3-glucoside</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>α-Glucose</td>
<td>Blackcurrant, blackberry</td>
<td>Goiffon et al. (1991); Freytag et al. (1998); Slimestad and Solheim, (2002); Wu et al. (2004); Jordheim et al. (2007)</td>
</tr>
<tr>
<td>Cyanidin-3-sophoroside</td>
<td>OH</td>
<td>H</td>
<td>α-Glucose-L-glucose</td>
<td>Raspberry, redcurrant, boysenberry</td>
<td>Cooney et al. (2004); Wu et al. (2004)</td>
</tr>
<tr>
<td>Cyanidin-3-galactoside</td>
<td>OH</td>
<td>H</td>
<td>α-Galactose</td>
<td>Blackberry</td>
<td>Goiffon et al. (1991); Protegente et al. (2002); Fan-Chiang and Wrolstad (2005)</td>
</tr>
<tr>
<td>Cyanidin-3-arabinoside</td>
<td>OH</td>
<td>H</td>
<td>α-Arabinose</td>
<td>Blackberry</td>
<td>Goiffon et al. (1991)</td>
</tr>
<tr>
<td>Cyanidin-3-sambubioside</td>
<td>OH</td>
<td>H</td>
<td>α-Galactose</td>
<td>Blackberry</td>
<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>Cyanidin-3-xyloside</td>
<td>OH</td>
<td>H</td>
<td>α-Xylose</td>
<td>Blackberry</td>
<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>Petunidin-3-glucoside</td>
<td>OCH₃</td>
<td>H</td>
<td>α-Glucose</td>
<td>Blackcurrant, redcurrant</td>
<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>Delphinidin-3-xyloside</td>
<td>OH</td>
<td>H</td>
<td>α-Xylose</td>
<td>Blackcurrant, redcurrant</td>
<td>Wu et al. (2004)</td>
</tr>
<tr>
<td>Petunidin-3-rutinoside</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>α-Glucose-L-rhamnose</td>
<td>Blackcurrant</td>
<td>Freytag et al. (1998); Slimestad and Solheim (2002)</td>
</tr>
<tr>
<td>Malvidin-3-rutinoside</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>α-Glucose-L-rhamnose</td>
<td>Blackcurrant</td>
<td>Slimestad and Solheim (2002)</td>
</tr>
</tbody>
</table>

Fig. 14.3. Continued
Ribes and Rubus

Caffeic acid (HCA)

\[
\begin{align*}
\text{Blackcurrant (217.6 ± 14.0 µg/g DW)}^a \\
\text{Blackberry (165.5 ± 4.0 µg/g DW)}
\end{align*}
\]

Chlorogenic acid (HCA)

\[
\begin{align*}
\text{Ester of caffeic and quinic acid} \\
\text{Raspberry (0.5–2.9 µg/g DW)}^c
\end{align*}
\]

Ellagic acid (HBA)

\[
\begin{align*}
\text{Red raspberry FEA (765±142 µg/g DW)}^b \\
\text{Red raspberry TEA (900 µg/g DW)}^g
\end{align*}
\]

Fig. 14.4. Chemical structure of phenolic acids (hydroxycinnamates (HCA) and hydroxybenzoic acids (HBAs)) and flavonols and their reported occurrence in certain Ribes and Rubus berries. FEA = free ellagic acid; TEA = total ellagic acid after acid hydrolysis. \(^a\)Zademowsky et al., 2005; \(^b\)Jakobek et al., 2009; \(^c\)Zhang et al., 2010; \(^d\)Olsson et al., 2004; \(^e\)Daniel et al., 1989; \(^f\)Häkkinen et al., 2000. Values were transformed to dry weight (DW) basis based on 10% dry matter content. Values given for coumaric acid correspond to the \(m\)-isomer.
(Ri. uva-crispa L.) contained up to ten different anthocyanins, with a higher proportion of aromatic acylated anthocyanins, than seen in other commercially available berries (Jordheim et al., 2007) (Fig. 14.4). In blackcurrants and blackberries, anthocyanin content is well correlated with berry colour, since the deeper the colour of the fruit, the higher the anthocyanin content. Recently, the anthocyanin profile has been proposed as a valuable tool to distinguish between different Rubus species (Mertz et al., 2007). Similarly, by combining the anthocyanin profile with multivariate data analysis, recent work has been able to discriminate between different blackcurrant cultivars (Gine Bordonaba and Terry, 2008). Besides variations between cultivars and degrees of maturities (Rubinskiene et al., 2006; Gine Bordonaba and Terry, 2008; Giné Bordonaba and Terry, unpublished data), anthocyanin content in Ribes and Rubus species depends on the harvest season and agroclimatic conditions (Fan-Chiang and Wrolstad, 2005; Rubinskiene et al., 2006). Over the last decade, a vast number of publications have referred to the bioavailability or health-related properties of anthocyanins, including those of Ribes and Rubus fruit. So far, anthocyanins, including those commonly found in Ribes and Rubus fruit, have exhibited anti-inflammatory, antioxidant, vasomodulatory and antihaemostatic (Rechner and Kroner, 2005) activities when assessed in vitro. In addition, the beneficial effect of these compounds on the treatment of retinopathies has been known since the early 1980s (Scharrer and Ober, 1981). In earlier works, Matsumoto et al. (2001) observed that, despite the low bioavailability of these flavonoids, anthocyanins were absorbed directly, distributed to the blood and excreted in urine as their glycosilated forms. Similar findings have since been reported by other authors when working with Ribes or Rubus berry extracts as anthocyanin sources (Netzel et al., 2001; Mülleder et al., 2002; McGhie et al., 2003; Hollands et al., 2008).

As mentioned earlier, all flavonoids except flavanols from Ribes and Rubus species are found in glycosylated forms, which clearly affects their absorption (Scalbert and Williamson, 2000). Absorption in the stomach is possible for some flavonoids in their aglycone form, but not for their glycosides. It has been postulated that glycoside forms may resist gastric hydrolysis and therefore arrive in the duodenum as intact molecules. Similarly in the small intestine, absorption is limited to aglycones and some of their glucosides. As a result, most flavonoid molecules linked to rhamnose or other glycoside moieties need to be hydrolysed by the colon microflora prior to their absorption (Scalbert and Williamson, 2000). Anthocyanins, though, may represent an exception, since intact glycosides have been recovered from urine or identified as the main form in blood. In contrast, there is little evidence of anthocyanin aglycones in human blood or urine (Kay, 2006), which may be related to the poor stability of such compounds in neutral pH conditions. This dichotomy has resulted in the mechanisms involved in anthocyanin metabolism and absorption still not being understood fully. For instance, Passamonti et al. (2003) suggested that glycosides of anthocyanins might be transported by bili-translocase at the gastric level, whereas Wu et al. (2004) proposed that these molecules might be converted into glucuronides by uridine 5'-diphosphate (UDP) glucose dehydrogenase. Generally, the urinary excretion of anthocyanins reported is very low, ranging from 0.016 to 0.13% of dosage within the first 2–8 h after consumption (Nielsen et al., 2003). Nevertheless, recent evidence strongly suggests that anthocyanin metabolites may be overlooked with the current identification methods and hence the absorption of these compounds may have been underestimated dramatically (Felgines et al., 2003). In the particular case of blackcurrants, Nielsen et al. (2003) studied the absorption and excretion of blackcurrant anthocyanins and found that the rutinoside forms were detected in urine, from both Watanabe heritable hyperlipidaemic rabbits and healthy humans, in higher concentrations (per cent excretion from 0 to 4 h; 0.058 ± 0.033) than the anthocyanin glucosides (0.046 ± 0.043). The authors suggested that this was due probably to the cleavage of the glucoside forms, but not of the rutinosides, in the small intestines, by β-glucosidases. Interestingly, blackcurrant berries are especially rich in both cyanidin and delphinidin rutinoside (Giné Bordonaba and Terry, 2008). Other studies have found larger proportions of ingested
Ribes and Rubus

269

delphinidin glycosides than of ingested cyanidin glycosides in blood (Matsumoto et al., 2001) or that the concentration of anthocyanin glycosides in plasma increases and decreases more rapidly compared with their respective rutinoside forms. Similarly, the plasma concentration:dose ratio in pigs after ingestion of marionberry freeze-dried powder was greater for cyanidin-3-rutinoside than for cyanidin-3-glucoside (Wu et al., 2004). Bioavailability and fate of anthocyanins are, however, also influenced by the food matrix and few studies to date have focused on this issue. Nielsen et al. (2003) studied different food matrices and found that rabbits fed blackcurrant juice showed higher plasma level of anthocyanins than animals fed with purified anthocyanins in an aqueous citric acid matrix. However, results from the same study showed that, in human subjects, the concentration of anthocyanins in plasma was not affected by the additional ingestion of a highly carbohydrate-rich meal (Nielsen et al., 2003). Further research should address the role that the food matrix may have on the bioavailability of anthocyanin and other flavonoids.

Phenolic acids

Phenolic acids, including hydroxybenzoic or hydroxycinnamate acids, are non-flavonoid polyphenolic compounds that are of significant importance in berries from the Ribes and Rubus genera (Häkkinen et al., 1998, 1999) (Fig. 14.4). Nevertheless, significant discrepancies exist between different published works found in the literature regarding phenolic concentrations within these berries. Most of these variations may be due not only to the different cultivars or agroclimatic conditions assessed, but also to the techniques used to extract and quantify the different phenolic fractions (Gine Bordonaba and Terry, 2008).

Hydroxybenzoic acids have a general structure derived directly from benzoic acid, with variations in the hydroxylation or methylation or the aromatic ring, whereas hydroxycinnamic acids tend to occur naturally as conjugated forms, being esters of hydroxy acids such as quinic, shikimic and tartaric acids or the corresponding sugar derivatives (Naczk and Shahidi, 2006). For instance, ellagic acid (Fig. 14.4), a type of hydroxybenzoic acid derivative, is known to be present in both Ribes and Rubus berries, but particularly in raspberries, where it accounts for approximately 88% of the total phenolic acids (Häkkinen et al., 1999; Amakura et al., 2000; Olsson et al., 2004). Raspberries and blackberries contain three times more ellagic acid than walnuts and 15 times more than other fruit and nuts (Tomás-Barberán and Clifford, 2000). Accordingly, ellagic acid concentration in raspberries was reported as 765 ± 142 µg/g DW, while this compound was not detected in any of the other berries analysed (Olsson et al., 2004). Other studies have detected 400 µg/g DW in freeze-dried raspberries when extracted with methanol (Daniel et al., 1989). In the same study, after acid hydrolysis, concentrations rose up to 1900 µg/g DW, indicating that most of the ellagic acid present in raspberries was encountered as ellagitanins (Daniel et al., 1989). Similarly, in raspberry cvs. Zeva, Heritage and Williamet ellagic acid was detected together with six other ellagic acid derivatives (Tomás-Barberán and Clifford, 2000). The corresponding ellagic acid concentrations in blackberry pulp and seeds were reported as 2.43 mg/g DW and 3.37 mg/g DW (Wang et al., 1996). Hydroxycinnamic acids (113 ± 43 µg/g DW), quercetin (28 ± 9 µg/g DW), quercetin-glycosides (99 ± 35 µg/g DW) and other flavonols (50 ± 34 µg/g DW) were detected by Olsson et al. (2004) in blackcurrant berries using an HPLC coupled to a diode array detector (DAD). Häkkinen et al. (1999) found large quantities of p-coumaric and caffeic acids in blackcurrant berries when a large set of different berries was screened for their phenolic content. When assessing variation in the phenolic content of different small, Polish grown berries, including blackcurrant and blackberries, Zadernowski et al. (2005) reported up to 14 different phenolic compounds. m-Coumaric acid derivatives were the principal phenolic compounds, with concentration over threefold higher in blackcurrants (1872.9 ± 145 µg/g DW) than in blackberries (596.6 ± 75.1 µg/g DW).

Although phenolic acids are the major polyphenols ingested by humans, the bioavailability of these compounds has not yet
received the same attention as that of flavonoids (Lafay and Gil-Izquierdo, 2008). The limited information so far reveals that, for instance, absorption of ferulic acid (Fig. 14.4) takes place mainly in the small intestine, with a urinary excretion of 40% of the ingested dose, whereas ferulic acid conjugates are absorbed principally in the large intestine (Kern et al., 2003a,b) and with lower recoveries. Gallic acid (Fig. 14.4) is absorbed fairly well in the upper part of the gut, with urinary excretions ranging from 36 to 40% of the ingested dose, depending very much on the food source (Shahrzad et al., 2001).

Similarly, when hydroxycinnamic acids are ingested, they are absorbed rapidly, which indicates an absorption in the upper part of the gut (Lafay and Gil-Izquierdo, 2008).

14.2.2 Tannins and stilbenes

Tannins are also important components of berry fruit (Szajdek and Borowska, 2008) and are responsible for the astringent taste of some fruit from different Rubus and Ribes species. Basically, the tart taste of certain berries can be attributed, in part, to the interactions between this type of polyphenol and proteins. Tannins include both condensed non-hydrolysable tannins (namely, proanthocyanidins) and hydrolysable tannins (namely, esters of ellagic and gallic acids, also known as ellagitannins and gallotannins, respectively). Although hydrolysable tannins are encountered more rarely in berries (Szajdek and Borowska, 2008), Mertz et al. (2007) has described two different ellagitannins detected in blackberry extracts, the first one consisting of lambertianin C and the second one identified tentatively as sanguin H-6, which has been previously identified in Ribes species (Määttä et al., 2003). Recently, McDougall et al. (2008) identified similar mixtures of ellagitannin components and ellagic acid in a tannin-enriched extract profile from raspberry and cloudberry fruit. Similarly, Nohynek et al. (2006) found similar concentrations of ellagitannins in both raspberry and cloudberry, while these components were not detected in blackcurrants.

In blackcurrants, all the cultivars investigated by Wu et al. (2004) had a similar proanthocyanidin profile containing both procyanidins and prodelphinidins. In the same study, polymeric proanthocyanidins with a degree of polymerization superior to ten were the main proanthocyanidins detected (80% of 1.21–1.66 mg/g FW) (Wu et al., 2004). Similarly, earlier works established that the average degree of polymerization in blackcurrant proanthocyanidins was 38.7 (Gu et al., 2003). In a range of gooseberry cultivars and redcurrants cv. Red Lake, proanthocyanidins with a high degree of polymerization (> 10) also accounted for most of the total concentrations of these compounds (0.45–1.34 and 60.8 mg/g FW, respectively) (Wu et al., 2004).

Both condensed tannins and hydrolysable tannins show a greater free radical scavenging capacity than vitamin C or other types of polyphenol (Szajdek and Borowska, 2008), and hence their potential role in ameliorating oxidative stress related to many diseases. The bioavailability and metabolism of these types of polyphenols have been studied extensively during the past years and the results indicate, for instance, that ellagitannins are metabolized primarily by the intestinal flora rather than being absorbed directly in the human body (Cerdá et al., 2005). Similarly, in vitro studies using human colonic microflora have demonstrated, to a certain extent, that polymeric proanthocyanidins are almost completely degraded in 48 h (Déppez et al., 2000).

Although initial studies showed that proanthocyanidins were metabolized and absorbed in both mice and rats (Santos-Buelga and Scalbert, 2000), more recent studies have failed to corroborate such findings (for the interested reader, see the excellent review by Beecher, 2004).

14.2.3 Ascorbic acid (vitamin C)

Ascorbic acid (AsA) is one of the most important water-soluble vitamins. Most plants and animals are able to synthesize this compound; however, apes and humans lack the enzymes
required and therefore AsA has to be supplemented, mainly through the consumption of fruit and vegetables (Naidu, 2003).

Similarly to that described for polyphenolic-type compounds, vitamin C concentration in berries from Ribes and Rubus species depends on several factors such as genotype, cultivation techniques, agroclimatic conditions, ripeness and postharvest storage and time (Hancock et al., 2007; Giné Bordonaba and Terry, 2008; Chope, Giné Bordonaba and Terry, unpublished data). In blackcurrant, the synthesis and role of AsA have been elucidated recently (Hancock et al., 2007). Variation in AsA content exists among blackcurrant cultivars (Viola et al., 2000; Giné Bordonaba and Terry, 2008) and such variation has been suggested as being established during the initial development stages (Viola et al., 2000). Recently, a wide range of UK grown blackcurrant cultivars was screened for several quality and health-related components (Giné Bordonaba and Terry, 2008) and the concentrations of AsA detected ranged from 1.922 to 5.415 mg/g FW, and therefore were far higher than those found in other common berry fruit, in which AsA content is commonly <1 mg/g FW. Similarly, other studies also found that AsA content in blackcurrant samples was much higher than that in the other berries analysed (Remberg et al., 2007). In other Ribes and Rubus fruit, concentration of AsA varies from 0.15 to 0.17 in blackberries, 0.15 to 0.32 in raspberries and 0.17 to 0.21 mg/g FW in redcurrants (Hägg et al., 1995; De Ancos et al., 2000; Haffner et al., 2002; Benvenuti et al., 2004).

Consumption of products naturally rich in AsA is associated with multiple health benefits. For instance, both ascorbate and dehydroascorbate delay the initiation of low-density lipoprotein (LDL) oxidation (Retsky and Frei, 1995), which is a process related to the formation of atherosclerosis. In addition, vitamin C plays an important role in the biosynthesis of certain vital constituents (namely, collagen, carnitine, neurotransmitters) and also stimulates immunological resistance, and can act as a detoxicant for certain mutagenic and carcinogenic compounds (Coulter et al., 2006). In this context, extensive clinical, animal and in vitro studies have been conducted during the past decades, trying to elucidate such health-promoting properties (for further information, see the review by Naidu, 2003). Nevertheless, a study by Olsson et al. (2004) failed to demonstrate any prevention of cancer cell proliferation using an ascorbate standard alone (Olsson et al., 2004). In the same study, a correlation was found between inhibition of cancer cell growth and AsA content between the different Ribes and Rubus extracts analysed and, therefore, the authors speculated that such a phenomenon was most probably the result of a synergistic effect of vitamin C with other bioactives present in the extracts studied (Olsson et al., 2004).

### 14.2.4 Fatty acids

Research over the past two decades has been carried out on the metabolism of polyunsaturated fatty acids (PUFAs) in general, with special emphasis on that of n-3 fatty acids (Simopoulos, 1999). This is due, in part, to the early evidence indicating that reducing the ratio of n-6 to n-3 fatty acids might play a role in decreasing the risk of heart disease and cancer. Nevertheless, numerous health-promoting properties are also reported for certain n-6 fatty acids (Ruiz del Castillo et al., 2002) that are encountered relatively rarely in nature. Today, it is known that fatty acids are essential for normal growth and development, and also may have a crucial role in the prevention and treatment of coronary and metabolic diseases, as well as inflammatory and autoimmune disorders and cancer (Simopoulos, 1999). Within the 30 different blackcurrant genotypes studied, Ruiz del Castillo et al. (2002) found that γ-linolenic (n-6) acid (GLA) ranged from 11 to 19% of the total fatty acid fraction, whereas two other fatty acids, stearidonic and α-linolenic (n-3), varied from 2 to 4% and 10 to 19%, respectively (Ruiz del Castillo et al., 2002). Few natural products are such rich sources of GLA as blackcurrant seeds. This fatty acid, in particular, is transformed to dihomo-γ-linolenic acid (DGLA; 20:3 n-6), the intermediate precursor of prostaglandin E1, which is recognized for its anti-inflammatory and immunomodulating properties (Leventhal et al., 1994). Supplementation with GLA has been shown to be a
satisfactory remedy for a diverse range of conditions, including rheumatoid arthritis and atopic eczema. In the Rubus genus, blackberries are an exceptionally rich source of omega-3 (α-linolenic acid; n-3) and other PUFAs, owing in part to their numerous and large seeds (Bushman et al., 2004). Seeds, however, tend to pass intact through the alimentary canal and hence any bioactives contained in this part of the fruit may not be assimilated. The preparation of berry extracts may overcome this limitation by enabling a better homogenization of the different components distributed in the whole fruit rather than specific tissues. Cold-pressed black raspberry, marionberry and boysenberry seed oil had 32.4, 15.8 and 19.5%, respectively, of α-linolenic acid and 53.0, 62.8 and 53.8%, respectively, of linoleic acid (Parry et al., 2005). In the same study, boysenberry seed oil showed the highest scavenging activity against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and peroxy radicals induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), followed by red raspberry and marionberry (Parry et al., 2005).

14.3 Chemopreventive Activity and Bioavailability

14.3.1 Introduction

Generally, it is accepted that a correct balance between oxidants and antioxidants is synonymous with good health and that alterations to this balance are associated with certain pathologic conditions such as ageing, cancer and cardiovascular diseases. In this context, most of the health benefits associated with the intake of berries from Ribes and Rubus species have been linked largely with the high antioxidant capacity of these fruit, as assessed in vitro or in cell-free systems. However, the health-related properties of these berries may not be limited to the presence of antioxidant compounds. For example, several studies have reported the benefits derived from blackcurrant seed oil (BSO) due to its high content of GLA (Noli et al., 2007), as well as the health benefits derived from the intake of blackcurrant polysaccharide fractions (Takata et al., 2005). Moreover, when considering recent studies and taking into account the low bioavailability of certain phytochemicals such as flavonoids, it appears that the health benefits associated with these berries may be the result of more complex biological processes rather than simply their capacity to scavenge free radicals. Williams et al. (2004) suggested that flavonoids might act as modulators of intracellular signalling processes, which could modify cellular redox status. Others (Seeram, 2008) suggested a synergistic effect among different berry bioactives as being responsible for many of the reported health-promoting properties.

Fruit and other parts from Ribes and Rubus plants have been used extensively as remedies for many diseases, and the relevant data can be traced back to as early as the 16th century (Dai et al., 2007). Nowadays, there is a plethora of scientific reports available that describe the beneficiary role these berries may have on cardiovascular diseases, brain dysfunction and ageing, eye care, urinary tract health, and antimutagenic, anticarcinogenic, antibiotic and anti-inflammatory processes (Tables 14.3 and 14.4). Some of the most relevant information is summarized in the following sections.

14.3.2 Cancer studies

The anticarcinogenic effects derived from the intake of Ribes and Rubus species are well documented (Table 14.3). Bioactive compounds in the berries play different roles in cancer prevention, such as protection against oxidative DNA damage and the formation of DNA adducts, enhancement of DNA repair mechanisms and modulation of signalling pathways involved in different crucial cellular processes (namely, cell proliferation, apoptosis, inflammation, angiogenesis and arrest of the cell cycle) (Stoner et al., 2008). ROS-induced DNA damage may be recognized as the possible first step involved in the complex process of carcinogenesis. Several studies (Table 14.3) have demonstrated in vitro or even in vivo (using animal models) the effects of Ribes and Rubus bioactives in
Table 14.3  Reported anticarcinogenic properties of fruit from *Ribes* and *Rubus* species.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of cancer cell proliferation in a dose-dependent manner</td>
<td>HepG2 human liver cancer cells</td>
<td>Extract equivalent to 50 mg/ml of raspberry extracts</td>
<td>Raspberry extracts from four different cultivars (Heritage, Kiwigold, Goldie and Anne)</td>
<td>Liu et al. (2002)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of the growth of premalignant and malignant human oral cell lines</td>
<td>Human oral epithelial cell lines; malignant (83-01-82CA), premalignant (SC-83-01-82)</td>
<td>50-200 pg/ml twice over 6 days</td>
<td>Different fractions from freeze-dried black raspberry extracts (namely, ferulic acid, β-sitosterol) from cv. Jewel</td>
<td>Han et al. (2005)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Preventing cell proliferation</td>
<td>Human colon cancer cells (CaCo-2) and human cervical cancer cells (Hela)</td>
<td>25–75 μg of GAE/ml</td>
<td>DigestedΔ raspberry extracts (cv. Glen Ample)</td>
<td>McDougall et al. (2005)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of tumour induction by N-nitroso-methylbenzylamine</td>
<td>Mouse epidermal (JB6 C1 41) cells</td>
<td>50 and 100 μg/ml of bioactive fractions</td>
<td>Different bioactive fractions from freeze-dried black raspberries cv. Jewel</td>
<td>Hecht et al. (2006)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Scavenge ultraviolet induced OH and O₂ radicals</td>
<td><em>In vitro</em>: JB6 cells</td>
<td>3.5 μM C3G/mouse</td>
<td>Cyanidin-3-glucoside from blackberry</td>
<td>Ding et al. (2006)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of human colon tumour cell growth</td>
<td>HT 29 human cancer cells</td>
<td>13.6–49.2 pg anthocyanins/ml</td>
<td>Blackberry (cv. Hull) extracts</td>
<td>Dai et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Suppression of interleukin-12 release</td>
<td>Mouse bone marrow-derived dentritic cells</td>
<td>0–40 pg anthocyanins/ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Continued*
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of cancer cell proliferation in a dose-dependent manner</td>
<td>Human cervical cancer (He La) <em>in vitro</em></td>
<td>17.5 µg/ml GAE</td>
<td>Ellagitannin-rich fraction from raspberry cv. Glen Ample</td>
<td>Ross <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Preventing cell proliferation</td>
<td>Human colon cancer cells (CaCo-2) and human cervical cancer cells (He La)</td>
<td>EC₅₀ 25–40 µg polyphenols/ml</td>
<td>Different polyphenolic fractions from various berry extracts (lingonberry, raspberry, etc.)</td>
<td>McDougall <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of N-nitrosomethylbenzylamine-induced tumours in the rat oesophagus</td>
<td>Sprague–Dawley male rats</td>
<td>Different treatments containing either 5% anthocyanin fractions of black raspberry at different concentrations or freeze-dried black raspberry extract</td>
<td>Freeze-dried black raspberry extracts</td>
<td>Wang <em>et al.</em> (2009)</td>
</tr>
</tbody>
</table>

*Notes: *Whenever possible sample tissue or the cultivars used are specified; *samples were digested chemically, mimicking the conditions that occur in the gastrointestinal tract. GAE = gallic acid equivalents.
Table 14.4. Miscellaneous health-promoting properties of *Ribes* and *Rubus* species reported in the literature.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antirheumatoid</td>
<td>Reduction in signs and symptoms of disease activity in rheumatoid arthritis patients</td>
<td>Human subjects</td>
<td>1.05 g/day</td>
<td>Blackcurrant seed oil (BSO)</td>
<td>Leventhal <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Supression of both cellular and fluid phases of inflammation as induced by monosodium urate crystals</td>
<td>Sprague-Dawley rats</td>
<td>ND</td>
<td>BSO (γ-linolenic and α-linolenic acid)</td>
<td>Tate and Zurier (1994)</td>
</tr>
<tr>
<td>Cardiovascular health</td>
<td>Inhibition of blood pressure (BP) over 40% and reduction in diastolic BP</td>
<td>Human subjects</td>
<td>6 g BSO/day over 8-week period</td>
<td>BSO</td>
<td>Défense and Leeds (1996)</td>
</tr>
<tr>
<td>Cardiovascular health</td>
<td>Favourable blood pressure lowering effect of gammalinolenic acid</td>
<td>Spontaneous hypertensive rats</td>
<td>11% by weight of BSO</td>
<td>GLA-enriched BSO (17% GLA)</td>
<td>Engler and Engler (1998)</td>
</tr>
<tr>
<td>Eye health</td>
<td>Preventing myopic refractory shift during visual tasks and promoting visual recovery</td>
<td>Double-blind, placebo-controlled crossover study with healthy human subjects</td>
<td>12.5, 20 and 50 mg/subject</td>
<td>Blackcurrant anthocyanoside concentrate</td>
<td>Nakaishi <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>Antiurolithiasis</td>
<td>Alkalizing effect in urine (greater pH and oxalic acid and citric acid in urine) which could support the metaphylaxis and treatment of urolithiasis</td>
<td>Human subjects</td>
<td>330 ml blackcurrant juice in three loading phases/person</td>
<td>Blackcurrant juice</td>
<td>Keßler <em>et al.</em> (2002)</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular health</td>
<td>Endothelium-dependent vasorelaxation effect</td>
<td>Thoracic aorta from male Sprague-Dawley rats</td>
<td>10–30 μg/ml blackcurrant concentrate</td>
<td>Blackcurrant concentrate</td>
<td>Nakamura et al. (2002)</td>
</tr>
<tr>
<td>Antiviral activity</td>
<td>Anti-influenza virus (IV) activity</td>
<td>Confluent monolayers of MDCK cells infected with IV A and IV B</td>
<td>10–100 μg/ml IC&lt;sub&gt;50&lt;/sub&gt; = 3.2 μg/ml</td>
<td>Blackcurrant extract (Kurokarin extract)</td>
<td>Knox et al. (2003)</td>
</tr>
<tr>
<td>Antiatherosclerosis</td>
<td>Increase plasma cholesterol and LDL cholesterol</td>
<td>Watanabe heritable hyperlipidaemic rabbits</td>
<td>100 mg anthocyanins/100 g std diet</td>
<td>Purified anthocyanins from blackcurrant and blackcurrant juice</td>
<td>Nielsen et al. (2005)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Inhibition of α-amylase and α-glucosidase (insulin-like effects)</td>
<td>In vitro assays</td>
<td>10–1500 μg GAE/ml (α-amylase assay)</td>
<td>Blackcurrant</td>
<td>McDougall et al. (2005)</td>
</tr>
<tr>
<td>Antimicrobial activity</td>
<td>Inhibition of human pathogenic bacteria</td>
<td>Liquid cultures of selected pathogenic bacteria (staphylococcus and Salmonella)</td>
<td>1–5 mg/ml extracts or 2–10 mg/ml dry berry powder</td>
<td>Raspberry and blackcurrant extracts</td>
<td>Puupponen-Pimiä et al. (2005)</td>
</tr>
<tr>
<td>Immunostimulatory effects and anticarcinogenic</td>
<td>Macrophage stimulating activity. Especially interleukin (IL) inducing activity and retardation of tumour growth when tested in vivo</td>
<td>In vitro; Earlich carcinoma-bearing mice</td>
<td>Juice 10 ml/kg body weight</td>
<td>Polysaccharide-rich fraction from blackcurrant juice</td>
<td>Takata et al. (2005)</td>
</tr>
<tr>
<td>Antimicrobial activity</td>
<td>Inhibited growth of <em>Staphylococcus aureus</em> DSM 799 and <em>Enterococcus faecium</em> DSM 2918</td>
<td>Microbial culture</td>
<td>0.22–1.224 mg/ml anthocyanins</td>
<td>Blackcurrant concentrate, blackcurrant juice and blackcurrant powder</td>
<td>Werlein et al. (2005)</td>
</tr>
<tr>
<td>Effect</td>
<td>Description</td>
<td>Model/Condition</td>
<td>Concentration/Unit</td>
<td>Source(s)</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>-------------------------------------------</td>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Antioxidant effect</td>
<td>Protection against induced $H_2O_2$ toxicity and protection of DNA damage from HL-60 human promyelocytic cells</td>
<td>SH-SY5Y human neuroblastoma cells</td>
<td>Within human physiological range</td>
<td>Anthocyanins and polyphenolic fractions from blackcurrant and boysenberry</td>
<td>Ghosh et al. (2006)</td>
</tr>
<tr>
<td>Antimicrobial activity</td>
<td>Inhibition of human pathogenic bacteria</td>
<td>Liquid culture of selected microbial pathogens</td>
<td>1 mg/ml dry berry extract</td>
<td>Raspberry, cloudberry and blackcurrant</td>
<td>Nohynek et al. (2006)</td>
</tr>
<tr>
<td>Protection against neurotoxic effect</td>
<td>Protective effect and restore the calcium buffering ability of cells subjected to oxidative stress by dopamine and amyloid $\beta_{1-35}$</td>
<td>COS-7 cells</td>
<td>100–500 $\mu$g/ml</td>
<td>Anthocyanin and phenolic fractions from blackcurrant</td>
<td>Ghosh et al. (2007)</td>
</tr>
<tr>
<td>Antioxidant and anti-inflammatory</td>
<td>Decreased plasma antioxidant activity and reduced plasma malondialdehyde</td>
<td>Human intervention study in an elderly population</td>
<td>Daily consumption per patient</td>
<td>Blackcurrant and boysenberry drink</td>
<td>McGhie et al. (2007)</td>
</tr>
<tr>
<td>Antidermatitis</td>
<td>Increased concentration of both $\gamma$-linolenic and dihomo-$\gamma$-linolenic acid in serum and improvement of atopic dermatitis</td>
<td>Dogs with atopic dermatitis</td>
<td>0.1 ml/kg body weight daily</td>
<td>BSO</td>
<td>Noli et al. (2007)</td>
</tr>
<tr>
<td>Antiobesity</td>
<td>Inhibition of pancreatic lipase activity and hence influence fat digestion and affect energy intake</td>
<td>$In vitro$ using lipase from porcine pancreas type II</td>
<td>50 $\mu$g/ml GAE</td>
<td>Different polyphenol-rich berry extracts including blackcurrant, raspberry, lingonberry, etc.</td>
<td>McDougall et al. (2009)</td>
</tr>
</tbody>
</table>
protection against oxidative DNA damage. However, probably most of the information available pertains to in vitro-based studies showing the inhibitory effect of berry extracts on different types of cancer cell lines (Liu et al., 2002; Olsson et al., 2004; Han et al., 2005; Ross et al., 2007; McDougall et al., 2008), as well as the ability of extracts to scavenge ROS (Jiao et al., 2005; Hecht et al., 2006).

Recently, McDougall et al. (2008), when assessing the inhibitory effect of a wide range of berry extracts on human cervical cancer and colon cancer cell lines, found that particularly those from the Rubus family were the most effective in preventing cell proliferation. Raspberry extracts from cv. Glen Ample, digested previously in conditions similar to those that occur in the upper gastrointestinal tract, were shown to reduce the population of human HT29 cancer cells in the G1 phase of the cell cycle (Coates et al., 2007). In the same study, the authors observed a protective effect against DNA damage in the HT29 cancer cells due to the same berry extract. In another study, raspberry extracts, from cvs. Heritage, Kiwigold, Goldie and Anne, inhibited HepG2 cell proliferation satisfactorily in a dose-dependent manner (> 10 mg/ml) (Liu et al., 2002). In this case, the authors could not explain the inhibitory effect as a result of the phenolic/flavonoid fraction of the different extracts investigated, therefore suggesting that most probably other phytochemicals were involved. Similarly, Ross et al. (2007) found that ellagitannin content in raspberry extracts (cv. Glen Ample) was strongly correlated with the inhibition of cell proliferation and therefore concluded that antiproliferative activity from raspberries was associated predominantly with the content of these bioactives. In this, as in many other studies, anthocyanin and other polyphenolic fractions were purified from interfering compounds by solid-phase extraction (Fig. 14.5).

Seeram et al. (2006) also demonstrated the antiproliferative properties of different berry extracts, including blackberry and black and red raspberry, yet the applied dose (200 μg/ml) was probably far superior to that which could be supplied in vivo (Stoner et al., 2008). That said, this seminal work clearly demonstrated the following: (i) that significant differences exist between the efficacy of different berry extracts on different cells (oral, breast, colon and prostate human cancer cell lines) and (ii) that extracts from black raspberry resulted in a significant induction of cell apoptosis (Seeram et al., 2006). Others (Dai et al., 2007; Wu et al., 2007) also concluded that extracts from different berries, including

![Fig. 14.5](example.png)

**Fig. 14.5.** Example of fractionation of polyphenolic compounds from berries into anthocyanins and other polyphenolic compounds using C18 cartridges (■ anthocyanins, □ non-anthocyanin polyphenols and △ sugars, acids and other water-soluble compounds).
blackberry (cv. Hull), inhibited cancer cell proliferation and, in specific cases, increased certain markers of cancer cell apoptosis. Certain bioactive fractions from black raspberry and blackberry extracts have also been reported as inhibitors of tumours induced by N-nitrosomethylbenzylamine (Hecht et al., 2006) or potent scavengers of ultraviolet light-induced 'OH and O₂ radicals when assessed in vitro, in mouse epidermal JB6 cells.

The health benefits associated with the intake of berry-derived products have also been investigated with the aim of developing new foodstuffs with added nutritional value. Recently, raspberry seed flour was shown to inhibit cell proliferation in human colon cancer cells (Parry et al., 2006a,b). The authors from the latest study highlighted the potential benefits of these berry-derived products in the formulation of new products. However, in an intervention study, blackcurrant seed press residue, a by-product without specific commercial value, consumed as part of a 250 g/day bread meal (containing 8% of the press residue), failed to reduce, and rather increased, oxidative stress markers in the stools and urine of 36 women (Helbig et al., 2009). Despite the fact that serum and stool total tocopherol concentrations were increased as a result of the blackcurrant seed press residue, Helbig et al. (2009) pointed out that consumption of ground berry seed might not represent any health advantage.

Although there is still a paucity of information from in vivo trials or intervention studies, as compared with that from in vitro studies, work has also been conducted using both animals and human subjects to try to elucidate the effect of Rubus and/or Ribes extracts on cancer cell proliferation. For instance, freeze-dried black raspberry extracts (cv. not specified) inhibited tumour-induced development in the rat oesophagus, by inhibiting the formation of DNA adducts and reducing the proliferation of prenoplasic cells (Chen et al., 2006). In the same study, the authors determined the possible mechanisms of action by which raspberry extracts inhibited tumour development. In addition, a polysaccharide-rich fraction from blackcurrant juice was shown to retard tumour growth when tested in Etarlich carcinoma-bearing mice (Takata et al., 2005). Clinical data from human studies also exist; for instance, Stoner’s group showed that after consumption of lyophilized black raspberry extract (60 g/day) by 50 subjects with colorectal cancer and/or polyps, proliferation and angiogenesis biomarkers were diminished, while apoptosis was enhanced (Stoner et al., 2007). Kresty et al. (2006) also showed that lyophilized black raspberry extract (32 or 45 g/day) diminished urine markers of oxidative stress in patients (n = 10) with Barrett’s oesophagus. In contrast to all these positive and encouraging results, there are also cases of human intervention studies that failed to demonstrate any beneficial effects of berry intake (Moller et al., 2004). This dichotomy or contradiction should be appreciated, as sometimes the positive benefits of fruit and vegetables are overexaggerated. Therefore, it is evident that further research is required at all levels (in vitro, in vivo and intervention studies) to understand the mechanisms by which consumption of Ribes and Rubus fruit may help against cancer.

14.3.3 Cardiovascular and metabolic diseases

In certain pathologic conditions (namely, hypertension, diabetes and atherosclerosis), the endothelium-dependent vasorelaxation caused by various vasodilator agonists is restrained considerably. Such a phenomenon is associated directly with a decrease in the release of NO, which certainly is crucial for the regulation of vasomotor tone and structure under certain physiological conditions. Given this, the development of vasodilator compounds with the ability to restore NO levels potentially could contribute to the treatments of some diseases. Nakamura et al. (2002) showed that blackcurrant concentrate could have an endothelium-dependent vasorelaxation effect when tested in vitro with rat thoracic aorta tissues. The authors found that increased levels of NO were one of the mechanisms involved in the vasorelaxation caused by the blackcurrant concentrate. In another study, purified anthocyanins from blackcurrant berries or blackcurrant juice
demonstrated an antiatheroesclerotic effect when tested in Watanable heritable hyperlipidaemic rabbits (Nielsen et al., 2005), suggesting the potential of these extracts in the prevention of certain cardiovascular conditions. A recent study in which an elderly population was given blackcurrant and other berry drinks showed a statistical significant improvement in oxidative status, as measured by plasma antioxidant capacity (McGhie et al., 2007).

There is increasing attention over the positive effect that berry-derived bioactives, and specifically anthocyanins, have on blood vessel walls (namely, vasodilation, permeability, fragility, etc.) (Kähkönen et al., 2003). Concomitant to this, anthocyanins from blackcurrant had considerable antioxidant activity when tested in vitro in lipid environments such as methyl linoleate and human LDL (Kähkönen et al., 2003). Nevertheless, some of the positive effects from berries of Ribes and Rubus species on cardiovascular health (Table 14.4) may be associated with the fatty acid composition of the oil obtained from the seeds of those species. Certain disorders, and specifically hypertension, are related to abnormalities in tissue fatty acid metabolism, due in part to a reduction in desaturase activity. As discussed earlier in this chapter, BSO is a rich source of GLA, a PUFA with known health-related properties. Engler and Engler (1998) demonstrated more than a decade ago that oil enriched with GLA from blackcurrant has a significant blood pressure lowering effect when tested in spontaneous hypertensive rats.

Evidence from in vitro studies conducted with Ribes and Rubus species, as well as other sources of anthocyanin-rich compounds, suggests that certain compounds present in these fruit may mitigate certain metabolic diseases (Table 14.4), such as diabetes. Indeed, in vitro studies revealed that flavonoids modified the insulin-secreting capacity, reduced the NaF-induced apoptosis and modulated the cell proliferation of β cells (Pinent et al., 2008). As β cells are the pancreatic cells responsible for producing and releasing insulin, they control blood glucose levels. McDougall et al. (2005) showed that, when tested in vitro, blackcurrant and raspberry polyphenol-rich extracts (with phenolic concentrations ranging from 10 to 1500 µg) had an insulin-like effect since they inhibited both α-amylase and α-glucosidase significantly (these enzymes being responsible for hydrolysing complex carbohydrates into glucose and other simple sugars and hence elevating blood glucose levels). Whereas blackcurrant (cv. Ben Lomond) extracts inhibited the α-glucosidase better, α-amylase was inhibited more readily by the raspberry (cv. Glen Ample) extract (McDougall et al., 2005). Jayaprakasam et al. (2005) found that specifically cyanidin-3-glucoside and delphinidin-3-glucoside, both anthocyanins commonly present in Ribes and Rubus species (Table 14.4), were the most effective insulin secretagogues among several anthocyanins tested in vitro. Sugimoto et al. (2003) studied the protective effects of major boysenberry anthocyanins against oxidative stress in streptozotocin induced diabetic rats. Elevated concentrations of oxidative substances in the plasma and also in the liver fell back to the levels of those observed in control rats when a diet with the berry anthocyanins was given to the diabetic animals. Accordingly, the authors pointed out that boysenberry anthocyanins were effective in protecting the development of in vivo oxidation involved with diabetes. Nevertheless, few in vivo studies and clinical data are available yet in order to validate the in vitro observations.

14.3.4 Urinary tract health and inhibition of intestinal pathogens

During the late 1950s and 1960s, several studies verified the role of anthocyanins and other polyphenols in altering microbial activity. The results from those studies demonstrated that, for instance, anthocyanins had stimulatory as well as inhibitory effects on microbial growth. More recently, the influence of blackcurrant concentrates or isolated anthocyanins from the same berry on the growth of microorganisms has been evaluated (Werlein et al., 2005). The authors concluded that, while the anthocyanin fraction alone did not have significant effects on the growth of the microorganisms studied, blackcurrant
extract inhibited in vitro the growth of certain microorganisms (Staphylococcus aureus, Enterococcus faecium), as well as stimulated the growth of Saccharomyces cerevisiae (Werlein et al., 2005). Finnish researchers have demonstrated, in several in vitro studies (Puupponen-Pimiä et al., 2001, 2005), the inhibitory effect of berry extracts, including raspberry, artic bramble and cloudberry, on the growth of both Gram-positive and Gram-negative intestinal pathogens. Recent research on this (Table 14.4) also supports the notion that proanthocyanidins commonly found in Ribes and Rubus berries prevent the adhesion of certain pathogenic bacteria to uroepithelial cells (Foo et al., 2000).

14.3.5 Ageing and brain health

There are numerous motor and cognitive behavioural deficits that occur during ageing. Although many of the mechanisms involved still remain unclear, numerous researchers sustain that oxidative stress and inflammation are, in part, involved in the ageing process (Lau et al., 2006). Indeed, Lau et al. (2006) suggested that combinations of antioxidants and anti-inflammatory polyphenols from berries might be key compounds to prevent, suppress or inhibit age-related deficits. Studies conducted on animals showed that supplementation with dietary antioxidants improved cognitive function (Joseph et al., 1998). Even though little research has been conducted in this regard with fruit from Ribes and Rubus, it is assumed that similar results to those obtained with blueberries or other berries may be observed following the consumption of these fruit. Shukitt-Hale et al. (2009) recently examined the effect of a 2% blackberry-supplemented diet in reversing the age-related deficits of rats. Results indicated that the blackberry diet not only improved motor performance on various tasks but that blackberry-fed rats had significantly greater working, or short-term, memory performance than the control rats (Shukitt-Hale et al., 2009). Another of the few relevant studies conducted on Ribes berries is that by McGhie et al. (2007) in which the ability of blackcurrant-based drinks to improve measures of oxidative stress and inflammation in an elderly population was assessed and an improvement in plasma antioxidant capacity was observed. Nevertheless, after the blackcurrant intake, plasma antioxidant capacity was the only indicator, from a wide range of oxidative stress markers studied, that improved. Anthocyanins and other polyphenolic fractions, at concentrations from 100 to 500 µg/ml, of Rubus (boysenberry cv. Riwaka Choice) and Ribes (blackcurrant cv. Ben Ard) species, have been reported to offer protection against the cytotoxic or neurotoxic effect of dopamine and amyloid β25-35 in M1 muscarinic receptor-transfected COS-7 brain cells (Ghosh et al., 2007). Either dopamine or amyloid β25-35 can disrupt the Ca2+ buffer ability of brain cells, leading to further oxidative stress and cell degeneration associated with ageing. The mechanisms underlying the positive effects of berries and other fruit and vegetables on ageing have been reviewed recently by Shukitt-Hale et al. (2008) and Joseph et al. (2009).

14.3.6 Other health-promoting properties

BSO supplemented to patients suffering from rheumatoid arthritis during a 24-week trial resulted in a significant reduction of the signs and symptoms of disease activity (Leventhal et al., 1994). The authors concluded that BSO was a potentially effective treatment for rheumatoid arthritis. Similarly, other studies conducted in vivo with Sprague-Dawley rats demonstrated that BSO suppressed both the cellular and fluid phases of inflammation significantly (Tate and Zurier, 1994). In this context, later studies showed that purified anthocyanins from blackcurrant and other berries were responsible for the inhibition of nuclear factor-κB, which controls the expression of many genes involved in the inflammatory response, as well as the reduction of proinflammatory mediators, when tested in healthy adults (Karlsen et al., 2007).

Consumption of blackcurrant berries has also been associated with positive effects against kidney stone formation. Köfler et al. (2002) showed that blackcurrant juice could be used as a support treatment and metaphylaxis
of uric acid stones due to its alkalizing effects. Crude extracts from wild blackcurrant berries had antiviral effects against influenza virus in a study conducted by Suzutani et al. (2003). This antiviral activity was speculated to be related to the interaction between the combining site on the viral envelope and certain constituents, not identified, in the crude extracts.

BSO administered to dogs suffering from atopic dermatitis resulted in increased concentrations of both GLA and dihomo-linolenic acid in the serum of the animals, but, more importantly, an improvement in the dermatitis was also observed (Noli et al., 2007). Other health-promoting properties from Ribes or Rubus berries relate to eye vision. Nakaishi et al. (2000) demonstrated, in a double-blind placebo-controlled crossover study with healthy human subjects, that blackcurrant anthocyanins at doses of 12.5, 20 or 50 mg had a positive effect, preventing myopic shift during visual tasks and promoting visual recovery. In the same study, oral intake of blackcurrant anthocyanins was found to decrease the dark adaptation threshold in a dose-dependent manner.

A few years later, Matsumoto et al. (2006), revealed the ocular distribution of blackcurrant anthocyanins in rats and rabbits after the oral, intravenous or intraperitoneal administration of anthocyanins isolated from blackcurrants. This study revealed, for the first time, that blackcurrant anthocyanins were absorbed and distributed in ocular tissues as intact forms and passed through the blood-aqueous barriers and blood-retinal barriers in both of the animals investigated. In summary, the above-mentioned studies may have demonstrated that oral intake of purified anthocyanins or anthocyanin-rich extracts from Ribes and Rubus species may be used therapeutically for the treatment of certain ophthalmological conditions.

14.4 Effect of Preharvest, Postharvest and Processing

The level of secondary metabolites in plants from Ribes and Rubus is regulated by both environmental and genetic factors. Plants produce a wide range of bioactive compounds as a result of survival or adaptive strategies. These bioactive compounds are plant secondary metabolites produced for defence, protection and cell-to-cell signalling as a response to exposure to certain environmental stresses. Although the environmental mechanisms responsible for enhanced bioactive content in Ribes and Rubus species still remain unclear, cultivation of plants under certain stress conditions is one of the means by which the content of these berry bioactives can be enhanced. In addition, extensive research is being done, through several breeding programmes worldwide, to develop improved varieties with enhanced content of phytochemicals in combination with low-input cropping systems (Brennan et al., 2008).

Blackberries, blackcurrants and raspberries, like other berries from the Ribes and Rubus genera, are not only available fresh but are distributed mainly as frozen and thermally processed products (namely, jams, jellies, juices, purees, cobblers and pies). For instance, most of the blackcurrant market in the UK is designated for the production of blackcurrant juice (e.g. Ribena®). After harvest, the quality of both Ribes and Rubus fruit declines dramatically, making postharvest storage at chilling temperatures (around 0°C) a requirement for the industry, generally for periods no longer than 3 weeks (Harb et al., 2008). Controlled atmosphere (CAs) are also used occasionally to extend storage life, not only for blackcurrants but also for many other perishable berry fruit (Agar et al., 1997; Terry et al., 2009), when prolonged storage is required. However, both nutritional value and quality of berries are known to be affected negatively by postharvest storage conditions. For example, the concentration of AsA in berries tends to decrease with increased storage time and temperature (Roelofs et al., 1993; Agar et al., 1997; Kalt et al., 1999; Häkkinen et al., 2000; Viola et al., 2000; Antunes et al., 2003). In particular, Roelofs et al. (1993) showed that AsA content was reduced significantly when redcurrant berries were stored for 25 days at either 1°C or at fluctuating temperatures between 10 and 20°C. Similarly, a reduction of 40% in AsA content was observed in blackcurrant berries stored for 10
days at 10 or 20°C (Viola et al., 2000). Even greater reductions in AsA, up to 50% of the initial content, were reported by Antunes et al. (2003) in blackberries stored at 20°C. Generally, the decline in AsA, and, indeed, in overall acid concentrations, during storage is accompanied by a darkening of the berry (Chope, Giné Bordonaba and Terry, unpublished data). This change in coloration has been related to an increase in anthocyanin concentration (Robbins et al., 1989; Kalt et al., 1999), which occurs in a temperature- and time-dependent manner. Raspberries stored at 0°C for 24 days contained 70% more anthocyanins than the initial values after harvest (Robbins et al., 1989). Conversely, another study (Chanjirakul et al., 2006) showed that, in raspberries stored for 7 or 10 days at 10°C, the concentration of anthocyanins was reduced considerably as compared with initial values before storage. Other bioactives are also affected by storage temperature. Ellagic acid content in red raspberry was reduced by 30% after 9 months of storage at ~20°C in a study conducted by Hakkinen et al. (2000). As mentioned earlier, CAs, and in particular those with high CO₂ concentrations, may be used to extend the shelf life of many berries, including blackberry, raspberry and currants (Terry et al., 2009). However, under these storage conditions, berries from Ribes (namely, black- and redcurrants) and Rubus (blackberry and raspberry) tend to suffer considerable reductions in their AsA content (Agar et al., 1997). Little research has been conducted on elucidating the effects of CA storage on other common bioactives from Ribes and Rubus species.

The effect of postharvest processing treatment is also well documented. In all thermally processed blackberry-derived products, the concentration of monomeric anthocyanins, as well as the antioxidant activity of the products, declined dramatically compared with those seen in non-treated products (Hager et al., 2008). In the same study, juice processing resulted in the greatest losses, whereas canned products were the least affected by processing. Most of the anthocyanin losses occurred during blanching and enzymatic treatment of blackberry juice (34% loss in total monomeric anthocyanins). In contrast, total phenolic concentration of blackcurrant juices stored at 4°C tended to decline from day 0 (1919.8 ± 149.5 mg/ml GAE) to day 15 (1309.6 ± 107.8 mg/ml GAE), but returned to their initial values after 29 days of storage (Piljac-Žegarac et al., 2009). However, antioxidant capacity, as measured by the Trolox equivalent antioxidant capacity (TEAC) assay, was diminished significantly. Postharvest storage of processed blackberry products also resulted in significant losses of monomeric anthocyanins, but had little or no significant effect on the antioxidant activity of most of the products. In another study, processing blackcurrant-derived products also reduced the content of total anthocyanins dramatically (to 0.05-10.3% of the levels in fresh fruit) but did not enhance the urinary yield in human subjects (Hollands et al., 2008).

14.5 Conclusions and Future Research Needs

A considerable number of recent studies advocate that a high intake of Ribes and/or Rubus fruit may offer a number of health benefits against degenerative diseases and can promote longevity. Based on the survey of the literature presented herein, there is no doubt that most Ribes and Rubus berries are particularly rich sources of biologically active compounds (i.e. they have high levels of anthocyanins, proanthocyanidins, quercetin, myricetin, phenolic acids, etc.). In addition, blackcurrants are one of the richest sources of vitamin C, contributing, together with bioactive phenolics, to the high antioxidant activity of the berries. The array of health-promoting properties of these berries includes the inhibition of the development of certain cancers, cardiovascular and metabolic disorders and inflammation-related diseases. Besides, Ribes and Rubus fruit may be used therapeutically to treat urinary infections, ophthalmological diseases and even fight against ageing-related conditions. Blackcurrant was demonstrated recently to provide effective neuroprotection against oxidative stress-induced neuronal
damage in human cell cultures. Among the bioactives of these berries, anthocyanins have received much more attention than the other polyphenols or non-polyphenol-type compounds, and hence further research should clarify the health-promoting properties of Ribes and Rubus bioactives other than anthocyanins.

As indicated, most Ribes and Rubus berries are consumed as derived products rather than fresh. However, most of the information related to health-promoting properties refers to fresh berries or purified berry fractions rather than the products that the consumer normally ingests. Only a limited number of studies have shown the detrimental effect of processing on the concentration and bioavailability of certain Ribes and Rubus bioactives (Hollands et al., 2008).

Despite all the positive effects mentioned earlier, robust animal and human intervention trials are still necessary in order to substantiate any claims of human health benefits.

References


15 Strawberry

Jordi Giné Bordonaba and Leon A. Terry

15.1 Introduction

Strawberry fruit (*Fragaria × ananassa* Duch.) is one of the most widely consumed fruit worldwide, as fresh fruit, processed products or even as dietary supplements, and represents an overall cultivated area greater than 200,000 ha (Liu *et al*., 2007). As fresh fruit or derived products, strawberries constitute a rich source of diverse bioactives with an array of known health-promoting properties. Recently, within a large set of 1113 food samples obtained from the US Department of Agriculture National Food and Nutrient Analysis Program, strawberries were ranked among the top three regarding their antioxidant content (3.584 mmol/serving) (Halvorsen *et al*., 2006). In a following study, Wolfe *et al.* (2008) reported that strawberry fruit were among the largest suppliers of cellular antioxidant activity from 25 different fruit and vegetables consumed by the American population. In agreement with others, strawberries were also the top source of antioxidants from fruit and vegetables (FAV) in the Scottish population studied by Haleem *et al.* (2008).

15.1.1 The strawberry fruit

Strawberry is a perennial plant with rooting runners that usually bears red fruit once it is developed. Botanically, a strawberry fruit is in fact a ‘false fruit’, being described as a modified receptacle with one-seeded fruits or achenes located on the outer surface (Perkins-Veazie *et al*., 1995). Whereas most crops were domesticated some 10,000 years ago, the first strawberry species can only be tracked to Roman times (approximately 2200 years ago), when wild species were grown for their appealing flavour and fragrance. Cultivation of strawberry fruit in Europe did not start until many years later, in the 14th century, when first the French, followed rapidly by the English, started to see strawberry plants not only as ornamental plants but as an intriguing food source. Towards the end of the 16th century, three European *Fragaria* species were commonly referred to – *F. vesca* (diploid), *F. moschata* (hexaploid) and *F. viridis* (diploid) – and shortly after, a new wild strawberry, discovered in the eastern part of North America (*F. virginiana*; octoploid), was introduced in European gardens, due mainly to its intense fragrance. Later, another American species, *F. chilonensis* (octoploid), characterized by good-size fruit, reached Europe after its discovery on the Pacific coast of the American continent (Medina-Minguez, 2008).
Due to the sterility of *F. chilensis* plants when introduced in Europe, *F. moschata* and *F. virginiana* grown rapidly in between *F. chilensis* plants, leading to a new strawberry hybrid that was later named *F. x ananassa* by Duchesne, in about 1780. The generation of this octoploid hybrid only 230 years ago was the origin of most of the cultivated strawberries now grown worldwide. Since then, hundreds of strawberry cultivars (*F. x ananassa* Duch.) have been grown, with cv. Elsanta nowadays probably being the predominant cultivar in north-western Europe.

Through history, strawberries have been appreciated not only for their particular flavour but also for their medicinal properties. Indeed, in the 13th century, when medical books were filled with botanical remedies, a Greek doctor, named Nicholas Myrepsur, detailed the medicinal properties of strawberries for the treatment of several illnesses (Medina-Minguez, 2008). Nowadays, the popularity of the strawberry may be attributed to its characteristic flavour and taste, which is defined in part by the balance between sugars and acids within the fruit, and to its known potential health-promoting properties.

### 15.1.2 Economic importance

Worldwide, the production of strawberries has grown steadily during the past 40 years, with most of the production in the northern hemisphere (> 95%). The USA is, in official numbers, the leading producing nation, followed by Spain, Turkey and the Russian Federation (Fig. 15.1). However, no official statistics are available for the size of the strawberry industry in China, even though it is accepted that China is nowadays a direct competitor for most of the major strawberry producing regions, with estimated values for the period 2001–2003 of c.1.5 million tonnes (Mt) (Carter et al., 2005). In addition, strawberry production is a major part of the European soft fruit industry, accounting in the UK, for instance, for a production value of £96 million in 2003 (Defra, 2003).

### 15.2 Identity, Role and Bioavailability of Strawberry Bioactives

#### 15.2.1 Introduction

Certainly, strawberry fruit have long been recognized as one of the main sources of vitamin C, folic acid and dietary fibre, as well as an excellent source of polyphenols in the diet (Table 15.1). Of all the types of bioactives present in strawberry fruit, polyphenols are without doubt the ones that have received most attention. In strawberries the main polyphenols are ellagic acid, ellagic acid glycosides, ellagitannins, gallotannins, flavanols, flavonols, anthocyanins and coumaroyl glycosides (Hanum, 2004; Zhang et al., 2008), which occur with non-polyphenol bioactives such as folate and vitamin C (Tulipani et al., 2009). Up to 40 different phenolic compounds have been detected recently by Aaby et al. (2007) in strawberry fruit cv. Senga Sengana, using high performance liquid chromatography (HPLC) coupled to various detectors (namely, diode array, mass spectrometer and coulometric array).

The following sections describe in detail the main bioactive compounds present in strawberry fruit, as well as their bioavailability.

#### 15.2.2 Flavanols

Flavanols, not to be confused with flavonols, are a type of flavonoid that may play an important role in the prevention of certain pathologies (Santos-Buelga and Scalbert, 2000; González-Paramás et al., 2006). In vitro studies have endeavoured to reveal the different biological activities of these compounds, some of which are antioxidants, scavengers, of free radicals, inhibitors of tumour growth and development or antibacterial agents (Pascual-Teresa et al., 2000). The content of flavanols in strawberries was described in detail by Pascual-Teresa et al. (2000), who identified ten different compounds, catechin-(4,8)-catechin (10.1 µg/g FW), catechin (15.7 µg/g FW) and epicatechin-3-O-gallate (6.6 µg/g FW) being the main ones. Studies on flavan-3-ols showed that their bioavailability was mainly...
Fig. 15.1. (a) Twenty highest strawberry producing countries (tonnes) in 2007. * = FAO estimates or unofficial figures for production values. (b) Worldwide strawberry production over the past 45 years (source: FAO, 2009).

dependent on, and linked intimately to, their chemical structure. For instance, catechin and epicatechin monomers are supposed to be some of the most bioavailable polyphenols, with urinary excretions ranging from 1 to 30% of the ingested amounts (Tomás-Barberán, 2008). None the less, further studies are required on other flavan-3-ols to reach any conclusion on their metabolism and differential adsorption in the human body.

Proanthocyanidins are better known as condensed tannins; they are mixtures of
Table 15.1. Nutrient and bioactive composition of strawberry fruit based on data available in the literature. Values are presented as mg or μg/g of fresh fruit (FW).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (mg)</td>
<td>53–125</td>
<td>Terry et al. (2007); Tulipani et al. (2008); Giné Bordonaba and Terry (2009)</td>
</tr>
<tr>
<td>Fibre (mg)</td>
<td>23</td>
<td>ESHA food database</td>
</tr>
<tr>
<td>Sugars (mg)</td>
<td>61.95–110.45</td>
<td>Terry et al. (2007); Giné Bordonaba and Terry (2009)</td>
</tr>
<tr>
<td>Glucose</td>
<td>18.01–31.00</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>22.54–36.10</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>21.40–43.35</td>
<td></td>
</tr>
<tr>
<td>Organic acids (mg)</td>
<td>5.96–14.29</td>
<td>Terry et al. (2007); Giné Bordonaba and Terry (2009)</td>
</tr>
<tr>
<td>Ascorbate (vitamin C)</td>
<td>0.24–0.74</td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>4.2–10.1</td>
<td></td>
</tr>
<tr>
<td>Malate</td>
<td>1.52–3.45</td>
<td></td>
</tr>
<tr>
<td>Proanthocyanidins (flavanols) (mg)</td>
<td>4.47</td>
<td>Hosseinian et al. (2007)</td>
</tr>
<tr>
<td>Monomers (epicatechin, B2)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Dimers</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Oligomers</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>Polymers (up to hexamer)</td>
<td>2.22</td>
<td></td>
</tr>
<tr>
<td>Anthocyanins (μg)</td>
<td>66–571</td>
<td>Wang et al. (2003); Kosar et al. (2004); Määttä-Riihinen et al. (2004); Skupienń and Oszmianński (2004); Terry et al. (2007)</td>
</tr>
<tr>
<td>Cyanidin-3-glucoside</td>
<td>4.5–34</td>
<td></td>
</tr>
<tr>
<td>Pelargonidin-3-glucoside</td>
<td>53–441</td>
<td></td>
</tr>
<tr>
<td>Pelargonidin derivatives</td>
<td>8.4–95.9</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration (µg)</td>
<td>References</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>19.9-522</td>
<td>Gil et al. (1997); Häkkinen and Törrönen (2000); Terry et al. (unpublished data)</td>
</tr>
<tr>
<td>Folate (vitamin B)</td>
<td>0.13-0.96</td>
<td>Strålsjö et al. (2003); Tulipani et al. (2008)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>0.12-2a</td>
<td>Wang et al. (2007)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>3-40</td>
<td>Gil et al. (1997); Häkkinen and Törrönen (2000)</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>2-13.7</td>
<td>Gil et al. (1997); Häkkinen and Törrönen (2000)</td>
</tr>
<tr>
<td>Total phenolicsb</td>
<td>0.86-3.75</td>
<td>Terry et al. (2007); Giné Bordonaba and Terry (2009)</td>
</tr>
<tr>
<td>Antioxidant capacityc</td>
<td>6.2-17.8</td>
<td>Terry et al. (2007); Giné Bordonaba and Terry (2009)</td>
</tr>
</tbody>
</table>

Notes: aValues for pulp and achenes, respectively; bmeasured by the Folin-Ciocalteu assay and results expressed as mg gallic acid equivalents (GAE)/g FW; cmeasured by the FRAP assay and results expressed as µmol Fe II/g FW.
oligomers and polymers built up of flavan-3-ol units and principally held together by C4-C8 bonds (Gu et al., 2003). Recent evidence suggests that certain proanthocyanins occur not only in red wines but also in several food-stuffs, including strawberries (Pascual-Teresa et al., 2000; Fossen et al., 2004; González-Paramas et al., 2006). Proanthocyanidins are supposed to contribute to the ‘French paradox phenomenon’ by exerting several health-promoting properties, such as antioxidant, anti-inflammatory and anticarcinogenic activity (Santos-Buelga and Scalbert, 2000). The proanthocyanidin concentrations in strawberries and other berries have been described recently by Hosseinian et al. (2007). When the whole fruit was considered, strawberry (4.47 mg/g FW) ranked second after raspberry (5.05 mg/g FW) in terms of total proanthocyanidin concentration. In the same study, 0.31, 0.39, 1.55 and 2.22 mg/g FW corresponded to monomers, dimers, oligomers and polymers of the total anthocyanidin fractions encountered in the fruit.

15.2.3 Flavonoids

Flavonoids are a type of polyphenolic compound that can be divided into different subclasses, including flavans, flavonols, flavones, flavanones, isoflavones and anthocyanidins. Within the group of flavonoids, most attention has probably been paid to the anthocyanins. These are water-soluble, flavonoid-type polyphenols widely expressed in the plant kingdom. Anthocyanins have been reported to have anticarcinogenic, anti-inflammatory, vasoprotective and anti-obesity properties (McGhie and Walton, 2007). In addition, research suggests that anthocyanins may play a role in enhancing vision and improving memory (Joseph et al., 1998). Recent reports estimate an anthocyanin consumption in the USA of c.12.5 mg/day (Wu et al., 2006), a value that is exceeded in certain European countries (Mullen et al., 2008). Yet, greater consumption does not always result in greater bioavailability and/or effect. Generally, these pigments are extracted using aqueous mixtures of ethanol, methanol or acetone (Terry et al., 2007; Giné Bordonaba and Terry, 2008); however, discrepancies exist in whether acetone- or methanol-based solvents appear more efficient for the extraction of these compounds from different FAV (Giné Bordonaba and Terry, 2008). Separation and quantification of anthocyanins are generally achieved using reversed phase HPLC coupled to various detection systems (usually a photo diode array (PDA) or mass spectrometry (MS)) and acetonitrile is generally as the mobile phase of choice, due to its elution strength, low viscosity and good miscibility with water.

Anthocyanin concentration in strawberry fruit varies greatly between cultivars and is also influenced by growing conditions and maturity at harvest (Wang and Lin, 2000; Lopes da Silva et al., 2007; Terry et al., 2007; Crespo et al., 2010). In contrast to some other fruit, a vast array of information is available detailing the anthocyanin profile and concentration of strawberry fruit, all of the relevant studies identifying pelargonidin-3-glucoside as the main anthocyanin, representing over 80% of the total anthocyanin pool (Fig. 15.2).

Lopes da Silva et al. (2007) detected, through a detailed study of strawberry pigments (by means of HPLC coupled to PDA and MS detection), up to 25 different anthocyanins within the five different strawberry cultivars analysed. The authors highlighted the notable variability among anthocyanin content in samples of the same variety and harvest, and therefore pointed out the strong influence of degree of maturity, edaphic-climatic conditions and postharvest storage on the concentration of these pigments. Terry et al. (2007) identified three major anthocyanins (namely, cyanidin-3-glucoside, pelargonidin-3-glucoside derivative and pelargonidin-3-glucoside at concentrations of 2.165, 33.56 and 121.54 µg/g FW, respectively) in strawberry cv. Elsanta fruit grown in a glasshouse and subjected to full or deficit irrigation (Fig. 15.3). In the same work, the authors demonstrated that differences existed in anthocyanin content between primary and secondary fruit from the same primary truss (Fig. 15.3). Similar concentrations were later reported by Crespo et al. (2010) when studying anthocyanin concentrations in fruit from four different cultivars grown at different Swiss production sites.
Fig. 15.2. Anthocyanin profile (a) and chemical structure (b) of the main anthocyanins identified in strawberry fruit (cv. Matis). Peaks [1] and [2] are pelargonidin derivatives.
Fig. 15.3. Effect of different water irrigation regimes (ml/day) on the concentration of main anthocyanins (cyanidin-3-glucoside (Cya-3-gluc), pelargonidin-3-glucoside (Pg-3-glucoside) and pelargonidin glucoside derivative (Pg-gluc derivative)) in strawberry (cv. Elsanta) primary and secondary fruit. Results are expressed on a fresh weight (FW; upper panel) and dry weight (DW; lower panel) basis. The different letters above the columns indicate significant differences between fruits from different irrigation regimes.
In addition to fruit position, anthocyanin profiles differ spatially with different tissues/locations within the fruit. Aaby et al. (2005) showed the different anthocyanin profiles in receptacle tissue and achenes from two different strawberry cvs. (Totem and Puget Reliance). Both cultivars had pelargonidin-3-glucoside as the main anthocyanin in the flesh, whereas similar amounts of this anthocyanin and cyanidin-3-glucoside were detected in the achenes of both cultivars. Almeida et al. (2007) later demonstrated that anthocyanin levels increased during ripening, whereas flavan-3-ols decreased, in both cvs. Queen Elisa and Korona. Indeed, the authors demonstrated that, whereas, at the white stage, flavan-3-ol content was associated mainly with vascular epidermal tissue and pith, it was limited mostly to vascular tissues at more advanced development stages. Recently, Zhang et al. (2008), when studying the antioxidant properties of different strawberry fractions, found that anthocyanins including cyanidin-3-glucoside (7156 pM Trolox/mg), pelargonidin (4922 pM Trolox/mg) and pelargonidin-3-rutinoside (5514 pM Trolox/mg) gave greater antioxidant activities, as determined by the popular Trolox equivalent antioxidant capacity (TEAC) assay (see Chapters 18 and 19 of this volume), than other purified fractions from the same berries. In the same study, the authors highlighted that strawberry extracts and their purified compounds had antiproliferative activity in a dose-dependent manner when assessed in different lines of human cancer cells (oral, colon and prostate).

The biological activity of strawberry fruit is due in part to the biological activity of its polyphenols, including anthocyanins. Therefore, it is crucial to determine the bioavailability of these compounds by means of human and animal studies designed to: (i) obtain information on target organ and organ tissue distribution; (ii) assess the concentrations reached in the organs, tissues or fluids; and also (iii) assess the potential toxic effects, if any. Although not much information exists in this regard, it is evident that the body of literature regarding the bioavailability of strawberry bioactives is currently growing, and data are available on the bioavailability of ellagic acid, ellagitannins, anthocyanins, procyanidins and flavonols (Tomás-Barberán, 2008). In the particular case of strawberry anthocyanins, it has been reported that consumption of 200 g of strawberries per person resulted in a mean concentration of pelargonidin-3-glucuronide in blood plasma of 274 nmol/1 (Mullen et al., 2008). Another human study, with six healthy volunteers, three from each gender, was that conducted by Felgines et al. (2003). In this particular case, results showed that after consumption of 200 g of strawberries by each volunteer, pelargonidin-3-glucoside, its aglycon and three monoglucuronides, as well as a sulfo-conjugate were detectable as urinary metabolites. Yet, the total urinary excretion of anthocyanin metabolites represented no more than 1.80 ± 0.29% of the total pelargonidin-3-glucoside ingested. Hollands et al. (2008) concluded that strawberry anthocyanins were partially bioavailable, with a linear relation between intake and urine excretion (each additional unit of dose ingested resulted in 0.0166 units excreted). Similar findings were shown by Carkeet et al. (2008) where the linear response doses were reported to be in the range of 15-60 μmol. In all cases, pelargonidin, the main anthocyanin detected in strawberry fruit, seems to be better absorbed and excreted (with recoveries in urine of 0.58% over 24 h) than any other anthocyanins commonly present in strawberry fruit (0.084–0.087%) (Carkeet et al., 2008). Although anthocyanins are absorbed mainly in the duodenum, with very little or no absorption taking place in the small intestine (Tomás-Barberán, 2008), Andres-Lacueva et al. (2005) have shown that these compounds can even be detected in the brain. For the interested reader, an excellent review on the bioavailability and absorption of anthocyanins is that published by McGhie and Walton (2007). Regardless of the poor bioavailability, it is accepted that the little portion that is absorbed may be very biologically active (Carkeet et al., 2008).

Other types of flavonoids are flavonols, which are present in strawberries as glucosides and glucuronides of quercetin and kaempferol aglycons (Seeram et al., 2006c). Epidemiological data suggest an inverse relationship between intake of flavonols and the incidence
of cardiovascular disease or certain cancer types (Knekt et al., 2002). In this context, Häkkinen et al. (1999) studied the flavonol content of different berries, including those from strawberry cvs. Senga Sengana and Jonsok, and found both quercetin (8 μg/g FW) and kaempferol (5–8 μg/g FW) in relatively low concentrations in the strawberries. In another study, Seeram et al. (2006c) identified quercetin-rutinoside, quercetin-glucoside, quercetin-glucuronide and kaempferol-glucuronide as the main flavonols in strawberry fruit. However, according to the literature, flavonol content in strawberries varies drastically. These great variations may be explained, in part, by differences in the genotypes studied, the growing conditions and the sample preparation and extraction methodology used (Häkkinen, et al., 1999). In addition, as observed for anthocyanins (Terry et al., 2007), flavonol concentrations may also vary according to fruit position on the cymose inflorescence. The bioavailability of flavonols has been investigated too, and, so far, there seems to be enough scientific evidence to suggest that monoglucosides are absorbed better than their corresponding aglycones, which in part seems to be related to the presence of glucose transporters in the intestinal wall (Tomás-Barberán, 2008).

There is a paucity of research data available describing the effect that different food matrices may have on the absorption and metabolism of strawberry bioactives in general, and flavonoids in particular. However, Mullen and co-authors demonstrated that consumption of strawberries with 100 ml of double cream delayed absorption of pelargonidin-3-glucoside by more than 1 h, but had no effect on the Cmax, as compared with fruit ingested without cream (Mullen et al., 2008).

15.2.4 Ellagic acid, ellagitannins and derivatives

Ellagic acid is a polyphenol occurring naturally, in both the free form and esterified to glucose in water-soluble, hydrolysable tannins, in certain fruit and nuts. Although concentrations of ellagic acid for strawberry differ markedly according to the literature (19.9 and 522 μg/g FW; Gil et al., 1997, and Häkkinen and Törrönen, 2000, respectively), strawberry fruit are one of the richest sources of this polyphenol, especially if compared with other commonly consumed fruit (Williner et al., 2003). Fruit tissue distribution and variability among cultivars have been highlighted by several works (Maas et al., 1991; Atkinson et al., 2006). Ellagic acid content was shown to decrease during fruit development in all of the five cultivars studied by Williner et al. (2003). For instance, cv. Chandler had 2.07 ± 0.10 mg/g DW at white stage, which decreased to 1.08 ± 0.11 and 0.46 ± 0.07 mg/g DW, respectively, in 50% and 100% red stage fruit (Williner et al., 2003). Ellagic acid, per se, has been associated with numerous health-promoting properties and up to now many data support the role of this phenolic compound as a chemopreventive agent (Hannum, 2004). Häkkinen et al. (2000) reported that ellagic acid in strawberries accounted for approximately 51% of the phenolic profile from this berry; besides, significant differences in ellagic acid concentrations existed between cultivars (396 pg/g FW in cv. Senga Sengana but 522 pg/g FW in cv. Jonsok). Genotypic differences in ellagic acid concentrations (60–341 μg/g FW) and in the ratio between conjugated ellagic acid and free ellagic acid were also seen by Atkinson et al. (2006). Häkkinen et al. (2000) detected other selected phenolic acids in strawberries at lower proportions than those described for ellagic acid (namely, kaempferol, 3.1%; quercetin, 6.0%; myrecitin, 1.6%; p-coumaric acid, 34.3%; p-hydroxybenzoic acid, 4%). Whereas, for most berries, ellagic acid seems to be concentrated in the seeds, Daniel et al. (1990) found that most of the ellagic acid in strawberries was to be found in the pulp (95.7%). In the same study, ellagic acid concentrations (~63 μg/g FW) in strawberry fruit were below the concentrations found in other berries (raspberry and blackberry: ~150 μg/g FW) but were far superior to those found in the other fruit analysed. The higher concentration in pulp than achenes was later highlighted as the possible explanation for the greater bioavailability of ellagic acid from strawberries compared with that from other
fruit (Hannum, 2004). In contrast, in a more recent study conducted by Aaby and collaborators, the achenes from two different strawberry cultivars were the main source of ellagic acid (cv. Totem 87.3 ± 14.6 mg/100 g FW and cv. Puget 34.4 ± 3.7 mg/100 g FW) or ellagic acid derivatives as compared with the flesh (cv. Totem 0.3 mg/100 g FW and cv. Puget 0.2 mg/100 g FW) (Aaby et al., 2005). In this context, the discrepancies encountered between the study of Aaby et al. (2005) and that of Daniel et al. (1990) are due most probably to variations in the methodologies used, such as hydrolysis conditions, extraction solvents used, etc.

Aaby et al. (2005) not only reported greater amounts of ellagic acid and anthocyanins in achenes than pulp but also found up to 20-fold greater total antioxidant activities and 10 times larger total phenolic values in achenes than in the flesh of the different strawberry cultivars investigated. In agreement, Terry (2002) and Terry et al. (2004) also showed, by thin layer chromatography (TLC), that strawberry achenes contained larger amounts of antifungal compounds, many of them being phenolic compounds, than other fruit tissues (Fig. 15.4).

Nevertheless, most of the achenes from the fruit tend to pass intact through the alimentary canal, ending up in the faeces, and hence any bioactives present in this fruit tissue are probably unavailable.

Other strawberry compounds derived from ellagic acid are ellagitannins, which are water-soluble polyphenols belonging to the hydrolysable tannins class. This type of compound can occur in complex polymeric forms of high molecular weight. Ellagitannins can be quantified directly or indirectly by hydrolysing the polymer with an acid or base, to yield ellagic acid (Häkkinen et al., 2000), thus resulting in the concept of free ellagic acid or conjugated ellagic commonly found in the literature (Fig. 15.5).

Seeram et al. (2006c) identified sanguin H-6 and ellagic acid glycosides among the major hydrolysable tannins present in strawberry fruit, whereas other types of ellagitannins were reported by Cerdà et al. (2005). Cerdà and collaborators studied the metabolism of ellagitannins from strawberries and identified urolithin B, a previously identified antiangiogenic and hyaluronidase inhibitor compound, as a suitable biomarker for ellagitannin consumption in healthy humans. After strawberry (cultivar not specified) intake, urolithin B excretion was c.2.8% of the ingested dose and varied considerably between individuals. Similar findings were observed by Seeram et al. (2006c) when studying the pharmacokinetic parameters of ellagic acid after ingestion of pomegranate juice. Despite the limited absorption of either ellagic acid or ellagitannins (Tomás-Barberán, 2008), these compounds and metabolites have been detected in the kidneys and liver of rats, as well as the prostate gland of mice (Cerdà et al., 2005; Seeram et al., 2006b; Seeram and Heber, 2007).

15.2.5 Ascorbate, folate and other bioactives

With constant advances in analytical sciences, new strawberry-derived compounds have been identified and associated with potential health-promoting properties. Resveratrol, a compound commonly found in grapes and synthesized from cinnamic acid derivatives, has been identified in strawberry fruit by Wang et al. (2007). During the past decades, several studies have shown the potential health-promoting properties of resveratrol (namely, antioxidant, anticarcinogenic, anti-inflammatory, cardioprotection) (for the interested reader, see the review by Aggarwal et al., 2004), and, as a result, increasing interest has been raised to identify new sources of this phytoalexin among fruit and vegetables. Even though there was evidence of this compound in other berries, the first detailed study on the compound in strawberries was probably that conducted by Wang et al. (2007). The authors not only studied resveratrol content of strawberry cvs. Kent and Earliglow grown in a glasshouse but also detailed the effect that different preharvest factors (namely, genotype, fruit maturity, cultural practices and environmental conditions) had on that content. Resveratrol concentration was greater in fully ripe than in early ripe berries, as well as being higher in
Fig. 15.4. (a) Section of a strawberry fruit and differentiation between main fruit tissues. (b) TLC *Cladosporium cladosporioides* bioassay of cv. Elsanta fruit crude ethanol extracts (100 ml, 0.2 μl/g FW), from different tissues of Green I strawberry fruit, run in hexane:ethyl acetate:methanol (60:40:10 v/v/v) (Terry, 2002; Terry *et al.*, 2004).
Fig. 15.5. (a) Chromatographic profile of ellagic acid in strawberry fruit (cv. Camarosa) prior to and after 90 min hydrolysis. (b) General scheme for the hydrolysis of ellagitannins from strawberry fruit; before hydrolysis the principal components correspond to ellagic acid glycosides, simple ellagitannins (B₁) and complex oligomeric ellagitannins that, when hydrolysed, give rise to ellagic acid (B₂), methyl gallate and methyl sanguisorboate.

achenes (~ 2 µg/g DW) than in pulp (~ 0.12 µg/g DW). Overall, values for resveratrol concentrations in strawberry fruit were far below the ~ 10 µg/g FW that could be found in the skin of red mature grapes cv. Napoleon (Cantos et al., 2000).

Another compound found in strawberry fruit with reported anti-inflammatory, antiproliferative and antiangiogenic (Sung et al., 2007) as well as memory enhancer properties (Maher et al., 2006) is fisetin. Fisetin is a flavonoid occurring naturally in certain fruit and vegetables, with concentrations ranging from 2–160 µg/g (Arai et al., 2000). The mechanism by which fisetin exerts its anticarcinogenic effects still remains unclear, although recent research has shown that it inhibits cyclin-dependent kinase 6 and downregulates nuclear factor-κB-regulated cell proliferation (Sung et al., 2007).

In addition to all the polyphenols mentioned earlier, strawberry fruit are one of the
richest natural sources of two water-soluble vitamins, folate and ascorbate. Inadequate folate status in humans has been associated with an increased risk of the chronic diseases that may affect the elderly population in particular (Rampersaud et al., 2003; Tulipani et al., 2009). Similarly, ascorbate deficiency is known to be related to detrimental health effects (see Chapter 14 of this volume). For the determination of folate content in strawberry fruit, both a microbial assay and a radio-protein binding assay have been developed and validated recently in berry fruits (Stråsljö et al., 2003; Tulipani et al., 2008). In a recent study, total folate content from nine Italian strawberry cultivars ranged from ~0.2 to ~1.0 μg/g FW (Tulipani et al., 2008) and was in agreement with earlier studies conducted on Swedish grown strawberries (0.73–0.99 μg/g FW; Stråsljö et al., 2003). In this context, it is generally accepted that 250–350 g of berries (~125 μg folate) can provide almost the totality of European daily intake recommendations (200–300 μg/day) (Bailey and Gregory, 1999), making strawberries one of the most appealing sources of this vitamin. Supplementation with folate to individuals with homocysteinaemia has been shown to reduce levels of homocystein in plasma, and therefore reduce the risk of heart disease (Spiller and Dewell, 2003).

Ascorbic acid content in strawberry fruit varies drastically among different genotypes (Fig. 15.6) and agroclimatic conditions (Cordenunsi et al., 2005; Atkinson et al., 2006; Terry et al., 2007; Giné Bordonaba and Terry, 2009, 2010; Crespo et al., 2010), with reported concentrations ranging from 0.2–0.9 mg/g FW (Atkinson et al., 2006; Terry et al., 2007; Giné Bordonaba and Terry, 2009; Crespo et al., 2010).

15.3 Chemopreventive and Health-related Properties

15.3.1 Introduction

The antioxidant properties of strawberries are well documented (Wang and Lin, 2000; Terry et al., 2007; Wolfe et al., 2008). Since oxidative stress has been suggested to play an important role in the development of certain conditions, including cancers, it was expected initially that the antioxidant capacity of the fruit would correlate well with its antiproliferative properties. Nevertheless, Meyers et al. (2003) demonstrated that antioxidant activity from eight different strawberry cultivars was not related to their antiproliferative properties. Nowadays, a plethora of research studies has shown that berries, including strawberry, as well as berry purified phenolic compounds, inhibit cancer cell proliferation, regulate cell cycle arrest and, in some cases, induce apoptosis by multimechanistic means of actions beyond antioxidation (reviewed by Seeram and Heber, 2007). Besides, little or no cytotoxic effect was observed when strawberry extracts were tested on normal cells, resulting in no doubts about the potential anticancer effects of strawberry fruit (Seeram, 2008). As for many other fruit and vegetables, most of the reported anticancer activity of strawberry fruit is based on in vitro studies rather than in vivo or intervention trials. Among the limitations of in vitro tests, several authors (Roques et al., 2002; Kern et al., 2007) have pointed out that the results of in vitro assays may be an artefact from the generation of hydrogen peroxide in the culture media by the antioxidants tested, and hence it is evident that results from this type of assay should be interpreted cautiously.

In this context, the following sections aim to describe the latest scientific evidence obtained from in vitro and in vivo experiments or intervention studies regarding the beneficial effects that strawberry fruit or their extracts (namely, freeze-dried powders, concentrates, etc.) may have on preventing or fighting against cancer as well as other illnesses (Table 15.2).

15.3.2 Cancer studies

Several studies have demonstrated that strawberry extracts inhibit the growth of human carcinoma cells when tested in vitro (Table 15.2). For instance, strawberries effectively inhibited, by different mechanisms, the growth of oral, breast and prostate (Seeram et al., 2006a) or liver cancer cell lines (Ramos et al., 2005) in a dose-dependent manner. Ramos et al. (2005) proved that whole strawberry extracts arrested...
Fig. 15.6. (a) Ascorbic acid (AsA) concentrations (mg/g FW) in a range of UK grown strawberry cultivars (Gine Bordonaba and Terry, 2009). (b) Reported AsA concentrations (mg/g FW) in strawberry fruit from different locations in Europe (NB: values correspond to average concentrations from different cultivars and different harvest years obtained from various literature).

the G1 phase, therefore showing proapoptotic effects. In a more recent study, Wu and collaborators (2007) investigated whether strawberry and other berry extracts had any effect on cell viability and expression of apoptotic cell markers in human HT29 colon cancer cells. The results suggested that berry extracts inhibited cell proliferation through the cyclin kinase inhibitor pathway, p21WAF1 (Wu et al., 2007). Other in vitro studies with extracts from two different strawberry cultivars (namely, Sweet Charlie and Carlsbad) showed the potential of both cultivars to inhibit breast cancer and cervical cancer cell proliferation (Wedge et al.,
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract typea</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibit the initiation and promotion of oesophageal cancer</td>
<td>Rat</td>
<td>Diet (AIN76) containing 5 and 10% of strawberries (- ellagic acid concentration was 0.34 and 0.67 mg/kg diet, respectively)</td>
<td>Freeze-dried strawberry puree</td>
<td>Stoner et al. (1999)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Reduction of tumour multiplicity in oesophageal cancer</td>
<td>Rat</td>
<td>Diet (AIN76) containing 5 and 10% of strawberries</td>
<td>Freeze-dried strawberry</td>
<td>Carlton et al. (2001)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Protective effect against endogenous generation of carcinogens</td>
<td>27 male and 13 female volunteers (ten healthy subjects in each group)</td>
<td>300 g on the 4th day of a 4-day trial</td>
<td>Fresh strawberries</td>
<td>Chung et al. (2002)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of cancer cell proliferation by inhibiting transcription factors and activating protein-1 (AP-1) and nuclear factor kappa B (NFκB)</td>
<td>In vitro: human lung epithelial cancer A549 cells and mouse epidermal JB6 P+ cell lines</td>
<td>–</td>
<td>Filtered and diluted strawberry homogenates</td>
<td>Wang et al. (2005)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Growth inhibition of liver cancer cells</td>
<td>In vitro: HepG2 cell cultures</td>
<td>0.1–0.8 mg/ml</td>
<td>Lyophilized strawberry extract</td>
<td>Ramos et al. (2005)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Growth inhibition of oral, breast and prostate cancer cells</td>
<td>In vitro: cell cultures</td>
<td>25–200 μg/ml</td>
<td>Polyphenolic enriched strawberry extract (sugars and acids removed by C18)</td>
<td>Seeram et al. (2006a)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Reduced cell proliferation through the cyclin kinase inhibitor pathway p21/WAF1</td>
<td>In vitro: HT29 colon cancer cells</td>
<td>0–60 mg/ml</td>
<td>Homogenized strawberry extract</td>
<td>Wu et al. (2007)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of nuclear factor of activated T cells (NAFT) and tumour necrosis factor (TNF)</td>
<td>In vitro: mouse epidermal JB6 Cl 41 cell lines</td>
<td>1–100 μg/ml</td>
<td>Lyophilized strawberry extract</td>
<td>Li et al. (2008)</td>
</tr>
<tr>
<td>Anticarcinogenic</td>
<td>Inhibition of tumour formation of oral cancer cells</td>
<td>Hamster</td>
<td>Diet (AIN76) containing 10% of strawberries or strawberries enriched with selenium (0.5 ppm)</td>
<td>Lyophilized strawberry or selenium-enriched lyophilized strawberries</td>
<td>Warner et al. (2008)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Endothelium-dependent vasorelaxation through the activation of PI3 kinase/akt</td>
<td>Rabbit aorta</td>
<td>0.1–10 mg/ml</td>
<td>Freeze-dried strawberry powder</td>
<td>Edirisinghe et al. (2008)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-------------</td>
<td>---------------</td>
<td>--------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Greater reduction in oxidative damage to low-density lipoproteins</td>
<td>Human intervention study on 28 hyperlipidaemic subjects</td>
<td>454 g/day in a randomized 1-month crossover study with a 2-week washout</td>
<td>Fresh berries</td>
<td>Jenkins et al. (2008)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Antiplatelet activity</td>
<td>Mice</td>
<td>~ 11 ml/kg</td>
<td>Strawberry filtrate</td>
<td>Naemura et al. (2008)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Borderline significant, multivariate 14% lower likelihood of an elevated C-reactive protein of ≥ 3 mg/l</td>
<td>Cohort study</td>
<td>At least two servings/week</td>
<td>–</td>
<td>Sesso et al. (2007)</td>
</tr>
<tr>
<td>Anticardiovascular disease</td>
<td>Hypocholesterolaemic effects and reduced lipid peroxidation</td>
<td>Women suffering metabolic syndrome</td>
<td>25 g/day</td>
<td>Freeze-dried strawberry powder</td>
<td>Basu et al. (2009)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Inhibition key inflammation enzymes (COX1)</td>
<td>Cell culture</td>
<td>125 μg/ml</td>
<td>Lyophilized crude anthocyanins from strawberries cv. Honeoye</td>
<td>Seeram et al. (2001)</td>
</tr>
<tr>
<td>Antineurodegenerative</td>
<td>Reversion of the ageing process by protecting against decrease in mental performance</td>
<td>Rat</td>
<td>9.5 g/kg of standard diet for 6 months</td>
<td>Lyophilized strawberry powder</td>
<td>Joseph et al. (1998)</td>
</tr>
<tr>
<td>Antineurodegenerative</td>
<td>Reduction of oxidative stress-induced neurotoxicity</td>
<td>Neuronal cells</td>
<td>100–2000 μg/ml</td>
<td>Phenolics extracted from 10 g of dried sample</td>
<td>Heo and Lee (2005)</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>Limiting post-meal blood glucose levels by reducing α-amylase</td>
<td>In vitro assays</td>
<td>0–500 μg/assay</td>
<td>Phenolic-rich fractions from breeding variety 932034 and cv. Elsanta from local growers</td>
<td>McDougall et al. (2005)</td>
</tr>
<tr>
<td>Antiobesity</td>
<td>Increased weight gain</td>
<td>Mice</td>
<td>Diet containing 10% freeze-dried strawberry powder</td>
<td>Whole fruit</td>
<td>Prior et al. (2008)</td>
</tr>
</tbody>
</table>
| Antiobesity | Reduced weight gain | Purified anthocyanins from strawberries
Purified anthocyanins from strawberry given in the water | – | – | – |

Note: *Most strawberry extracts used for in vitro studies are based on acidified methanol aqueous extraction followed by an acetone-water extraction, evaporation of the solvents and resuspension of the extracts in water prior to being deposited on to the cell cultures. Whenever data are available, the cultivars used are specified. (-) concentration dose not specified.
Li et al. (2008) not only demonstrated the anticarcinogenic properties of strawberry extracts but also elucidated that they specifically inhibited nuclear factor of activated T cells (NFAT) and tumour necrosis factor (TNF). Results from the same study suggested that the chemopreventive properties of black raspberry and strawberry bioactives might be targeted through different signalling pathways. In an earlier study, Wang et al. (2005) showed that strawberry extracts suppressed cancer cell proliferation and transformation by means of inhibiting the transcription factors, activating protein-1 (AP-1) and nuclear factor kappa B (NFκB). In the same study, the authors postulated that the antioxidant properties and the ability to reduce oxidative stress most probably were related to the ability of the same extracts to block ultraviolet B (UVB) and TPA-induced AP-1 and NFκB activation.

Besides the earlier highlighted mechanisms of action, strawberry fruit are thought to possess anticarcinogenic effects by inhibiting possible mutations. In this context, Hope et al. (2004) pointed out that strawberry tannin fractions were very effective in inhibiting mutations caused by both methyl methane-sulfonate and benzopyrene.

Whereas animal and human studies are still limited, many research groups are currently investigating the possible role of strawberry fruit in preventing not only cancer but also several other diseases. The dietary intake of products rich in ellagitannins, such as strawberries, has been shown to inhibit the initiation and promotion of oesophageal cancer (Stoner et al., 1999). Stoner and collaborators included 5 or 10% of freeze-dried strawberries into the diet of rats prior to the induction of oesophagus cancer with N-nitrosomethylbenzylamine (NMBA) and observed an inhibition effect depending on the dose concentration. Using a similar approach, the chemopreventive effect of strawberry lyophilized extracts on NMBA-induced rat oesophageal carcinogenesis was studied by Carlton et al. (2001). They proved that although the berry extract had no effect on tumour incidence, it reduced tumour multiplicity significantly compared with that seen in control or nontreated cells. Yet an earlier study by Stoner et al. (1999) did not detect any effect of strawberries in the reduction of lung cancer in rats, induced by other carcinogenic compounds. In a different study conducted by Chung et al. (2002), the effect of strawberries against the endogenous generation of carcinogens in healthy individuals consuming a diet with excessive nitrates was evaluated. Results from that study demonstrated that consumption of 300 g of strawberry resulted in a reduction of 70% in the urinary concentration of the carcinogen, NMBA. Recently, Warner et al. (2008) demonstrated that lyophilized strawberries or selenium-enriched lyophilized strawberries satisfactorily inhibited tumour formation by 43 and 59%, respectively. Overall, the authors concluded that, based on their results using a hamster cheek pouch model, strawberries and strawberries with selenium could prevent or delay the development of oral cancer (Warner et al. 2008).

15.3.3 Cardiovascular disease

Epidemiological data suggest that consumption of fruit and vegetables may lower the risk of cardiovascular disease (CVD). Again, antioxidant activity has been cited as the possible mechanisms by which strawberries or specific polyphenols found in strawberries may exert their beneficial effects. In this context, it is postulated that the antioxidant activity of strawberry is crucial in the prevention of atherosclerosis, since the oxidation of low-density lipoproteins (LDL) is a key phenomenon associated with the development of such conditions (Diaz et al., 1997; Edirisinghe et al., 2008).

In addition, specific compounds found in strawberries (anthocyanins for instance) are known vasodilators and help towards reducing the incidence of coronary diseases. Edirisinghe et al. (2008) showed, for the first time, that not only did freeze-dried strawberry powder from California strawberries cause endothelium-dependent relaxation in the rabbit aorta (EDR) but also that this was achieved through the activation of PI3 kinase/akt (Table 15.2). The major phenolic compounds present in the strawberry extracts were pelargonidin-3-O-glucoside, coumaryl-3-O-glucoside and trans-cinnamoyl-O-glucoside.
Previous studies also demonstrated that ascorbate had EDR effects at concentrations similar to those found in strawberry fruit, and therefore the authors pointed out the possible synergistic effect between ascorbate and polyphenols. The loss of proper endothelial function is frequent in people suffering from diabetes mellitus, hypertension and other chronic conditions that can therefore increase the risk of heart disease. The role that certain polyphenols present in strawberry fruit (namely, kaempferol, catechin and anthocyanins) have in inhibiting the formation of atheroma plaque, and therefore reducing the incidence of thrombosis, has been demonstrated (Rein et al., 2000). In a cohort study conducted by Sesso et al. (2007), higher strawberry intake (more than two servings/week) was associated with a reduced borderline but significant likelihood of having elevated C-reactive protein (CRP) levels. However, in the same study, no association was found between the risk of incidence of CVD, lipids, or CRP in middle-aged and older women and the consumption of strawberry fruit. The authors pointed out that additional epidemiological data were needed to clarify any role of strawberries in CVD prevention.

Jenkins and co-workers (2008) assessed the effect of adding strawberries as a source of antioxidants to improve the antioxidant effect of a cholesterol-lowering diet on 28 hyperlipidaemic subjects. Results from this study revealed that supplementation with strawberry fruit resulted in a greater reduction in oxidative damage to LDL, while preserving reductions in blood lipids and enhancing diet palatability (Jenkins et al., 2008).

In another study conducted by Naemura et al. (2008), an in vitro platelet function test (haemostatometry) was used to screen different strawberry cultivars. In the same study, those cultivars showing significant antiplatelet function were further examined in vivo by means of a laser-induced thrombosis test in mice. Results suggested that strawberry varieties KYSt-4 (Nohime), KYSt-11 (Kurume 1H-1) and KYSt-17 (Kurume 58) showed significant antiplatelet activity both in vitro and in vivo.

Recently, the effect of freeze-dried strawberry powder supplementation has been evaluated on women suffering metabolic syndrome (Basu et al., 2009). Short-term supplementation of a strawberry drink, consisting of 25 g of freeze-dried strawberry powder (unspecified cultivar), one cup of water, artificial sweeteners and vanilla essence, resulted in hypocholesterolaemic effects and decrease in lipid peroxidation (Basu et al., 2009) at 4 weeks as compared with baseline values. When a similar study was undertaken with hyperlipidaemic subjects each ingesting 453 g of fresh strawberries daily for 4 weeks, no differences were observed in lipid levels (Jenkins et al., 2008). In the same study, however, strawberry consumption was associated with reduction of lipid oxidative damage.

### 15.3.4 Other beneficial effects

#### Anti-inflammatory

It is possible that certain phenolic compounds from strawberry fruit may exert positive effects on the immune system. For instance, Seeram et al. (2001) reported on the inhibitory effect of berry anthocyanins on cyclooxygenase (COX). COX is a key enzyme in inflammation and its inhibition is the target mechanism of different drugs, including the common aspirin. Strawberries have been proven to be very effective in inhibiting COX2, though not so effective against COX1. Given that inflammation is a process involved in the aetiology of several pathologic conditions, including cancer, cardiovascular, Alzheimer’s etc., the findings by Seeram et al. (2001) highlighted the potential of strawberries for the treatment of multiple conditions (Tomás-Barberán, 2008).

#### Anti-neurodegenerative

Scientific evidence suggests that strawberries (Table 15.2) and other berries may have a role in delaying or even overturning age-related degenerative diseases. Already, studies performed during the last decade have shown the ability of diets supplemented with strawberries to retard and reverse the ageing process in rats (Joseph et al., 1998; Shukitt-Hale et al., 1999; Bickford et al., 2000). In the study carried out by Joseph and collaborators, rats...
of 6 months of age were fed with strawberries for 8 months. Results revealed the potential of strawberry fruit in preserving cerebral function and protecting against the decrease in mental performance associated with ageing (Joseph et al., 1998). Other studies conducted on aged rats showed that strawberries improved the rodents’ motor and learning skills, and reduced the deficit in the cognitive capacity of the animals (Bickford et al., 2000). More recent data, generally obtained from in vitro studies, reveals the potential of strawberry fruit, as well as their anthocyanins or other polyphenols, to reduce oxidative stress-induced apoptosis in neuronal cells. In the study conducted by Heo and Lee (2005), strawberries reduced oxidative stress-induced neurotoxicity significantly in neuronal cells in a greater manner than observed for banana or orange (Heo and Lee, 2005). Accordingly, Shukitt-Hale et al. (2007) suggested that combinations of antioxidants and anti-inflammatory polyphenols from berries might be key compounds to help prevent, suppress or inhibit age-related deficits by several mechanisms. Recently, Shukitt-Hale et al. (2007) have investigated whether strawberry and other berry extracts can mitigate the oxidative stress in rats exposed to irradiation. Exposing young rats to irradiation enhances oxidative stress and disrupts the dopaminergic system in a way similar to that observed in aged rats (Shukitt-Hale et al. 2007). In this context, the authors demonstrated the ability of strawberry extracts to protect against spatial deficits.

15.4 Effect of Preharvest and Postharvest Continuum

Strawberry growth and development are characterized by changes in colour, texture and flavour, with four or five different stages commonly described in the literature that are based on the development of non-ovarian receptacle tissue (Terry et al., 2004). These stages include small green, large green, white and full red. The contents of certain bioactives that account for the potential health-promoting properties of the fruit vary markedly, depending on developmental stage. Changes in anthocyanins and other bioactives during strawberry fruit development have been described in detail recently (Carbone et al., 2009). In addition, during fruit growth and development, exposure of the plant to certain abiotic and biotic conditions may result in enhancing oxidative stress, and therefore generation of reactive oxygen species (ROS). It is believed that under such conditions the plant responds by increasing bioactive-related gene expression, thus enhancing the production of ROS scavengers, mainly antioxidants that may counteract ROS at different levels. It has been demonstrated that growing strawberry plants under different agroclimatic conditions may result in fruit with different contents of health-promoting components (Atkinson et al., 2006; Terry et al., 2007). Recent studies have elucidated that exposing the plant to different stress conditions (namely, deficit irrigation, salinity, etc.) resulted in enhanced content of specific bioactives (Terry et al., 2007; Keutgen and Pawelzik, 2008; Table 15.3). Specifically, Terry et al. (2007) showed that anthocyanin content, total phenolics and antioxidant capacity were greater in cv. Elsanta plants irrigated with 50 ml/day than in the plants receiving greater amounts of water (100 or 200 ml/day) (Fig. 15.3).
Table 15.3. Effect of preharvest factors (a) and postharvest treatments (b) on the health-related composition of strawberries.

<table>
<thead>
<tr>
<th>(a) Preharvest factors</th>
<th>Effect on bioactives</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional versus organic cultivation systems</td>
<td>No effect on total phenolics</td>
<td>Häkkinen and Törnönen (2000)</td>
</tr>
<tr>
<td>Growing temperature</td>
<td>Strawberry grown at higher temperatures (°C) showed higher concentrations of bioactive compounds</td>
<td>Wang and Zheng (2001)</td>
</tr>
<tr>
<td>Cultural system (hill plasticulture versus matted row)</td>
<td>Hill plasticulture systems resulted in higher content of phenolics, flavonoids and ascorbate</td>
<td>Wang et al. (2002)</td>
</tr>
<tr>
<td>Ozone exposure</td>
<td>No significant effect on antioxidant activity or bioactives</td>
<td>Keutgen and Pawelzik (2007)</td>
</tr>
<tr>
<td>Salinity stress</td>
<td>Moderate salinity resulted in increase antioxidant activity and bioactives</td>
<td>Keutgen and Pawelzik (2007)</td>
</tr>
<tr>
<td>Deficit irrigation</td>
<td>Higher content of certain anthocyanins, total phenolics and antioxidant activity</td>
<td>Terry et al. (2007)</td>
</tr>
<tr>
<td>Inoculation with <em>Botrytis cinerea</em></td>
<td>No effect on strawberry (cv. Elsanta) bioactives or antioxidant activity</td>
<td>Terry et al. (2007)</td>
</tr>
<tr>
<td>Organic and conventional nutrient amendments</td>
<td>No significant differences between treatments on antioxidant activity</td>
<td>Hargreaves et al. (2008)</td>
</tr>
<tr>
<td>Methyl jasmonate (MeJa) applied in fully or deficit irrigated plants</td>
<td>Higher concentrations of anthocyanins found in MeJa-treated plants and changes in fruit and leaves antioxidant capacity</td>
<td>Giné Bordonaba and Terry (unpublished data)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Postharvest treatment</th>
<th>Effect on bioactives</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-MCP</td>
<td>Lower accumulation of phenolic anthocyanins in cv. Everest treated fruits</td>
<td>Jiang et al. (2001)</td>
</tr>
<tr>
<td>Heat treatment before refrigerated storage</td>
<td>Treated fruits (cv. Selva) showed less anthocyanin accumulation than controls when held at 20°C</td>
<td>Vicente et al. (2002)</td>
</tr>
<tr>
<td>Exogenous abscisic acid (ABA) application</td>
<td>Anthocyanin and phenolic contents and PAL activity increased during storage of ABA-treated strawberry fruit (cv. Everest) more rapidly than in non-treated fruit</td>
<td>Jiang and Joyce (2003)</td>
</tr>
</tbody>
</table>

(Continued)
Table 15.3. Continued

<table>
<thead>
<tr>
<th>(b) Postharvest treatment</th>
<th>Effect on bioactives</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage temperature</td>
<td>Higher temperatures (5° and 10°C) resulted in greater antioxidant capacity, total phenolics and anthocyanins of the fruit (cv. Chandler) as compared with fruit stored at 0°C</td>
<td>Ayala-Zavalá et al. (2004)</td>
</tr>
<tr>
<td>UV-C (4.1 kJ/m²) and heat treatment (45°C, 3 h in air) either separately or combined</td>
<td>All treatments reduced the accumulation of anthocyanins in strawberries cv. Seascape</td>
<td>Pan et al. (2004)</td>
</tr>
<tr>
<td>Strawberry wrapping with polyvinyl chloride and stored at 1°C</td>
<td>Wrapped fruit (cv. Oso Grande) suffered lower water loss and maintained better anthocyanin and other soluble phenolics as compared with unwrapped fruit</td>
<td>Nunes et al. (2005)</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>Lower temperatures affected anthocyanins and ascorbate negatively but had no effect on flavonols, ellagic acid and total phenolics and antioxidant capacity</td>
<td>Cordenunsi et al. (2005)</td>
</tr>
<tr>
<td>Superatmospheric storage conditions</td>
<td>High oxygen atmospheres (&gt; 40 kPa) resulted in fruit (cv. Chandler) with higher antioxidant capacity, total phenolics, less decay and longer postharvest life than those stored in air</td>
<td>Ayala-Zavalá et al. (2007)</td>
</tr>
</tbody>
</table>

Note: aAt anthesis of primary flower.
The antiproliferative effect of strawberry fruit grown conventionally or organically was assessed recently by Olsson et al. (2006). The organically grown strawberries showed greater antiproliferative activity, which the authors related to the higher content of secondary metabolites found in those berries and which in turn might be associated with the exposure of the plants to greater stress from pathogens when grown under organic cultivation systems. In contrast, others (Hargreaves et al., 2008) could not find differences in several bioactive constituents between organically and conventionally grown strawberries, and hence further research is required to elucidate whether or not organic production may result in 'healthier' berries.

Clearly, metabolism is known to continue beyond fruit harvest; however, this is often ignored or, more commonly, overlooked or not appreciated. The concentration of certain metabolites is expected to change during postharvest storage and through the supply chain. Given this, however, few works have studied the role that postharvest treatments have on strawberry bioactives. In addition, the effectiveness of any postharvest treatment is dependent on whether the treatment is focused on preserving appearance or maintaining the health-related composition of the fruit. Earlier works have shown that postharvest treatments focused on maintaining appearance do not correlate with, for instance, better maintenance of certain bioactives (Pelayo et al., 2003). Postharvest storage temperature (5 or 22°C) did not have a significant effect on ellagic acid content in strawberries stored for 24 h (Häkkinen et al., 2000). Under longer storage conditions, Gil et al. (1997) reported that content of ellagic acid increased over the course of 10 days in fruit stored at 5°C and pointed out that such a phenomenon probably was the result of degradation of ellagitannins (Fig. 15.5). In another study, Cordenunsi et al. (2005) evaluated the chemical composition and antioxidant activity changes of different strawberry cultivars (namely, Dover, Campineiro and Oso Grande) stored at 6, 16 and 25°C for 6 days. The authors concluded that low temperatures affected anthocyanins and ascorbic acid negatively, whereas it had no significant effect on the content of flavonols, ellagic acid or total phenolics. Antioxidant activity, on the other hand, was similar between cultivars and in all cases decreased after harvest, independent of temperature. Interestingly, the observed increase in ascorbate in all three cultivars during storage (10% greater than initial values) was experienced only at 16°C and therefore was in disagreement with previous studies (Nunes et al., 1998; Cordenunsi et al., 2003), which highlighted that low temperatures and high humidity during storage might retard ascorbate degradation (Nunes, 2008). Nunes et al. (1998) showed that, in strawberry fruit stored for 8 days at 1 or 10°C, as well as 4 days at 20°C, ascorbic degradation was greater at the higher temperatures. In the same study, postharvest shelf life was enhanced and ascorbic acid degradation reduced by 7.5-fold in fruit stored at 1°C. In this context, Cordenunsi et al. (2005) postulated that ascorbic acid synthesis might occur during postharvest storage and that it was indeed affected by lowering temperature. Häkkinen et al. (2000) also demonstrated that quercetin content increased markedly in strawberries or strawberry jams stored for 9 months, whereas ellagic acid tended to decline in fresh fruit but not in jams from the same berry. Similarly, others also reported that flavonol content increased in strawberries or other fruit stored under refrigerated conditions (Gil et al., 1997). Controlled atmosphere (CA) during storage may reduce the rate of accumulation of anthocyanins normally observed in strawberry fruit after harvest (Zheng et al., 2007). Nevertheless, Gil et al. (1997) showed that enriched CO₂ atmospheres had a minimal effect on anthocyanin concentrations of cv. Selva fruit. In the particular case of anthocyanins, it is likely that changes in other components within the fruit (i.e. organic acids) may affect the pH, and hence the stability, co-pigmentation and spectra of these pigments within the fruit (Terry et al., 2009), thus accounting for the reported changes during postharvest storage. In addition, given the variability in colour within different genotypes (Gine Bordonaba and Terry, 2009), it may be feasible to speculate that changes in anthocyanins and other pigments, as a result of any postharvest treatment, may
be genotype-dependent, and this should be investigated further.

The effects that certain non-conventional treatments have on preharvest or postharvest strawberry bioactives have also been studied increasingly over the last decade. As an example, Zabetakis et al. (2000) studied the effect that treatment with high hydrostatic pressure and further storage at different temperatures had on strawberry anthocyanins. High hydrostatic pressure may be an alternative to conventional heat treatments for preservation of strawberry-derived products. The authors observed that samples pressurized under 800 MPa for 15 min (the greatest of the different treatments applied) resulted in the lowest losses of anthocyanins. Furthermore, after high hydrostatic pressure treatments, storage at 4°C also resulted in the best maintenance of both pelargonidin-3-glucoside and pelargonidin-3-rutinoside. Accordingly, treatment with 400 and 600 MPa resulted in greater enzymatic activity involved in anthocyanin degradation than seen with 800 MPa (Zabetakis et al., 2000).

In a different study, Wang et al. (2007) showed that strawberries treated postharvest with essential oils inhibited human HT-29 colon cancer cell proliferation better than those not treated. The application of essential oils preharvest (methyl jasmonate for instance) resulted in marked changes in anthocyanin concentrations in different strawberry cultivars (Gine Bordonaba and Terry, unpublished data).

15.5 Future Research Needs and Conclusions

Unlike many other fruit and vegetables, there appears to be substantial scientific evidence to confirm that strawberries are one of the main sources of vitamin C, folic acid and dietary fibre, as well as an excellent source of dietary polyphenols. From the survey of the literature presented herein, anthocyanin pigments, together with hydrolysable and non-hydrolysable tannins, are among the main compounds with reported health-promoting properties (namely, anticarcinogenic, anti-cardiovascular and anti-neurodegenerative diseases, anti-inflammatory, etc). However, in order to understand fully the health-promoting properties of this berry, more studies are still required to elucidate further not only the heterogeneity in bioactive compounds among strawberry genotypes as affected by preharvest/postharvest continuum, but also to broaden investigative research on the bioavailability of specific strawberry bioactives in different food matrices.

In vitro studies must continue, since they provide vital information on the mechanisms and actions of specific bioactives, but it is clear that such studies may present certain limitations and the results cannot be translated in vivo. Consequently, further in vivo and intervention studies must be conducted to sustain the information obtained so far, as well as to study the long-term beneficial or toxic effect, if any, derived from strawberry consumption.

Daily consumption of dietary polyphenols can vary from a few hundred mg to almost 2 g per capita. As described throughout this chapter, only a very minor fraction of the polyphenols found in strawberry fruit is absorbed directly. Generally, most polyphenols pass the stomach and come across the gut microflora, resulting in a diverse range of metabolites, most of them still unknown, which undoubtedly may have potential health-related properties. Further work should aim to clarify whether the metabolites generated by the interaction between gut microflora and polyphenols exert health-promoting properties.

References


Skupień, K. and Oszmianański, J. (2004) Comparison of six cultivars of strawberries (Fragaria x ananassa Duch.) grown in northwest Poland. European Food Research and Technology 219, 66–70.


16 Tomato and Other Solanaceous Fruits

Amarat H. Simonne, Cecilia do Nascimento Nunes and Jeffrey K. Brecht

16.1 Main Introduction

The Solanaceae or nightshade plant family is one of the major families of plants supplying vegetable and staple food crops in the world (Swiader and Ware, 2001). Tomato (Solanum lycopersicum L.) is the second most important crop in this family, after potato (S. tuberosum L.) (see Chapter 12 of this volume). Tomatoes and peppers (Capsicum annuum L.) are the Solanaceous fruit crops representing the most diverse fruit morphology (Paran and van der Knaap, 2007). Tomatoes, peppers and aubergines (eggplants; S. melongena L.) are grown worldwide, while other Solanaceae such as tomatillo (Physalis philadelphica Lam) and uchuva, or cape gooseberry (P. peruviana L.), are grown in limited areas of the world. The current tomato world production is 126 million tonnes (Mt)/year, while the annual world production of other significant Solanaceous fruit, aubergines and peppers, is 32 and 28 Mt, respectively. The production statistics for peppers include dried chilli peppers as well as fresh chilli peppers and bell peppers (FAOSTAT, 2009). Although tomatillo and uchuva or cape gooseberry are also listed among some other commercially grown Solanaceous fruit, due to their small production volumes, current world production figures for these crops are not readily available. However, production of tomatillo has been reported to be significant in Mexico and Central America (Moriconi et al., 1990; Hernández Bermejo and Leon, 1994), while the main production areas for uchuva or cape gooseberry include Asia, Africa and South America (Morton, 1987). The fruit from Solanaceae plants are important food sources as they provide important bioactive nutrients to the human diet, as well as many secondary metabolites with reported medicinal properties.

16.2 Tomato

16.2.1 Introduction

Cultivated tomato fruit come in many sizes, shapes and colours, resulting from a long history of genetic improvement of the plant for various usages, as well as for improving disease resistance and other desirable traits (Figs. 16.1 and 16.2). Despite the diverse genotypes, the majority of domesticated tomatoes are the results of crosses among the cultivated species and their wild relatives. Although experts agree that the genetic base of the cultivated tomato is narrow, more than 75,000 S. lycopersicum germplasm accessions are maintained by countries around the world (Robertson and Labate, 2007). Per capita consumption of tomatoes varies around the world, with countries considered as having high, average or low per capita consumption
Fig. 16.1. Large tomato types. Top from left to right: red round vine ripe green house tomatoes, red round field grown tomatoes; red heirloom ugly tomato cv Mortgage Lifter grown in a shaded condition, and OSU P20 (AraR atvav) with anthocyanin expression (photo is a courtesy of James R. Myers from Oregon State University); bottom from left to right: orange round field grown tomatoes, yellow round field grown tomatoes, red heirloom field grown ugly tomatoes.

Fig. 16.2. Small tomato types. Top from left to right: heirloom cherry tomatoes cv Brown Berry, red grape tomatoes cv Chiquita and Honey Bunch, yellow grape tomatoes cv. Morning Light; bottom from left to right: heirloom yellow plum tomatoes cv. Cream Sausage, red plum tomatoes and cherry tomatoes.

Countries with high tomato per capita consumption include the USA, Italy, Canada and Algeria, while others with average tomato per capita consumption include the UK, France, the former USSR and Germany, and those with low per capita consumption include Japan and Brazil (Bieche and Covis, 1992). Tomato consumption patterns in recent years show increases in both processed and fresh consumption, with mostly processed tomato products being consumed in the USA and mostly fresh tomatoes being consumed in the EU (Harvey et al., 2003), but these changes are by no means monolithic. In Europe, for example, annual per capita tomato consumption in 2007–2008 varied from 30 kg or less in Germany and the Netherlands, mostly as processed products, to 82 kg in Greece, of which 75% was fresh tomatoes (Eurostat, 2006).

16.2.2 Identity and role of bioactive compounds

Tomato contributes significant dietary components to human health due to its popularity, availability and high per capita consumption (Stommel, 2007). A 100 g portion of fresh or
raw tomatoes supplies 93–95 g of water, 15–23 calories, 1–2 g of fibre, 9–23 mg of ascorbic acid, 9–30 μg of folate, less than 1 g of fat and no cholesterol (USDA, 2008). In addition to traditional nutritional components such as vitamins and minerals, tomato is rich in other bioactive components including carotenoids (lycopene, β-carotene, α-carotene, lutein, zeaxanthin, unique lycopene metabolite) (Burri et al., 2009), and phenolic compounds (Jones et al., 2003). Several other tomato components may also influence human health, including flavonoids, folic acid and the tocopherols, or vitamin E (Dorais et al., 2001).

Lycopene is the pigment responsible for the red colour of ripe tomato fruit, while other carotenoids in tomato fruit are either yellow or colourless. The physiological function of carotenoids in plants relates primarily to their photoprotective and chemoprotective role as antioxidant free radical scavengers, counteracting the damaging effects of reactive oxygen species (Demmig-Adams et al., 1996). Carotenoids are also precursors of some important aroma volatile compounds in tomato (Lewinsohn et al., 2005).

Phenolic compounds have numerous roles in plants, including cell wall structure, pigmentation and protection against microbial pathogens (von Roepenack-Lahaye et al., 2003), and, like carotenoids, they also play roles as antioxidants and free radical scavengers. Phenolic compounds in Solanaceous plants (as well as in other plants) are classified into many groups depending on the complexity of their structures, including the numbers of aromatic rings (one, two or more) and the substitutional groups on those rings. To date, there is no one perfect way to group them and, therefore, phenolic compounds such as flavonoids, anthocyanins, ascorbic acid or quercetin may not be classified under the same heading, despite their general classification as phenolic compounds (Shahidi, 2005; Shahidi and Ho, 2005; Slimestad and Verheul, 2009). Although phenolic compounds in tomatoes are present in lower concentrations than other phytonutrients, such as lycopene in mature and ripe fruit, the concentrations of these compounds depend on genotype, maturity and location within the fruit, with the highest concentrations found in the skin and placental tissues (Buta and Spaulding, 1997; Slimestad and Verheul, 2009). These phenolic compounds serve to protect the fruit from oxidative and other stresses (Winkel-Shirley, 2002). A recent comprehensive review on flavonoids and other phenolic compounds in fruit of different tomato cultivars revealed fast-growing interest in the subject, and the authors concluded that choices of cultivar as well as growing environment affected the quantity and quality of phenolics in tomatoes (Slimestad and Verheul, 2009).

**Carotenoids (lycopene, β-carotene, lutein)**

Tomato fruit are generally known to contain high levels of lycopene, which is responsible for the typical characteristic deep-red colour of ripe tomato fruit and the red colour of tomato products. Lycopene comprises 80–90% of the pigments in red tomatoes and a typical red tomato fruit may contain 0.01–0.2 mg of lycopene/g on a fresh weight (FW) basis (Shi and Le Maguer, 2000). Furthermore, tomato varieties with the crimson gene tend to have extremely high levels of lycopene (Thompson et al., 2000), but the distribution of different types of carotenoids in fact determines the fruit colour (Fig. 16.3). In addition to lycopene, red tomatoes also contain very small amounts of other carotenoids such as β-carotene, α-carotene, β-cryptoxanthin and lutein (Shi and Le Maguer, 2000). The content of these other carotenoids is relatively low in comparison with lycopene, but β-carotene is an important precursor of vitamin A. The major carotenoids in yellow (and some orange) tomatoes are β-carotene and lutein, but the contents of these compounds are often very low in comparison with lycopene content in red tomatoes. For example, Simonne et al. (2007) reported the β-carotene and lutein contents to vary from 0.6–0.9 and from 0.1–0.4 μg/g FW, respectively, in yellow tomato cv. Honey Bunch.

Tomato fruit constitute a generous source of vitamin C as well as other important bioactive compounds. At the red ripe stage, tomatoes contain on average 0.13 mg of vitamin C/g FW (USDA, 2008). However, depending on the type or cultivar, weather conditions, agricultural practices and postharvest environmental conditions, vitamin C content can range from 0.15–0.95 mg/g FW of tomato fruit.
Fig. 16.3. Typical chromatograms of tomatoes as a function of colours and types. First and second from the top are chromatograms of yellow and orange round tomatoes, respectively, with a prominent peak of β-carotene at six minutes retention time. The third, fourth, and fifth from the top are chromatograms of red ugly, red round and red grape tomatoes, respectively, all with a prominent lycopene peak at 25 minutes retention time and small β-carotene peak at six minutes retention time for red ugly and red grape tomatoes. These chromatograms were obtained from A.H. Simonne research laboratory with HPLC conditions in Simonne et al. (2007).

fruit (Abushita et al., 2000; Yahia et al., 2001; Dumas et al., 2003). Vitamin C content in tomatoes was, however, reported to be more reliant on the cultivar and maturity stage of the fruit than on seasonal variations (Shino-hara et al., 1982; Raffo et al., 2006).
Tomatoes have been documented as foods with potential chemopreventive activities against many chronic diseases because of the high levels of lycopene and other bioactive compounds (Giovannucci et al., 1995; Giovannucci, 1999, 2002). Experts agreed that, in order for the bioactive compounds from foods or supplements to exert any health effects, they must be consumed, absorbed and metabolized by the body and remain at certain concentrations in various tissues; however, data on absorption and metabolism of tomato bioactive compounds remain incomplete (Porrini et al., 1998). Therefore, the subjects of absorption and pharmacokinetic properties of bioactive compounds such as lycopene have been investigated by many researchers (Hadley et al., 2002; Divadkar-Navsariwala et al., 2003; Cohn et al., 2004; Gustin et al., 2004; Basu and Imrhan, 2007; Lindshield et al., 2006; Unlu et al., 2007a,b; Devraj et al., 2008; Burri et al., 2009). It is well accepted that uptake of lycopene progresses from intestinal mucosa into lymph, then the liver before deposition in various tissues (Schmitz et al., 1991; Stahl et al., 1992; Clinton et al., 1996; Bramley, 2000). Furthermore, lycopene may undergo changes in the human body post-consumption and absorption, due to the biochemical processes in the human body (Lindshield et al., 2006).

Overall, based on the current research, bioavailability of bioactive compounds depends on the type of compounds, food matrix (processing) and interactions with other food components, as well as the stage of gastrointestinal physiology. For example, bioavailability of lycopene in cooked or processed tomatoes is greater than in raw tomatoes because the processing treatments increase the bioaccessibility of the plant tissue (Gartner et al., 1997; Dewanto et al., 2002; Richelle et al., 2002). Also, bioavailability and absorption of lycopene is further dependent on the type of isomers, with cis-isomers being more bioavailable than the all-trans form (Failla et al., 2007; Unlu et al., 2007a), lipid levels (Gustin et al., 2004) and tomato variety (Unlu et al., 2007b; Burri et al., 2009).

### Cancer studies

After the research publication by Giovannucci et al. (1995) suggesting that tomato-based food may be beneficial in reducing prostate cancer risk, and because of the antioxidant properties of lycopene, tomato products containing high levels of lycopene have received much attention as potential cancer fighting foods. A comprehensive review of the epidemiological literature in English by Giovannucci (1999, 2002) revealed that intake of tomatoes and tomato-based products and lycopene levels in plasma were strongly negatively correlated with the risk of some types of cancers, such as lung, stomach and prostate gland, but only weakly correlated, if at all, with the risk of cancers of the cervix, breast, oral cavity, pancreas, colorectum and oesophagus. Another review by Miller et al. (2002), summarizing accumulated research on tomato products, lycopene and prostate cancer risk, recommended that consumption of one serving/day or five servings/week of tomato products, as a part of a healthy dietary pattern, might reduce the risk of prostate cancer or other chronic diseases. These reviews (Giovannucci, 1999, 2002; Miller et al., 2002) also revealed that, although the benefit of tomato and tomato products for reducing risk of certain cancers was often attributed to lycopene, there was no proof of direct benefit from ingesting lycopene alone. Giovannucci (1999) suggested ultimately that diets rich in a variety of fruits and vegetables, including tomatoes and tomato-based products, would provide health benefits.

After these reviews were published, many more research studies reported positive, neutral (inconclusive) or negative outcomes in attempts to link lycopene intakes and cancer. Among the positive outcomes, many studies have shown an inverse association between consumption of tomato and/or lycopene supplements and the risk of certain types of cancers, but others have only suggested that increased consumption of lycopene from tomatoes and tomato products may provide protection, while not enough information is available on the therapeutic use of lycopene (Chen et al., 2001; Stacewicz-Sapuntzakis and Bowen, 2005). The US Food and Drug Agency has approved the use of lycopene as a food additive for use as a colorant in foods such as canned tomatoes, sauces and salsas.
Administration evaluated two health claim petitions submitted in 2004 and found very limited evidence to support the association between tomato consumption and reduced risks of prostate, ovarian, gastric and pancreatic cancers (Kavanaugh et al., 2007). Another study, by Peters et al. (2007), examining the association between prediagnostic serum carotenoids (including lycopene) and the risk of prostate, lung, colorectal and ovarian cancers, revealed that high serum β-carotene increased risk for aggressive prostate cancer and that lycopene and other carotenoids were unrelated to prostate cancers. The authors further suggested that lycopene or tomato-based products would not be effective for prostate cancer prevention. Authors of other reviews (Etminan et al., 2004; Seren et al., 2008) examining the use of lycopene in cancer prevention and treatment came to the conclusion that, because there were not enough data regarding the benefit of lycopene supplementation, the best potential benefits could be obtained from consumption of lycopene-rich fruit and vegetables, including tomatoes. A comprehensive review of dietary lycopene in relation to its properties and anticarcinogenic effects revealed inconsistencies in the epidemiological data related to disease prevention by lycopene, and the pharmacokinetic properties of lycopene still remain poorly understood (Singh and Goyal, 2008). Another recent review (Amin et al., 2009), on the potential use of many natural products such as lycopene in cancer prevention, has shown that lycopene may decrease growth of some cancer cells, but could not define clear mechanisms of how this compound prevents cancer. Another recent study on lycopene and health claims (Cámara and Fernández-Ruiz, 2009) also concluded that more research was needed.

Because of the inconsistent outcomes in regard to consumption of tomatoes or tomato products (as a source of lycopene and other bioactive compounds) and different types of cancers, many researchers continue to focus on understanding the modes of action or basic mechanisms of action or roles of lycopene and bioactive compounds in oxidative stress (Porrini and Riso, 2000; Chen et al., 2001; Basu and Imrhan, 2007), carcinogenesis (Porrini and Riso, 2000; Sharoni et al., 2004; Wertz et al., 2004) and specific gene regulation (Zhang et al., 1992). Among the reported modes of action of lycopene are: DNA protection (Porrini and Riso, 2000), increased communication of gap-junction (Wertz et al., 2004), inhibition of IGF-1 (insulin-like growth factor I) signal transduction (Wertz et al., 2004), inhibited or reduced gene expression (Herzog et al., 2004; Wertz et al., 2004) and receptors of specific molecules (Wertz et al., 2004), gene transcription (Sharoni et al., 2004) and cell cycle regulation (Karas et al., 2000; Rao and Rao, 2007). Lindshield et al. (2006) suggested that tomato carotenoid metabolites (lycopenoids) may be responsible for reduced risks of prostate cancer in men who have consumed high levels of tomato products, but more research is needed.

Cardiovascular diseases

A comprehensive review of tomatoes and cardiovascular health (Wilcox et al., 2003) revealed that tomatoes and tomato products contained nutrients essential for cardiovascular health, namely lycopene and β-carotene, as well as other vitamins and bioactive compounds. Earlier studies revealed a strong association between diets rich in fruit and vegetables and reduced risk of cardiovascular diseases (CVD) (Clarke and Armitage, 2002; Bazzano et al., 2003; Hung et al., 2004; Omoni and Aluko, 2005; Dauchet et al., 2006). An extensive study on lycopene and myocardial infarction risk from ten European countries (Kohlmeier et al., 1997) eliminated the association of α- and β-carotene with myocardial infarction risk, but correlated it with lycopene, a carotenoid that is more common in food sources. Subsequently, basic research has revealed that the oxidation of low-density lipoproteins (LDL) increases the risk of CVD. A study by Fuhrman et al. (1997) showed the hypcholesterolaemic effect of lycopene and β-carotene, which suppressed cellular cholesterol synthesis from acetate, and that the inhibition occurred at the same time as the stimulation of LDL receptor activity on macrophages, leading to clearance of LDL from the plasma. Epidemiological observations also suggest that antioxidant vitamins (i.e. vitamins E and C) and carotenoids such as
lycopene and \( \beta \)-carotene may have protective effects against CVD (Kris-Etherton et al., 2002; Rao, 2002). These trends led to years of research to evaluate the effects of both pure supplements and specific foods on the prevention of CVD and cancers, but comprehensive reviews of the results have failed to confirm any protective effects of these supplements/foods against either cancer or CVD (Clarke and Armitage, 2002). Recent research results, however, continue to show that fruit and vegetable consumption is associated inversely with CVD (Dauchet et al., 2006), with tomato being one of the top nutrient-rich fresh produce types on the list. Possible mechanisms of action of tomatoes, tomato products and tomato constituents on the prevention of CVD have been attributed to antioxidative (e.g. reduced LDL oxidation) as well as non-oxidative effects (e.g. reduced HMG-CoA-reductase activity, reduced homocysteine levels in the blood and reduced platelet aggregation); however, many research reports continue to suggest that additional examination of the health benefits of tomato consumption is required (Wilcox et al., 2003).

Other beneficial/detrimental effects

Intake of tomatoes, cooked vegetables and fruit has been documented as being protective against wheezing in children (Farchi et al., 2003). Another review by Sies and Stahl (2004) revealed that dietary lycopene could protect against skin damage (erythema) due to sunlight. Shao and Hathcock (2006) systematically evaluated the risk of high intake of lutein and lycopene and found that intakes of up to 20 mg/day for lutein and 75 mg/day for lycopene did not result in any clear adverse effects, but they did not have enough data to determine long-term safety.

16.2.4 Effect of preharvest and postharvest continuum

Genotypes

Commercial tomatoes include several different types, including round tomatoes, which are most commonly used for fresh consumption, oblong ‘roma’ or ‘plum’ types, typically grown for processing, and small ‘cherry’ and ‘grape’ tomatoes, usually eaten fresh. Furthermore, while most ripe tomatoes are red, there are also cultivars that become yellow or orange during ripening. Research has shown that nutritional quality and bioactive compound levels may be different for various genotypes of tomatoes (Leonardi et al., 2000). For example, the different colours found in various tomato genotypes represent the relative amounts of red lycopene and yellow \( \beta \)-carotene pigments. The lycopene content of most red tomato cultivars is about 30–50 mg/kg, while deep red cultivars contain more than 150 mg/kg and yellow cultivars only about 5 mg/kg (Hart and Scott, 1995); the ranges of lycopene content found in fresh market tomato cultivars are similar to the levels measured in processing tomatoes (Garcia and Barrett, 2005). In recent years, efforts have also been made to enhance further the levels of bioactive compounds in tomatoes by examining tomato genotypes with the capacity to produce anthocyanins in the fruit (Jones et al., 2003) and ways to express genes related to anthocyanin production (Butelli et al., 2008). Other efforts have also been made to understand the upregulation of a number of tomato mutants with high pigment levels, as well as those with high antioxidant contents (Long et al., 2006; Kolotilin et al., 2007).

Production conditions

In general, as tomato fruit mature on the plant, vitamin C content tends to increase. For example, vitamin C content increased from about 0.13 mg/g FW at 18 days after fruit set to 0.95 mg/g FW at 74 days after fruit set, at the red ripe stage (Yahia et al., 2001). Consequently, vitamin C levels are usually higher in vine-ripe tomatoes than in fruit harvested at the mature-green stage (Yahia et al., 2001; Dumas et al., 2003; Slimestad and Verheul, 2005).

Dumas et al. (2003) and Dorais (2007) have reviewed the effects of environment and production conditions on the antioxidant content of tomato fruit. The amount of lycopene, the predominant antioxidant compound in tomato fruit, is influenced by the environmental conditions under which the fruit develop and
ripen. Red colour development and vitamin C content are limited by extreme high and low temperatures and solar radiation occurring during the time when the tomato fruit are ripening on the plant (Brandt et al., 2006; Dorais, 2007; Dorais et al., 2008). Furthermore, light exposure also contributes to vitamin C accumulation in tomato fruit (Dumas et al., 2003). While vitamin C synthesis in tomato fruit is stimulated by light exposure, lycopene synthesis is influenced negatively by light (Passam et al., 2007). The effect of UV-B irradiation on antioxidant potential in tomato fruit has been found to be either negligible or detrimental, depending on the tomato genotype; although depletion of UV-B radiation in the light growth conditions induced a significant increase in the vitamin C content of DRW 5981 and Esperanza tomato cultivars, vitamin C levels in HP 1 fruit tended to decrease (Giuntini et al., 2005). Similarly, Giuntini et al. (2005) reported that vitamin C accumulation was either negligible or was promoted, and carotenoid accumulation was either inhibited or promoted, in different tomato genotypes under such radiation. Luthria et al. (2006) found that reduced UV radiation, with no difference in photosynthetically active radiation, resulted in lower total phenolics, as well as lower concentrations of individual phenolic compounds in ripe tomato cvs. Oregon Spring and Red Sun fruit. In a season-long study in southern Spain, using greenhouse cherry tomatoes, Rosales et al. (2006) showed that lycopene and antioxidant levels in cv. Naomi tomato fruit were correlated negatively with temperature and overall solar radiation. In another greenhouse tomato study in New Zealand, Toor et al. (2006) showed similar trends for cvs. Excell, Tradiro and Flavourine, with the total phenolics and antioxidant activity (AOX) in the three cultivars being higher in summer than in spring and the lycopene contents being lower in the summer months; any effects of light intensity or temperature on vitamin C were unclear.

Water deficit during fruit development is well known to increase tomato soluble solids content, as well as increasing lycopene and vitamin C contents (Dumas et al., 2003). Lycopene content in tomato fruit can also be enhanced by the fertilization regime, with moderate N and increasing P and K levels resulting in greater carotenoid development (Dumas et al., 2003). A report by Taber et al. (2008) indicated that the response of tomato to a high K fertilization rate was genotype dependent, with a high-lycopene (crimson gene) variety developing greater carotenoid content in response to increasing K, while a normal variety showed no response. Excess N has also been reported to result in lower vitamin C in tomatoes (Mozafar, 1993; Kobryn and Hallmann, 2005). A report by Subbiah and Perumal (1990) indicated that Ca sprays applied to tomatoes during fruit growth increased the vitamin C content. Tomato fruit grown on organic substrates were reported to have higher vitamin C levels than fruit grown in hydroponic substrates (Premuzic et al., 1998).

Another recent review by Dorais et al. (2008) concluded that producing tomatoes with specific health attributes might have to be accomplished under unique growing conditions that might not result in the highest yield, and thus much more work will be needed to achieve any given specific benefit.

**Postharvest treatments and storage**

Lycopene content in ripening tomato fruit is influenced strongly by storage temperature, as lycopene synthesis is inhibited at temperatures at or above 30°C (Goodwin and Jamikorn, 1952; Tomes, 1963). Synthesis of β-carotene, however, continues up to at least 35°C (Hamauzu et al., 1998). Tomato ripening does not cease completely at 30°C, however, and high temperature inhibition of lycopene synthesis is reversible if the fruit are returned to a lower temperature to the extent that they remain viable to continue ripening.

Postharvest exposure of unripe or partially ripe tomato fruit to temperatures below 12°C also inhibits carotenoid synthesis due to chilling injury, which either inhibits ripening or results in abnormal colour development during ripening (Wang, 1993). Exposure of green tomatoes to ethylene initiates and accelerates ripening, including red colour development, but does not influence the final lycopene content of ripe fruit. Conflicting reports of differences in lycopene development in tomato fruit ripened on the plant
versus postharvest (Giovanelli et al., 2001; Wold et al., 2004) may be due to temperature differences, with the temperature regime that is more conducive to lycopene development resulting in greater levels of lycopene.

Vitamin C content of tomato fruit generally increases during ripening on the plant and declines during postharvest storage and handling (Bisogni et al., 1976; Betancourt et al., 1977; Soto-Zamora et al., 2000). The vitamin C content may increase during storage of tomatoes harvested at earlier stages of ripeness, but it never attains the levels found in vine-ripened fruit (Scott and Kramer, 1949). Although tomatoes ripened on the plant tend to accumulate more vitamin C than fruit ripened off the plant, the accumulation patterns in vine-ripened and postharvest-ripened fruit differ (Giovanelli et al., 1999). In vine-ripened tomatoes, vitamin C accumulation takes place during the first stages of fruit ripening and then decreases, whereas in postharvest-ripened tomatoes (20°C) vitamin C shows an initial decrease, followed by a significant increase in the last stages of fruit ripening (Giovanelli et al., 1999). Tomato fruit harvested at the breaker stage and ripened at 20°C contained only 43.6–62.9% of their potential vitamin C content if ripened on the plant to the red ripe stage (Betancourt et al., 1977). Tomatoes harvested green and ripened at 20°C contained about 55–65% of the vitamin C content relative to those harvested at the table-ripe stage (Kader et al., 1977).

Postharvest ethylene treatment to accelerate ripening results in higher vitamin C levels in the ripened fruit than seen in tomatoes ripened without ethylene, due to less vitamin C loss during the shorter time required for ethylene-treated fruit to reach the full ripe stage (Watada et al., 1976; Kader et al., 1978).

Environmental conditions during the postharvest period, namely temperature, may also contribute to increased or decreased vitamin C content of tomato fruit. For example, in tomatoes (cvs. Roma, Marglobe, Sioux, Best of All, Red Plum, Pusa Ruby, Ponderosa and H.S. 102) stored at 20°C, vitamin C content showed a tendency to increase during the first 8 days of storage but decreased afterwards (Syamal, 1990). In general, postharvest conditions or treatments such as ethylene or controlled atmospheres that either accelerate or delay ripening have indirect rather than direct effects on those tomato bioactive compounds that change in concentration during ripening.

Storage of tomatoes at chilling temperatures (i.e. below 10°C) increases the rate of vitamin C loss. Tomatoes harvested at the colour-break stage and stored for 10 days at 21°C showed the highest content of vitamin C with greatest retention during storage, compared with storage at the chilling temperatures of 1.6 or 10°C (Scott and Kramer, 1949). In tomato fruit stored at 4 or 10°C, vitamin C content increased initially but then decreased (Soto-Zamora et al., 2005).

Ripening of tomato fruit at high temperatures leads to a decrease in vitamin C content due to ascorbate oxidation (Dumas et al., 2003); however, heating cv. Rhapsody tomato fruit in air at 34°C for 24 h prior to storage at 10°C for up to 30 days reduced losses in antioxidant content compared with unheated fruit, and fruit colour developed adequately (Soto-Zamora et al., 2005). Exposure of mature green cv. Rhapsody tomato fruit to 34°C and 95% RH for 24 h promoted the tomato antioxidant system during ripening at 20°C (Yahia et al., 2007).

16.3 Peppers

16.3.1 Introduction

Although more than 30 species of peppers exist, commercial production around the world consists of the five main domesticated species: C. annuum (bell peppers, paprika, cayenne, jalapeños and the chiltepin), C. frutescens (tabasco peppers), C. chinense (naga, habanero, datil and Scotch bonnet), C. pubescens (South American rocoto peppers) and C. baccatum (wax peppers and berry-like South American aji peppers) (McLeod et al., 1982; Pickersgill, 1997; Lefebvre et al., 2001; Votava et al., 2005; Moscone et al., 2007; Paran and van der Knaap, 2007; Jarret and Berke, 2008). Among the five domesticated pepper species, C. annuum represents the largest group of peppers grown worldwide. The fresh non-pungent
peppers, which are known as bell peppers, are more economically and nutritionally important, because of their higher consumption level (Paran and van der Knaap, 2007), than those used as seasonings (paprika, dried pepper and chilli powder) (Pradeep et al., 1992; Wall et al., 2001).

16.3.2 Identity and role of bioactive compounds

Peppers are rich in traditional nutrients such as vitamin C and are a moderate source of bioactive compounds such as carotenoids, phenolics, flavonoids, vitamin E and capsaicin, to name just a few. While bioactive compounds in peppers may have some physiological function in the plants or fruit themselves, and serve as functional food for humans, some researchers have concluded that peppers are not as good a source of carotenoids (zeaxanthin, lutein, β-carotene), vitamin E (α-tocopherol) or the flavonoids quercetin and luteolin as some other fruit and vegetables (Lee et al., 2005).

Carotenoids

The fruit of the genus Capsicum, which includes both sweet (C. annum) and hot (C. frutescens) peppers, are rich in many typical carotenoids (e.g. provitamin A carotenoids, lutein and lycopene) and unique keto-carotenoids as well as allylic apo-carotenols. Pepper carotenoids have been well investigated in relation to paprika, which is commonly used as a natural colourant, as well as in terms of their vitamin A precursor activity (Gross, 1987). Pepper carotenoid profiles have been summarized comprehensively by Gross (1987). Carotenoid content of peppers varies in different cultivars (Simonne et al., 1997; Howard et al., 2000; Breithaupt and Bamedi, 2001; Wall et al., 2001; Deepa et al., 2006; Ha et al., 2007; Suzuki et al., 2007) and, in general, the total carotenoid and β-carotene contents increase with maturation and ripening (Howard et al., 2000; Deepa et al., 2006; Ha et al., 2007). Also, it was found that red peppers accumulated relatively high amounts of total carotenoids during ripening, while non-red peppers accumulated lower levels of total carotenoids of varying compositions (Ha et al., 2007). Many hot pepper varieties have been documented to have extremely high amounts of β-carotene (up to 1.2 mg/g dry weight) and total carotenoids (up to 10 mg/g dry weight) (Wall et al., 2001). However, given the extreme pungency of many hot peppers, the amount of consumption is quite low. Thus, in a practical sense, high levels of carotenoids in very hot peppers may be of limited nutritional interest because normally a large amount cannot be consumed.

Phenolic compounds and vitamin C

Bell peppers are one of the most important sources of vitamin C in the human diet, with some cultivars contributing almost 500% of the RDA (recommended daily allowance) for this vitamin per 100 g serving (Howard et al., 2000). Coloured bell peppers tend to have higher contents of vitamin C and carotenoids than green peppers; however, green, red and orange peppers have all been reported to have much higher contents of vitamin C and carotenoids than more unusually coloured peppers, such as black, purple or white (Simonne et al., 1997). Green bell peppers contain on average 0.15–0.80 mg of vitamin C/g FW, whereas red and yellow peppers may contain on average 127.7 and 315.3 mg of vitamin C/100 g FW, respectively (Osuna-García et al., 1998; Yahia et al., 2001; Geleta and Labuschagne, 2006; USDA, 2008). Green C. annum bell peppers and green hot peppers contain much higher levels of most polyphenols than the ripe fruit, but the amounts of some phenolic compounds increase during ripening (Marín et al., 2004; Materska and Perucka, 2005). Coloured peppers have, in general, a higher nutritional value than green fruit because they may contain three times more vitamin C and four times more vitamin E.

Vitamin C levels increase during ripening and peak at the full ripe stage of development (Howard et al., 1994, 2000; Luning et al., 1994; Simonne et al., 1997; Osuna-García et al., 1998; Márkus et al., 1999; Yahia et al., 2001). At the full ripe stage, peppers may contain 95% more vitamin C than at the mature green stage (Howard et al., 1994). Minimum vitamin
C levels (0.52 mg/g FW) were detected in bell peppers at 22 days after fruit set, increasing rapidly to 1.36 mg/g FW at 51 days after fruit set (Yahia et al., 2001). This trend was also observed in 43 pepper cultivars and breeding lines grown in Texas, which showed a substantial increase in vitamin C concentrations with maturation (Crosby et al., 2008). Similarly, vitamin E (α-tocopherol), also present in different chilli pepper varieties, increases during ripening, from the mature green to the fully red stages (Osuna-Garcia et al., 1998; Márkus et al., 1999).

**Flavonoids**

Peppers are documented to have moderate to high levels of flavonoids as per the definition provided by Peterson and Dwyer (1998), in which low, moderate and high flavonoid concentration ranges are 0.01–0.4, 0.04–0.1 and > 0.1 mg/g, respectively. Total flavonoid contents reported for various pepper species (C. annuum, C. frutescens and C. chinense) ranged from 0.002 mg/g (C. chinense habanero type cv. Red Savina) to 0.85 mg/g (C. annuum, long yellow-type pepper cv. Inferno), while the flavonoid content of a yellow bell pepper (C. annuum) was 0.32 mg/g (Howard et al., 2000). The levels of flavonoids in the peppers used in the latter study could not be predicted from maturity, as the flavonoid content of some fruit increased while that of others decreased during maturation, and it was suggested that extremely low levels of flavonoids in very hot peppers might reflect the interchange between flavonoids and capsaicin (Howard et al., 2000).

In another study, Saga and Sato (2003) examined immature and ripe Japanese hot and sweet peppers and found that the amount of total phenolics was higher in hot and ripe peppers than in sweet and immature peppers, but there was no difference in quercetin content between hot and sweet peppers during the fruit growing season. In a later study by Marin et al. (2004), of antioxidant constituents of sweet peppers (hydroxycinnamic acids, flavonoids, carotenoids and ascorbic acid), clear differences in individual and total phenolic contents were demonstrated for different maturity stages; immature green peppers exhibited the highest content of polyphenols, while red ripe fruit had the highest contents of ascorbic acid and provitamin A.

Based on many studies, it appears that flavonoid contents in peppers may depend on genotype (types of peppers), maturity stage, postharvest treatment, cooking and processing, as well as use. In general, the flavonoids reported most often in peppers are quercetin, luteolin (Howard et al., 2000), coumaric acid, caffeic acid derivatives, apigenin and luteolin derivatives (Materska et al., 2003; Materska and Perucka, 2005). However, specific information on the effects of cooking on these bioactive components remains conflicting; while some have reported no significant changes (Turkmen et al., 2005; Ornelas-Paz et al., 2010), others have suggested that losses are highly variable (Rickman et al., 2007).

**Capsaicin**

Capsaicin (trans-8-methyl-N-vanillyl-6-non-enamide) is a pungent (perceived as 'hot') compound found in various species of Capsicum and is classified as part of the alkaloid family along with other capsaicinoids (Thompson et al., 2006; Hayman and Kam, 2008; NPIC, 2009). Documented uses of capsaicin include as a flavouring agent (Mortensen and Mortensen, 2009), a pesticide and insect repellent (NPIC, 2009) and an analgesic (Hayman and Kam, 2008), as well as a tool in neurobiological research (Buck and Burks, 1986; Franco-Cereceda et al., 1987; Merck Index, 1996). Capsaicin has also been widely documented for use in topical pain management, diabetes and obesity control, cancer treatment, headache control and as an anti-inflammatory agent (Mortensen and Mortensen, 2009), as well as for control of overactive bladders (a condition of frequent urination) (Cronin, 2002). Other uses of capsaicin include pepper spray for personal protection (Mortensen and Mortensen, 2009). Although capsaicin is widely used topically and is well absorbed via the skin for various conditions including pain (Gilbert et al., 2007; Singh and Nulu, 2008), this chapter will focus only on the food and oral use of this compound. Despite
a long historic use of chilli peppers, the chemical investigation of these fruit did not begin until the 19th century and the chemical structure of capsaicin was identified only in the early 20th century (Barceloux, 2009). Furthermore, although the capsaicinoid family consists of more than 20 compounds, only three (6-ene-8-methyl capsaicin (6,8-C), 8-methyl dihydrocapsaicin (8-DC) and N-vanillylnonanamide (NVN)) are well characterized and commercially available in pure form (Thompson et al., 2006).

The highest level of capsaicin is present in the fruit placental tissues (which hold the seeds) of hot pepper fruit. Based on the pungency rating using Scoville heat units, bell peppers score 0–100, habanero peppers score 200,000–575,000, while pure capsaicin registers as 16 million Scoville units (Hayman and Kam, 2008; Mortensen and Mortensen, 2009). Characterization and quantification of capsaicin in hot peppers have been subjects of much research, and in a recent study by Garcés-Claver et al. (2006) it was found that two forms of capsaicin (capsaicin and dihydrocapsaicin) were the predominant contributors to pungency in hot peppers. To date, information concerning the levels of capsaicin in hot peppers is somewhat fragmented; however, Garcés-Claver et al. (2006) reported that the concentrations of capsaicin in different pepper genotypes ranged from 0.002–6.6 mg/g.

Other phytonutrients

Other phytonutrients, such as non-pungent capsaicinoids (capsiate) in sweet peppers, have been evaluated for chemopreventive and anticancer potential (Macho et al., 2003). Others have reported that the capsaicinoids cause an increase in body temperature (Ohnuki et al., 2001b), suppress fat accumulation (Ohnuki et al., 2001a) and block pathologic angiogenesis and vascular permeability caused by vascular endothelial growth factor (Pyun et al., 2008). Some efforts have been made to identify and characterize additional capsaicinoids (Kozukue et al., 2005; Thompson et al., 2005) for further assessment of their biological activities.

16.3.3 Chemopreventive activity and bioavailability

Chemopreventive properties of some pungent ingredients in red peppers have been described by Surh et al. (1998). For peppers, many of the chemopreventive properties identified have been attributed to capsaicin (Surh, 2002). Although red and green C. annuum peppers have high levels of antioxidative activity, a recent study has shown that methanolic extracts of pepper fruit tissue do not exhibit any antiproliferative activity in contrast to many other commonly consumed raw vegetables (Park et al., 2000). Bioavailability or absorbability of some bioactive components in peppers is limited compared with other vegetables or fruit such as tomatoes. Suresh and Srinivasan (2007) compared the absorbability of three spice active principles, curcumin, piperine and capsaicin, in an in vitro rat intestine model system. Although these three compounds were similar in structure, the researchers found that capsaicin (10–500 µg/10 ml) was absorbed the least for a given concentration and the amount absorbed did not increase proportionally with the concentration applied; however, absorption increased when the same compound was present in micelles rather than in its native form.

Cancer studies

Capsaicin in peppers has been at centre stage when it comes to cancer studies. Capsaicin has been documented to have both negative and positive effects on various types of cancers, but so far the results have not been conclusive. A case-control study that took place in Mexico City from 1989 to 1990, examining the relation between chilli peppers and gastric cancer, found that chilli pepper consumption was considered a possible risk factor for gastric cancer, but more studies are needed to prove this assumption (López-Carrillo et al., 1994). These authors later investigated an association of gastric cancer with capsaicin consumption and Helicobacter pylori infection, but they concluded that, in Mexico, chilli pepper consumption might be independent of gastric cancer (López-Carrillo et al.,
Another case-control study in Korea (Lee et al., 1995) revealed an increased risk of stomach cancer among those people with high consumption of soybean paste stew and hot peppers. Another study (Serra et al., 2002) revealed an association of chilli pepper consumption, low socio-economic status and longstanding gallstones with gallbladder cancer in a Chilean population, but the researchers also suggested additional studies needed to be conducted to examine the risk factors further.

Laboratory studies suggest that capsaicin in hot chilli peppers may be a carcinogen. In reviews by Surh and Lee (1995, 1996) examining capsaicin as a carcinogen, co-carcinogen or anticarcinogen, it was revealed that capsaicin might have dual effects on chemically induced carcinogenesis and mutagenesis, but the results were conflicting. Metabolism of capsaicin appeared to involve microsomal cytochrome P450-dependent monoxygenases, which are involved in both activation and detoxification of many chemical carcinogens and mutagens. Another review of both experimental and clinical data on capsaicin and stomach disease revealed that capsaicin at low concentrations protected rat gastric mucosa from injury by ulcerogenic agents via stimulation of the local defence system; however, in humans, the higher concentrations of capsaicin obtained from hot foods produced the opposite effects. The authors of this review also concluded that more studies were needed to support their findings (Abdel-Salam et al., 1997). Based on these reviews, it is possible that the positive or negative effects of capsaicin may depend on dosage (Surh and Lee, 1995, 1996; Abdel-Salam et al., 1997; Surh et al., 1998). Yet, it remains unclear whether capsaicin is carcinogenic or anticarcinogenic (Surh et al., 1998).

A review by Archer and Jones (2002) examining the association between capsaicin and ethnicity strengthened further the results of prior case-control studies on the association of stomach cancer with capsaicin in peppers; they found elevated stomach cancer incidence among five cultural groups with high usage of peppers in their cooking. In this study, a reduced colon cancer rate was found among high capsaicin pepper users, but the authors also suggested additional studies to test their findings further. Laohavechvanich et al. (2006) reported that four Thai hot chilli peppers (C. frutescens) contained mixtures of antimutagens, but could not establish an association between the antigenotoxicity and glutathione transferase activity tests with larvae of Drosophila melanogaster. The anti-tumour or anticancer action of capsaicin and luteolin in peppers may be due to apoptosis of tumour or cancer cells. Such results may have potential application for delaying cancer growth or for cancer treatment (Khan et al., 2006). Although many studies have revealed the anticancer effects of capsaicin and luteolin (Table 16.1), based on current knowledge there are not enough data to make a final conclusion because some of the negative or positive effects are dose dependent.

Cardiovascular diseases

Direct studies testing the potential benefits of peppers on CVD have not been documented. Many studies have, however, included peppers as one of the vegetables with potential properties against CVD due to the high content of many antioxidative compounds that may help prevent the oxidation of cholesterol and other blood lipids (Perucka and Materska, 2001; Suhaj, 2006; Sun et al., 2007; Antonious et al., 2009).

Other beneficial and detrimental effects

Peppers and their bioactive compounds have been documented to have antimicrobial properties against some foodborne pathogens and and chemoprotective effects against ethanol-induced injury of gastric mucosa, to be potential remedies for functional dyspepsia, to have antioxidative properties in brain tissues and to increase carbohydrate oxidation and metabolic rate in humans. Furthermore, capsaicin has also been reported to have a thermoregulatory effect in animals in both cold and warm environments (Szikszay et al., 1982) and thermogenesis (Mahmmoud, 2008).

Antimicrobial properties of chilli peppers as well as their uses in Mayan medicine have been recorded, with capsaicin and dihydrocapsaicin showing varying degrees of growth
<table>
<thead>
<tr>
<th>Activity</th>
<th>Action</th>
<th>System</th>
<th>Dose</th>
<th>Extract type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer</td>
<td>Induced programmed cell death by activating the peroxisome proliferator-activated receptor</td>
<td>HT-29 human colon cancer cells</td>
<td>0–300 fmol</td>
<td>Capsaicin</td>
<td>Kim et al. (2004)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Inhibit growth of prostate cancer cells</td>
<td>Prostate cancer cells (LNCap, PC-3 and Du-145 cells)</td>
<td>1 x 10^{-4} - 5 x 10^{-4} mol/l PC-3 cells and 1 x 10^{-4} - 5 x 10^{-4} mol/l LNcaP cells</td>
<td>Pure capsaicin</td>
<td>Mori et al. (2006)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Induce programmed cell death and inhibit xenograft prostate tumour growth</td>
<td>Prostate tumour PC-3 cells</td>
<td>IC_{50} = 20 fmol</td>
<td>Capsaicin</td>
<td>Sánchez et al. (2006)</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Block STAT3 activation pathway</td>
<td>Multiple myeloma cells</td>
<td>Varied</td>
<td>Capsaicin</td>
<td>Bhutani et al. (2007)</td>
</tr>
<tr>
<td>Antimicrobial</td>
<td>Inhibit growth of <em>Helicobacter pylori</em></td>
<td><em>In vitro</em>, 16 clinical isolates of <em>H. pylori</em></td>
<td>25–50 μg/ml</td>
<td>Capsaicin</td>
<td>Zeyrek and Oguz (2005)</td>
</tr>
<tr>
<td>Antioxidative</td>
<td>Protect against ethanol-induced oxidative injury by inhibiting cyclooxygenase-2</td>
<td>Gastric mucosa of rat</td>
<td>0.5–10 mg/kg</td>
<td>Capsaicin</td>
<td>Park et al. (2000)</td>
</tr>
<tr>
<td>Increased metabolism</td>
<td>Increase carbohydrate oxidation</td>
<td>Human subjects</td>
<td>10 g</td>
<td>Red hot peppers</td>
<td>Lim et al. (1997)</td>
</tr>
<tr>
<td>Increased body temperature</td>
<td>Increase O_{2} consumption</td>
<td>Human subjects</td>
<td>0.1 g/kg body weight</td>
<td>Sweet peppers: CH-19 and California-Wandar</td>
<td>Ohnuki et al. (2001a)</td>
</tr>
<tr>
<td>Increased metabolism</td>
<td>Increased energy metabolism and suppressed body fat accumulation</td>
<td>Mice</td>
<td>10 and 50 mg/kg body weight</td>
<td>Capsiate</td>
<td>Ohnuki et al. (2001b)</td>
</tr>
<tr>
<td>Cancer prevention</td>
<td>Antiproliferative and apoptotic activity</td>
<td>Human colon cancer cells (Hepa-1c1 x 7 and Sw480 human colon cancer cells)</td>
<td>1–20 μg/ml</td>
<td>Ixocarpalactone A (IxoA) from tomatillo</td>
<td>Choi et al. (2006)</td>
</tr>
</tbody>
</table>
inhibition of Bacillus cereus, B. subtilis, Clostridium sporogenes, C. tetani and Streptococcus pyogenes (Cichewicz and Thorpe, 1996). Pepper extracts (C. annuum, such as habanero, Serrano and pimiento morron peppers) were documented to be most effective against growth of Listeria and least effective against Salmonella, but the inhibitory effects were attributed to cinnamic and m-coumaric acids, not to capsaicin (Dorantes et al., 2000).

Red peppers were more effective than a placebo in decreasing the intensity of dyspeptic symptoms for people with functional dyspepsia (without gastro-oesophageal reflux disease and irritable bowel syndrome), and the decrease in symptoms was attributed to desensitization of gastric nociceptive C-fibres induced by capsaicin (Bortolotti et al., 2002). Water extracts from C. pubescens (tree peppers) were also found to inhibit lipid peroxidation induced by different pro-oxidant agents in rat brain, in an in vitro study (Oboh et al., 2007; Oboh and Rocha, 2008), and the antioxidative effect was attributed to the high phenolic content in the peppers.

In addition to beneficial effects, pepper capsaicin has been reported to have some adverse effects on humans, including a fatal case of pepper poisoning of an 8-month-old infant (Snyman et al., 2001). In recent years, capsaicin has been linked to arterial hypertensive crisis and acute myocardial infarction (with high thyroid-stimulating hormones), including the case of a 59-year-old male after ingestion of a large quantity of pepper and chilli peppers the day before (Patanè et al., 2008) and a case of arterial hypertensive crisis in a 19-year-old male (Patanè et al., 2009).

Additional information on some beneficial properties is provided in Table 16.1.

16.3.4 Effect of preharvest and postharvest continuum

Genotypes

The types and amounts of antioxidants in peppers vary by genotype and maturity and are influenced by growing conditions (Davies et al., 1970; Mejia et al., 1988; Lee et al., 1995; Daood et al., 1996; Simonne et al., 1997; Osuna-Garcia et al., 1998; Márkus et al., 1999; Deepa et al., 2006). Howard et al. (2000) measured the carotenoids, flavonoids, phenolic acids, ascorbic acid and AOX in bell pepper and tabasco- and habanero-type peppers. The habanero peppers contained much lower amounts of carotenoids and flavonoids than the bell pepper and tabasco types, but total soluble phenolic content, vitamin C and AOX were similar in all three pepper types. Capsaicinoid content is also affected by the types and varieties of peppers (Cruz-Perez et al., 2007; Hayman and Kam, 2008; Mortensen and Mortensen, 2009), as well as by the stages of development (Cruz-Perez et al., 2007); the capsaicinoids tended to decrease at later stages of ripeness (after 58–96 days) in most varieties of manzano hot peppers used in the latter study. Konisho et al. (2005) investigated the specific variation of capsaicinoid concentration in cultivated Capsicum species (145 accessions) from around the world and found negative correlations between capsaicinoid concentration and fruit size. Furthermore, the researchers found high levels of variation in capsaicinoid content and composition between the domesticated Capsicum species. A recent study by Antonious and Jarret (2006), examining 90 Capsicum accessions selected from the USDA Capsicum germplasm collection, revealed that capsaicin concentrations in peppers were typically higher than those of dihydrocapsaicin, and that the concentrations of total capsaicinoids were non-detectable in C. chinense and highest in the C. frutescens group.

Production conditions

The growing environment was shown by Lee et al. (2005) to affect the phenolic and carotenoid compositions of different pepper types produced in a greenhouse at College Station and in field plots at Uvalde and Weslaco, Texas, USA. The three growing environments differed in terms of soil or growth medium, temperature, humidity, rainfall and evapotranspiration. The best sources of β-carotene were mature, greenhouse-grown fruit of cvs. Fidel and C127. Mature, greenhouse-grown fruit of cvs. Tropic Bell and PI357509 had high
lutein, but Uvalde field-grown mature fruit of these lines had low levels. Cultivar MJ201 fruit had the highest zeaxanthin levels at both College Station and Uvalde. The best sources of quercetin over all locations were the yellow wax types, cv.s. Banana Supreme, PI357509 and Rio Grande Gold. Cultivars Fidel and Banana Supreme were the best sources of luteolin. Greenhouse-grown peppers at College Station contained more carotenoids than the field-grown peppers in Uvalde and Weslaco, but there were no significant differences among locations in terms of the flavonoid concentrations.

Bell peppers responded to increasing saline irrigation (15 or 30 mM NaCl) in a hydroponic production system with higher lycopene and hydrophilic and lipophilic AOX in red (ripe) fruit, but there were no significant effects on β-carotene, and ascorbic acid and total phenolic contents were reduced (Navarro et al., 2006). Limited information is available on growing conditions and hot pepper quality. Dorji et al. (2005) compared two irrigation practices designed to save water and found that reduced water irrigation could be used when the benefit of saving water outweighed the decrease in total fresh mass of fruit.

**Postharvest treatments and storage**

Peppers are non-climacteric fruit and as such do not ripen in terms of colour development in response to ethylene treatment, although some further colour development of partially ripe peppers can occur postharvest (González-Aguilar, 2004). The total AOX and vitamin C content of both green and red cv. California bell peppers increased during 19 days of storage at 20°C (Jiménez et al., 2003), but the vitamin C content of habanero peppers did not show a significant change during 20 days at 7°C and decreased after 35 days of storage at that temperature (González et al., 2005). Hot cayenne peppers accumulated higher levels of carotenoids, phenolics and capsaicin as they ripened and also developed higher AOX, but small green fruit exhibited the highest scavenging activity against DPPH• (2,2-diphenyl-1-picrylhydrazyl radical) (Conforti et al., 2007). Generally, the changes in composition that occur during postharvest storage of peppers are much less significant than the differences associated with horticultural maturity (stage of ripeness of the fruit) (Raffo et al., 2007). In solutions of protic solvents such as alcohol and water, vanillyl nonanoate (a capsinoid) tended to decompose, but it tended to be more stable in non-polar solvents (Sutoh et al., 2001) and the compound tended to be stable in the pepper tissues after various drying temperatures and times, as demonstrated in jalapeño peppers (Pordesimo et al., 2004).

**16.4 Other Solanaceous Fruit**

**[Aubergine, Cape Gooseberry, Tomatillo]**

### 16.4.1 Introduction

Aubergine (eggplant; *S. melongena* L.) is among the top ten vegetables in terms of the ability to absorb oxygen radicals, due to its high contents of many classes of phenolic compounds (Chanatsut and Rattanapanone, 2008; Raigón et al., 2008; Singh et al., 2009). Despite the potential antioxidative and other beneficial health effects for humans, phenolic compounds may have a potential drawback due to the negative quality effect of oxidative tissue browning when the fruit are cut (Prohens et al., 2007, 2008). Although aubergine is normally eaten cooked, except for some regions such as in Thailand (Chanatsut and Rattanapanone, 2008), most of the recent research has been conducted on raw aubergine.

### 16.4.2 Identity and role of bioactive compounds

**Phenolic compounds and vitamin C**

Depending on the cultivar and environmental conditions during development in the field and during the postharvest period, aubergines may contain between 0.02 and 0.05 mg vitamin C/g of fresh fruit (Floyd and Fraps, 1939; Aubert, 1971; Flick et al., 1977; USDA, 2008). These are low concentrations compared with those in many other fruit and vegetables, and may limit the role of aubergine vitamin C as an antioxidant (Hanson
Aubergine is also a poor source of β-carotene and vitamin E, with average values of 0.02 μg/g FW and 0.003 mg/g FW, respectively. On the other hand, purple aubergines are a good source of phenolic compounds and, at harvest maturity, the total phenolic content of aubergines may range from 3.45–78.96 μmol/g tissue dry weight, depending on the cultivar (Stommel and Whitaker, 2003; Whitaker and Stommel, 2003; Hanson et al., 2006).

The vitamin C content of aubergines was reported to increase during fruit growth until 42 days after fruit set, decreasing thereafter (Esteban et al., 1992). Likewise, total phenols increased during fruit development until day 42 and declined afterwards. The increases in vitamin C and total phenolic contents of three different aubergine cultivars (semi-round striped, purple long and black round) were coincident with the increase in total sugar content and, after 42 days from fruit set, the fruit were considered to be at their maximum visual and eating quality (Esteban et al., 1992).

For dark purple aubergine, coloration of the fruit epidermis is attributed to the relatively high concentration of anthocyanin pigments, which consist mainly of delphinidin derivates (Sakamura and Obata, 1963; Nothmann, 1986). One of these pigments has since been identified as nasunin, which consists of cis-trans isomers of delphinidin 3-[4-(p-coumaroyl)-L-rhamnosyl (1→6) glucopyranoside]-5-glucopyranoside (Ichiyanagi et al., 2005). Accumulation of anthocyanin pigments starts at the fruit apex, spreading gradually towards the base of the fruit (Nothmann, 1986). Maximum anthocyanin concentrations were observed in dark-coloured fruit, with a gradual decrease with declining intensity of purple colour. The highest concentrations of anthocyanins were accompanied by the highest chlorophyll levels. Dark-purple fruit contained more chlorophyll than dark-green fruit, but dark-green aubergines contained twice the amount of chlorophyll as light-green fruit. White aubergines, not surprisingly, contained almost no pigments (Nothmann et al., 1976). Nasunin from aubergine peel has been found to be a potent scavenger of oxygen radicals (Noda et al., 2000). In recent years, an effort has been made to recover anthocyanins from aubergine peel for potential food use (Todaro et al., 2009). After harvest, the anthocyanin content of aubergines (cv. Money Maker) decreased by 56% during the first 6 days of storage at 10°C and remained constant up to 15 days of storage, while the anthocyanin content of aubergines stored at 0°C decreased by 73% after 2 days and increased slightly afterwards (Concellón et al., 2007). Similarly, vitamin C content of aubergines (semi-round striped, purple long, and black round) reportedly decreased by 75% after 18 days of storage at 10 or 20°C, but at 5°C the reduction was less pronounced (Esteban et al., 1989). After 14 days at 10 or 12°C, vitamin C content of aubergine fruit (cv. Pala-49, Kaynas et al., 1995; cv. Tsakoniki as scion, with S. sisymbriifolium and S. torvum rootstocks, Arvanitoyannis et al., 2005) was reduced by approximately 43 and 58%, respectively (Kaynas et al., 1995; Arvanitoyannis et al., 2005).

Chlorogenic acid, the main phenolic compound identified in Japanese aubergines, decreased during storage for 2 days at 1°C, increased rapidly after 4 days, and decreased gradually thereafter. For aubergines stored for 2 days at 1°C plus 30 h at 20°C, chlorogenic acid content increased gradually during the 30 h after removal of the fruit from cold storage. Likewise, chlorogenic acid content increased steadily during a 10-day storage period at 20°C (Kozukue et al., 1979). Esteban et al. (1989) reported an initial increase in the polyphenol content of aubergines stored at 10 or 20°C, with an even greater increase observed at 5°C. The increased development of browning of aubergine fruit during storage was attributed to the rapid increase in chlorogenic acid, as the oxidative substrate, after 4 days of storage at 1°C and during storage at 20°C (Kozukue et al., 1979).

Although not many studies have been conducted on the quality and compositional attributes of cape gooseberry (P. peruviana L.), it is clear that this fruit is very rich in potassium, vitamins B, C and E, carotene and polyphenols (Morton, 1987; Hewett, 1993; McCain, 1993; van Wyk, 2005). As the fruit of cape gooseberry ripen, vitamin C content increases...
continuously until the full ripe stage. Cape gooseberries contain on average 0.02-0.03 mg of β-carotene and 0.30-0.54 mg of vitamin C/g fruit FW (Mazumdar and Basu, 1979; Morton, 1987; Sarkar and Chattopadhyay, 1993; Fischer et al., 2000; Ramadan and Morsel, 2005).

To date, no extensive research has been reported on cape gooseberry fruit and limited information can be found in the literature regarding fruit quality and composition, particularly when handled under different environmental conditions; however, compared with other temperatures, cape gooseberries maintain the longest postharvest life and best visual quality when stored at 10°C. Storage at 0 or 5°C reduced the postharvest life of the fruit due to the development of skin injury and discoloration on transfer to ambient temperature, which was probably the result of chilling injury, while storage at 15 or 20°C resulted in accelerated shrivelling and development of decay (Nunes, 2008).

Little was found in the literature regarding tomatillo or husk tomato (P. ixocarpa Brot.) quality and composition, namely vitamin C content during fruit development. An early study reported that vitamin C content of tomatillo was relatively low (0.03–0.04 mg/g FW) at early stages of development and remained unchanged during further development (Cantwell et al., 1992). At the ripe stage, tomatillo fruit may contain on average 0.12 mg of vitamin C/g FW (USDA, 2008).

16.4.3 Chemopreventive activity and bioavailability

Plant phenolics have been well documented as having multiple chemopreventive properties, but limited information is available specifically for aubergine, cape gooseberry or tomatillo. Until recent years, very little information on the health benefits of tomatillo had been published. Kennelly et al. (1997) revealed an induction of quinone reductase by withanolides isolated from tomatillo; withanolides are classes of steroids first isolated from Withania somnifera in the late 1960s (Su et al., 2004). According to a review by Su et al. (2004), several new withanolides have been isolated and characterized as inducers of quinone reductase enzymes, and previously withanolides have been documented as having many potential chemopreventive properties, including the antiproliferative and apoptotic properties of ixocarpalactone A in tomatillo (Choi et al., 2006). With regard to the bioavailability of bioactive compounds, one study revealed that steam cooking improved in vitro bile acid binding significantly in many vegetables, including aubergines, in comparison with uncooked ones (Kahlon et al., 2007).

Cancer studies

According to some folk medicines, some plants of the Solanaceae family have been implicated in anticancer effects (Kennelly et al., 1997; Su et al., 2004; Choi et al., 2006), but limited information is available on reasons why those members of the plant family have these effects. Historically, atropine (derived from (-)-hyoscyamine and -hyoscine) from Solanaceae species has been used clinically (Kanto and Klotz, 1987; Phillipson, 2001). A recent study by Lee et al. (2004) revealed that glycoalkaloids and metabolites found in potato, aubergine and tomato inhibited growth of human colon and liver cancer cells. A study by Yeh and Yen (2005) suggested that fruit and vegetables, including aubergines, containing components that had the ability to induce a detoxifying enzyme called phenol-sulfotransferase could play a role in the prevention of chronic diseases such as cancer. Azevedo et al. (2007) revealed that a purified anthocyanin (delphinin) from aubergines might have potential as a natural colorant that could prevent mutation, but the researchers suggested additional studies to confirm their findings.

Cardiovascular diseases

Phenolic compounds have been well documented as effective antioxidants and, although aubergines have been used in many areas of the world to control cholesterol, very little information is available on this specific subject. A study by Botelho et al. (2004)
revealed that aubergine extract increased oxidative stress in LDL receptor knockout mice (LDLR−/−), which meant that aubergines could be a risk factor for atherosclerosis. However, another study revealed an antiangiogenic activity of nasunin (an anthocyanin in aubergine peel) in in vitro models based on endothelial cells from human umbilical vein (Matsubara et al., 2005). From existing information, it is not possible to reach any conclusion on the relationship between CVD and this plant family.

Other beneficial and detrimental effects

Because of their high content of carotene, lutein and zeaxanthin, the consumption of cape gooseberry may help reduce the incidence of cataracts and macular degeneration (Lyle, 2006). Another study revealed that P. peruviana fruit juice exhibited a mild anti-inflammatory activity and this activity might relate to its ability to inhibit fibroblast growth (Pardo et al., 2008). However, a recent evaluation of some native Peruvian fruit for antihyperglycaemia and antihypertension potential, using in vitro models, did not show any significant potential for cape gooseberry for these two conditions; the researchers correlated high phenolic contents in some of the fruit to antioxidant activity (Da Silva Pinto et al., 2009). Kwon et al. (2008) reported that aubergine phenolics inhibited key enzymes (α-glucosidase and angiotensin I converting enzyme) for type II diabetes and hypertension in an in vitro study.

In addition to beneficial effects, aubergines have been reported recently to have some negative effects, including anaphylaxis in a patient with latex allergy (Lee et al., 2004) and other allergy reactions (Pramod and Venkatesh, 2004; Harish Babu et al., 2008).

16.5 Future Research Needs

Further research is needed on the potential effects of preharvest factors, such as irrigation, chemicals and light, on the bioactive components of Solanaceous fruit, as well as on the effectiveness of specific postharvest treatments, such as temperature, humidity or modified atmospheres, as a way to boost the nutritional value and bioactive compounds in fruit from Solanaceous plants.

Further research is also needed to confirm the bioavailability of specific bioactive compounds, namely lycopene, anthocyanins and other minor bioactive compounds, when consumed as part of a ‘fruit or vegetable cocktail’, as in real life, rather than individually. However, the effects of lycopene and other bioactive compounds used individually also need further investigation, in order to understand how they act in protecting the human body against some degenerative diseases.

Study in more detail is also needed for some specialty crops, such as cape gooseberry and tomatillo, in terms of their composition, postharvest requirements and potential health benefits when part of a balanced diet.

16.6 Conclusion

More collaborative research should be conducted that includes all those involved in the study of the Solanaceae family – namely, food scientists, postharvest physiologists and nutritionists – in an effort to understand better the characteristics, pre- and postharvest requirements and potential health benefits of Solanaceous fruit.

References


17 Tropical Fruit
[Banana, Pineapple, Papaya and Mango]

Thiruchelvam Thanaraj and Leon A. Terry

17.1 Introduction

Tropical fruit crops (banana, pineapple, papaya and mango) are a group of botanically unrelated plants. They are grouped together for ease in this chapter merely because of their tropical adaptation and global importance. Tropical crops form a substantial part of the export economy of several developing countries. Banana, pineapple and mango are categorized as major tropical fruit crops, while papaya is considered a minor crop (Galán Saúco, 1996).

Tropical fruit are commonly consumed fresh. Despite their generally relatively low calorific value (banana and plantain are the exceptions), tropical fruit play a major role in the human diet, mainly because of their high and diverse concentrations of vitamins, minerals, carotenoids and other bioactive components. The antioxidant activities of tropical fruit have been discussed in a few studies only, even though some of these fruits are rich in dietary antioxidants (Jimenez-Escrig et al., 2001; Bashir et al., 2003; Bennett et al., 2010). Mango and papaya are good sources of carotenoids (β-carotene) and vitamin C, while banana is rich in polyphenols (Arora et al., 2008; Vijayakumar et al., 2008; Bennet et al., 2010) and pineapple is rich in vitamin C (Cordenunsi et al., 2010). However, pineapple and papaya contain proteolytic enzymes, namely bromelain and papain, respectively. These enzymes have significant chemopreventive activities and are used in several industrial applications. Tropical fruit also contain high levels of pectin, fibre and cellulose, which are believed to promote intestinal motility. The relatively high organic acid content of many tropical fruit may also stimulate appetite and aid digestion (Martin et al., 1987).

In the main, tropical fruit are rich in health-promoting properties and are associated with several health benefits to humans; however, the published information is still very limited. Therefore, it is necessary to highlight the health benefits of tropical fruit, which may improve awareness among people, especially in the developing world, in selecting and consuming these fruit.

17.2 Banana

17.2.1 Introduction

Banana is a common name for the fruit of the genus Musa, which bears edible fruit of the family Musaceae. Edible fruit-bearing banana cultivars belong to the species endemic to South–East Asia (Musa acuminata and M. balbisiana) (Zhang et al., 2005). Banana usually refers to a soft, sweet dessert fruit but there are also green, firm and starchy fruit from a group
referred to as the plantains, which are also valued as a staple crop. Dessert banana fruit are imported in large quantities from the tropics to temperate regions. Banana cv. Cavendish is still the most important among commercial cultivars and accounts for a huge bulk of the bananas imported from the tropics (Arora et al., 2008). Total banana production was about 81.3 million tonnes (Mt) in 2007. India, Ecuador, Brazil and China are the leading banana producers; however, Ecuador, along with Costa Rica, the Philippines and Colombia are the leading banana exporting countries (FAO, 2005). Because of the dominance of cv. Cavendish, less is known about the vast plethora of other banana varieties (Arora et al., 2008).

Banana fruit dry matter consists mainly of sugars (glucose, fructose and sucrose), starch and fibre, making it an ideal immediate and slightly prolonged source of energy. Due to the fibre content, banana can help to restore normal bowel function. Banana fruit also contains pectin, a soluble fibre (hydrocolloid) that can help normalize movement through the digestive tract. Other than these beneficial effects, banana fruit is also a rich source of health-related compounds such as vitamins, phenolic compounds, carotenoids, minerals and certain amino acids. Though banana is low in primary antioxidants, it is rich in secondary antioxidants (Lim et al., 2007).

17.2.2 Identity and role of bioactives

Phenolic compounds

Total phenolic (TP) content of banana peel varies greatly among cultivars; for example, cv. Kluii Hom Thong, which has 3.0 mg gallic acid equivalents (GAE)/g fresh weight (FW), contains higher TP than cv. Kluii Khai (0.9 mg GAE/g FW) (Nguyen et al., 2003). The apparent TP content of Malaysian banana cv. Pisang-mas varies between 0.24 and 0.72 mg GAE/g FW depending on the extraction method (Lim et al., 2003). The apparent TP content of Malaysian banana cv. Pisang-mas varies between 0.24 and 0.72 mg GAE/g FW depending on the extraction method (Lim et al., 2007; Alothman et al., 2009). The TP and flavonoids contents correlate positively with antioxidant capacities, as measured using ferric reducing antioxidant power (FRAP) or 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Alothman et al., 2009).

Generally, flavonoids are believed to exhibit anti-inflammatory, antineoplastic and hepatoprotective activities and to reduce acid secretion from gastric parietal cells (Havsteen, 1983; Beil et al., 1995). Leucodelphinidin (a flavonoid) is more abundant in plantain than in banana pulp and has been reported to have an antiulcerogenic effect in humans, and also to have a protective effect against aspirin-induced damage of the gastric mucosa (Lewis et al., 1999). Galloctechin is more abundant in banana peel (1.58 mg/g dry weight (DW)) than in pulp (0.3 mg/g DW) and thus is probably not of importance (Someya et al., 2002). Bennett et al. (2010) showed recently that catechin, galloctechin, epicatechin and condensed tannins were present in soluble extracts of banana fruit pulp, and reviewed the chemical structures commonly found in various Musa spp.

Banana bracts are abundant edible residues of banana production and are consumed as a vegetable in most banana producing countries. Bracts contain various anthocyanins (~ 0.32 mg/g FW). Cyanidin-3-rutinoside is the prominent anthocyanin and contributes about ~ 80%; however, 3-rutinoside derivatives of delphinidin, pelargonidin, peonidin and malvidin also contribute in considerable quantities.

Vitamins

Both ascorbic acid (AsA) and dehydroascorbic acid (DHA) contribute to the total vitamin C concentration. Banana is a moderate source of vitamin C (0.33 mg/g FW) (Cano et al., 1997). Dwarf Brazilian (‘apple’) banana fruit contain about threefold higher levels of vitamin C (0.13 mg/g FW) than cvs. Williams and Pisang-mas (0.05 mg/g FW) (Wall, 2006; Lim et al., 2007). However, the vitamin C content of banana cv. Cavendish ranges between 0.02 and 0.19 mg/g FW (Leong and Shui, 2002; USDA-ARS, 2004). Vitamin A content of Dwarf Brazilian (‘apple’) banana fruit is around 12.4 μg retinol activity equivalents (RAE)/100 g FW, while cv. Williams has 8.2 μg RAE/100 g FW (Wall, 2006). These values are higher than the vitamin A content of cv. Cavendish fruit (4.5 μg RAE/100 g FW) (Wenkam, 1990). However, vitamin A content of banana is based mainly on the β-carotene (most active provitamin A
pigment) and α-carotene (less active provitamin A pigment) concentrations (Wall, 2006).

**Carotenoids**

Banana fruit are generally recommended for young children and pregnant/lactating mothers since they are rich in carotenoids that protect against vitamin A deficiency (Englberger et al., 2003). Banana fruit with orange-coloured flesh have higher concentrations of bioavailable carotenoids, such as β-carotene (9.4–27.8 μg/g FW), α-carotene (6.1–9.5 μg/g FW), lutein (0.4–1.0 μg/g FW) and zeaxanthin (0.1–0.2 μg/g FW), than yellow and more creamy-fleshed banana pulp (Englberger et al., 2003). Since the banana matrix is digestible, the bioavailability of β-carotene in banana fruit is relatively high (Englberger et al., 2003). The average lutein concentrations of Dwarf Brazilian banana fruit and cv. Williams were found to be 1.6 and 1.1 μg/g FW, respectively, which exceed the corresponding values for the provitamin A pigments such as β-carotene and α-carotene (Englberger et al., 2003; Wall, 2006). Arora et al. (2008) detailed the variation in β-carotene content in the pulp and peel of a number of Indian-derived banana cultivars and showed that there might be an opportunity to exploit these as a by-product as the higher carotenoid levels are found in the peel. The Red banana was ranked as one of the highest for total carotenoid levels found in both pulp and peel (Arora et al., 2008).

**Minerals**

Potassium (5.09 mg/g FW) is the prominent mineral found in banana, followed by phosphorus (0.59 mg/g FW), magnesium (0.38 mg/g FW) and calcium (0.38 mg/g FW) (Hardisson et al., 2001). The peel of some Cameroon banana cultivars has a relatively high content of minerals, namely potassium (50.0 mg/g DW), phosphorus (22.0 mg/g DW), magnesium (11 mg/g DW) and calcium (18 mg/g DW) (Emaga et al., 2007). Generally, calcium content is low in banana; however, Micronesian cv. Karat contains relatively high calcium content (Englberger et al., 2003). Banana contributes about 2.7% of the total potassium and fibre consumed by an average adult (USDA-HHS, 2004).

**Essential amino acids**

Dopamine is a strong, water-soluble antioxidant found in the peel (0.8–5.6 mg/g FW) and pulp (0.03–0.1 mg/g FW) of banana cv. Cavendish, and is one of the catecholamines that suppress the oxygen uptake of linoleic acid. Dopamine has similar antioxidant potency to strong antioxidants such as galloatechlin gallate and AsA (Kanazawa and Sakakibara, 2000). Bioactive amines such as putrescine, spermidine and serotonin have been identified in high concentrations from banana cv. Prata. The content of serotonin, which is responsible for regulating a number of important functions in humans, i.e. sleeping, thirst, hunger, mood and sexual activity, reduces during ripening, while that of some other amino acids is maintained (Coutts et al., 1986; Adão and Glória, 2005). Vetorazzi (1974), Marriott and Palmer (1980) and Coutts et al. (1986) reported that bioactive amines such as serotonin, dopamine, noradrenaline, octopamine, histamine, 2-phenylethylamine and tyramine have been identified in various banana cultivars.

**17.2.3 Chemopreventive activity and bioavailability**

Banana contains relatively high iron content. Frequent consumption of banana (four to six times/week) reduces the risk of kidney cancer and other disorders related to the kidney and urinary tract. Banana has relatively high potassium (3.5 mg/g FW) and very low sodium content, a good ratio for preventing high blood pressure and stroke. Consuming bananas regularly in the diet can reduce significantly the risk of death caused by strokes, by about 40%. Phytonutrients from the fruit generally stimulate natural detoxifying enzymes in the body; these enzymes reduce the risk of atherosclerosis and cancer (Ames et al., 1993). The ability of banana (9%) to bind to bile acids in vitro is higher than that for other fruit such as pineapple (5.9%), grape (5%), peach (6%) and pear (4.7%). Preventing the recirculation of bile acids reduces fat absorption, increases the excretion of cancer-causing toxic metabolites and increases cholesterol conversion to more bile acids (Kahlon
and Smith, 2007). Banana can cause a natural anti-acid effect in the human body; therefore, eating banana can provide a soothing relief in people suffering from heartburn. Plantain has long been recommended to treat digestive disorders in humans since it is believed to contain active antiulcerogenic agents (Goel et al., 1989; Dunjic et al., 1993).

### 17.2.4 Effect of preharvest and postharvest continuum

Variety, harvest maturity, state of ripeness, soil type, soil condition, fertilization, irrigation and weather are all important preharvest factors that affect the quality of banana fruit (Tahvonen, 1993). Bananas for export are transported in a preclimacteric state and thus need to be ripened in specialist ripening rooms near to the point of sale. Even though the banana dominates the fresh fruit export market, there has been little research conducted on detailing the temporal changes in health-promoting compounds after harvest and linking these with effects on human health. This is surprising considering the dominance of the banana compared with other frequently consumed fruit and may reflect the commoditization of the product.

### 17.3 Pineapple

#### 17.3.1 Introduction

The pineapple (*Ananas comosus* L.) is the most economically important member of the family Bromeliaceae and the only bromeliad bearing edible fruit. The family Bromeliaceae includes about 45 genera and over 2000 cultivars of pineapple, which have been cultivated around the world. Smooth Cayenne, Natal Queen, Red Spanish and Kona Sugarloaf are some of the prominent commercial cultivars. Among these cultivars, cv. Smooth Cayenne is popular around the world for its excellent processing characteristics, such as regularity of shape and size, high sugar and acid content, ability to withstand rough handling and an acceptable shelf life (Kelly and Bagshaw, 1993; Paull, 1993). Pineapple is ranked third among world tropical crops, preceded only by banana and citrus (Uriza-Avila, 2005). Although pineapple is thought to be native to the South Americas (Brazil, Hawaii, Paraguay, etc.), it was first discovered by Europeans, in 1493, in the Caribbean. The pineapple is now grown extensively in Hawaii, the Philippines, Ivory Coast, the Caribbean, Malaysia, Costa Rica, Taiwan, Thailand, Australia, Mexico, Kenya and South Africa. Pineapple has long been considered as one of the most popular non-citrus tropical and subtropical crops owing to its attractive flavour and refreshing sugar-acid balance (Bartolomé et al., 1996; Morse, 2008). Global production of pineapples was about 18.87 Mt in 2007. Thailand is the largest producer, accounting for 16% of global output, followed by the Philippines (12%) and Brazil (10%) (Rebolledo-Martinez et al., 2005; Fold and Gough, 2008).

#### 17.3.2 Identity and role of bioactives

**Bromelain**

Pineapple has been used extensively as a folk remedy for several health ailments, including digestive problems. Bromelain is a complex mixture of substances (collectively named as proteolytic enzymes or proteases) found mainly in the stem and core of the pineapple fruit. Bromelain is considered to be one of the best vegetable proteases, with numerous applications in the food industries, medicine and pharmacology (Devakate et al., 2009). Bromelain obtained from the stem is widely used in industries; fruit bromelain (from the core) is not available commercially but is believed to have possible digestion-related and anti-inflammatory benefits (Devakate et al., 2009).

**Vitamins and minerals**

The average vitamin C concentration in fresh pineapple flesh (0.15 mg/g FW) is higher than that in its juice (0.11 mg/g FW) (USDA, 1992; Morse, 2008). The manganese concentration (15–20 mg/l) of commercial pineapple juice is much higher than the concentrations
of chromium, iron and copper. However, the nutritional bioavailability of the manganese is uncertain (Beattie and Quoc, 2000). In addition to manganese, pineapple is also a good source of potassium, calcium, iron and magnesium (Nakasone and Paull, 1998). A combination of glucosamine, chondroitin sulfate and manganese may offer significant improvement of symptoms for those with mild to moderate osteoarthritis of the knee (Orlando, 2006).

**Carotenoids and phenolic compounds**

Total carotenoid concentration is proportional to the degree of yellow colour in pineapple flesh. Carotenoid content is higher in the flesh than in the juice of cvs. Del Monte Hawaii Gold (1.36 µg/g in flesh and 0.25 µg/g in juice) and Smooth Cayenne (0.45 µg/g in flesh and 0.07 µg/g in juice). β-Carotene is a primary provitamin A found in pineapple and contributes to about 35% of total carotenoids, its content also being considerably lower in juice than flesh, irrespective of cultivar (Paull, 1993; Ramsaroop and Saulo, 2007).

Phenolic compounds are associated with several health benefits. Total phenolics are one of the important antioxidants in pineapple and their concentration varies between 0.32 mg GAE/g FW and 0.52 mg GAE/g FW. Flavonoid content of pineapple ranges between 0.01 mg catechin equivalents (CEQ)/g FW and 0.04 mg CEQ/g FW (Alothman et al., 2009).

**17.3.3 Chemopreventive activity and bioavailability**

Therapeutic doses of bromelain are believed to reduce excessive inflammation, coagulation of the blood and certain types of tumour growth. Protein molecules from bromelain, such as CCZ and CCS, have been identified as powerful anticancer agents and could lead to a new class of cancer-fighting drugs. Antioxidant capacity of pineapple varied between 1.72 limo' Fe/g and 5.3 limo' Fe/g, while DPPH inhibition ranged between 12.7 and 90.8% (Alothman et al., 2009). Since pineapple is rich in fibre, fresh pineapple prevents constipation and also relieves constipation in those who already have it (Morse, 2008). Bromelain is also effective in treating sore throat pain, upper respiratory conditions and acute sinusitis. Fresh pineapple juice also speeds up the natural healing of warts (Orlando, 2006).

**17.3.4 Effect of preharvest and postharvest continuum**

Adequate soil nutrition (minerals) is crucial for good quality and productivity of fruits. Addition of potassium (K) to the soil can play a significant role in producing good quality pineapple, increasing total solids and producing fruit with a larger diameter. However, high concentrations of K in the soil may lead to very acidic fruit with pale and rigid pulps (Dull, 1971). Adequate K in the soil may improve pineapple flavour, increase stalk diameter and increase ascorbic acid concentrations. The ascorbic acid may prevent some degree of enzymatic browning, by inhibition of polyphenoloxidase activity (Soares et al., 2005). Acidic soil condition, although often associated with higher manganese levels, may lead to iron deficiency (Beattie and Quoc, 2000).

Natural flowering has been a long-lasting agronomic problem in pineapple cultivation, since it causes considerable postharvest losses and irregular market supply. Therefore, flowering is usually induced using various external induction agents (ethephon, ethylene gas, etc.) (Bartholomew et al., 2003; Wang et al., 2007). Pineapple fruit is commercially important and a major export crop for some countries. However, occurrence of internal browning (black heart or endogenous brown spots) presents challenges in maintaining adequate fruit quality standards and export potential. The quality of the fruit essentially depends on planting, harvesting and pre- and postharvest factors (Selvarajah and Herath, 1997; Selvarajah et al., 2001).

As the external colour of pineapple is not an exact predictor of ripeness, selecting the optimum harvest maturity or ripeness is a challenging task. Ripeness has traditionally been judged by sniffing at the stem end of the fruit or by looking for fresh deep green leaves.
Since carotenoids are very sensitive to heat and light, heat treatment during preparation of pineapple juice reduces the carotenoid concentration (Hodgson and Hodgson, 1993). Since bromelain is deactivated by high temperatures, the bromelain content may be lower in pineapple juice and canned pineapple than in fresh fruit.

17.4 Papaya

17.4.1 Introduction

Papaya (Carica papaya L.) is the major economically important species out of the 21 species within the genus Carica of the small dicotyledonous family Caricaceae. Most of the papaya cultivars are grown in tropical and subtropical countries, while cvs. Solo and Sunrise have widespread distribution beyond the tropics. Papaya is called by different names around the globe, namely ‘tepayas’ in East Malaysia, ‘betik’ in Peninsular Malaysia, ‘lechosa’ in Venezuela, ‘pawpaw’ in Sri Lanka and ‘papali’ in India (Fasihuddin and Ghazally, 2003). Tropical Central America (Mexico and Costa Rica) is believed to be the origin of papaya; however, it is now cultivated throughout the tropics and subtropics. The main papaya producing countries are Brazil, Nigeria, India, Mexico and Indonesia (Banerjee-Bhattacharya, 2002). Global production of papaya has increased continuously over the past decade; production reached around 6.94 Mt in 2007. Brazil is the major producing country and contributes about 24.6% of total production. The European Union countries are the most prominent papaya importers, with imports increasing by 50% between 2001 and 2003. Since papaya contains specialized cells called laticifers, it is referred to as a laticiferous plant (Azarkan et al., 2003). The papaya fruit is a melon-like, oval to nearly round, 15–20 cm long fleshy berry. A rich orange-coloured flesh with either yellow or pink hues is deliciously sweet, with musky undertones and a soft, butter-like consistency. The papaya is available year-round and the ripe fruit is a favourite breakfast and dessert. It can also be used to make fruit salad, refreshing drinks, jam, jelly, marmalade, candies and crystallized fruit, etc. (Banerjee-Bhattacharya, 2002).

Papaya fruit is highly accepted worldwide and is sought after for its health-promoting properties, flavour and digestive properties (Fernandes et al., 2006). Papaya fruit not only offers luscious taste and the sunlit colour of the tropics, but also is a good source of energy and antioxidant nutrients such as carotenoids, vitamins, minerals, flavonoids, anthocyanins, etc. Some of these nutrients improve the cardiovascular system and provide protection against colon cancer (Banerjee-Bhattacharya, 2002). The fruit and other parts of the papaya plant contain papain, an enzyme that is reported to help in the digestion of protein (Banerjee-Bhattacharya, 2002). However, this enzyme is especially concentrated in unripe fruit. Papain is extracted commercially to make digestive enzyme dietary supplements and is also used as an ingredient in some chewing gums.

17.4.2 Identity and role of bioactives

Carotenoids and phenolics

β-Carotene (2.32–5.98 µg/g FW) and β-caryophyllene (5.94 µg/g FW) are the prominent carotenoids in papaya; however, β-carotene is the more active provitamin A pigment. Red-fleshed papaya cultivars (Sunrise and SunUp) contain significant concentrations of lycopene (13.50–42.81 µg/g FW); however, lycopene has not been detected in yellow-fleshed papaya cvs. Formosa, Kapoho, Laie Gold, Maradol and Rainbow (Chandrika et al., 2003; Wall, 2006; de Oliveira et al., 2010; Rivera-Pastrana et al., 2010). Lycopene has the highest free radical scavenging ability, followed by β-caryophyllene and β-carotene (Miller et al., 1996). The concentration of β-carotene is also higher in red-fleshed papaya fruit (7.0 µg/g DW) than in yellow-fleshed fruit (1.4 µg/g DW); however, β-cryptoxanthin is in a more or less similar quantity (16.0 µg/g DW) in both red- and yellow-fleshed fruit (de Oliveira et al., 2010).

Papaya has a comparatively high primary antioxidant potential, similar to those of
guava and starfruit (see Chapter 8 of this volume). Total phenolic concentrations (0.54 mg GAE/g) of papaya are similar in the peel and flesh and across maturity stages (Kondo et al., 2005) and are lower than those of most other tropical crops, such as mango, guava and pineapple (Pathhamakanokporn et al., 2008). However, papaya cv. Soursop contains relatively high concentrations of flavonoids (10–30 µg/g). Rivera-Pastrana et al. (2010) reported that ferulic acid, caffeic acid and rutin were the most abundant phenolic compounds identified in mesocarp tissue.

Vitamins and minerals

Vitamin A concentration of papaya fruit ranges between 0.19 and 0.74 µg RAE/g FW. Papaya is a very rich source of vitamin C, yet the content may vary between 0.46 and 1.45 mg/g FW depending on the cultivar, ripening stage and handling after harvest (Firmin, 1997; Proulx, 2002; de Oliveira et al., 2010). According to Lim et al. (2007), papaya has a higher content of ascorbic acid (1.08 mg/g FW), than most other tropical fruit apart from guava. Ascorbic acid increases with maturity in both the peel and pulp (Kondo et al., 2005). In addition to vitamins A, C and E, folate is also found in considerable quantities in papaya.

Papaya fruit can also contribute a significant amount of minerals to the human diet, but the mineral composition of the fruit reflects the trace mineral content of soil and also varies with climate, maturity, cultivars, agricultural practices, etc. (Forster et al., 2002). Phosphorus (0.05–0.09 mg/g FW), potassium (0.90–2.21 mg/g FW), calcium (0.1–0.32 mg/g FW), magnesium (0.19–0.33 mg/g FW) and sodium (0.05–0.24 mg/g FW) are the important minerals identified in significant quantities from the prominent papaya cvs. Kapoho, Laie Gold, Rainbow, Sunrise and SunUp (Wall, 2006).

Papain

Unripe papaya fruit contains an enzyme called papain, which is a cysteine protease with an action similar to the pepsin in gastric juice. Papain is derived, for commercial use, from the latex of the unripe papaya fruit immediately after harvest. Chymopapain, caricain and papaya proteinase VI are the other cysteine proteases isolated from papaya latex (Morton, 1987a; Ohara et al., 1995). Papain has been used in several industrial applications, such as in beer as a clarifier, in meat as a tenderizer, in the preparation of protein hydrolysates and in the pharmaceutical industry (in the treatment of osteoporosis, arthritis, vascular diseases and cancer) (Sie Winnsky et al., 1996; Brömme et al., 2004). Unripe papaya peel has high levels of papain and chymopapain (Emeruwa, 1982; Osato et al., 1993) and has been used as an effective external treatment for skin wounds.

17.4.3 Chemopreventive activity and bioavailability

Preparation method, processing and cooking affect the total antioxidant capacity (TAC) of fruit and vegetables, as does genotype. According to Prior and Cao (2000), a study has revealed that cooking generally reduces the antioxidant capacity in fruit and vegetables by 15%. Antioxidant activities of papaya (measured as oxygen radical absorbance capacity or FRAP) are lower than for other tropical crops such as mango, guava and pineapple (Pathhamakanokporn et al., 2008). However, Lako et al. (2007) reported that Hawaiian papaya cv. Soursop contained higher TAC than other fruit grown in Fiji, and moderate levels of total polyphenol (TPP).

The bioavailability of carotenoids in red-fleshed papaya is under investigation. It has been noted that excess intake of red-fleshed papaya can cause a yellow-orange discoloration of the skin of the palm (lycopenaemia) among the peoples of Sri Lanka. No other yellow or red fruit in Sri Lanka causes this symptom (Chandrika et al., 2003). Lycopene has no provitamin activity as it misses a β-ionone ring; however, conjugated and non-conjugated double bonds make lycopene highly reactive towards oxygen and free radicals. Perspective and retrospective epidemiological studies have indicated that oral intake of lycopene is bioavailable and reduces prostate...
cancer risk. In addition, in vitro experiments also indicate that lycopene can induce apoptosis in cancer cells and inhibit their proliferation by producing cell cycle arrest (van Breemen and Pajkovic, 2008). Since lycopene is bound tightly to macromolecules within the food matrix, its bioavailability is relatively poor. Cooking or processing lycopene-rich food like tomato and papaya, however, releases lycopene from protein complexes and enhances its bioavailability. Consumption with lipids can also increase the bioavailability of lycopene, as it is highly lipophilic (Erdman et al., 1993; van Breemen and Pajkovic, 2008).

17.4.4 Other beneficial and negative effects

Different parts of the papaya fruit, such as the peel, flesh, seeds and latex, traditionally have been used to treat various ailments in humans. Papaya seed is a rich source of biologically active isothiocyanate (Nakamura et al., 2007); the seeds have also been used as an emmenagogue, thirst quencher, or carminative to alleviate pain from insect bites and stings (Wiart, 2006). Papaya latex is a rich source of four cysteine endopeptidases (papain, chymopapain, glycyl endopeptidase and caricain), the content of these enzymes varying in different parts of the plant. Since latex concentration decreases during ripening, it is commercially extracted from unripe papaya fruit (Azarkan et al., 2003). Latex is very efficient against gastrointestinal nematodes (Stepek et al., 2007) and is also used to treat eczema and psoriasis in Cambodia, Laos and Vietnam (Amenta et al., 2000). Papaya fruit also contains carpanie, which is an alkaloid with a strong depressant action on the heart (Hornick et al., 1978). The content of arginine, which is known to be essential for male fertility, and is very effective in treating nematodes such as roundworms (Ascaridilla galli) in humans, may explain why the latex of young fruit has an anthelmintic activity. Intestinal nematode infections may cause intestinal disorders, discomfort and loss of productivity through direct or indirect interference with host nutrition and metabolism in humans and animals (Satrija et al., 1995).

17.4.5 Effect of preharvest and postharvest continuum

The nutritional composition of a papaya fruit varies widely based on cultivar, maturity, climate, soil type and fertility. Carotenoids and ascorbic acid increase with maturation and ripening. Ascorbic acid concentration is also influenced by the availability of light to the crop and fruit (Wenkam, 1990; Lee and Kader, 2000). The reported concentrations of β-carotene in papaya vary with genotype,
quantification methods, etc. For example, cv. Khakdahm ripe fruit were reported to contain 4.71 µg/g of β-carotene along with 21.69 µg/g of lycopene (Charoensiri et al., 2009); however, β-carotene concentrations of 2.76 µg/g (Holden et al., 1999), 2.28 µg/g (Tee and Lim, 1991) and 4.40 µg/g (Setiawan et al., 2001) have also been estimated for the same cultivar using different methods. The ascorbic acid content of papaya is higher in first-harvested fruit (7.50 mg/g DW) than in second-harvested fruit (4.00 mg/g DW) (Lee and Kader, 2000; Proulx et al., 2005).

The quality of papaya fruit may be reduced by the adverse environmental and physical conditions encountered during transportation, distribution and retailing. Poor appearance, flavour and nutritional value may result from extreme or fluctuating temperatures and/or mechanical damage, combined with improper harvesting and handling practices. Papaya may have a shelf life of 4–6 days under ambient tropical conditions (25–28°C) or up to 3 weeks at lower temperatures (10–12°C) if it is handled properly after harvest (Paul et al., 1997). Chilling injury symptoms occur in mature green papaya after 14 days, and in 60% of yellow fruit after 21 days, at 7°C. Rivera-Pastrana et al. (2010) demonstrated that carotenoids (except β-carotene) in cv. Maradol papaya fruit were affected detrimentally by low temperature storage at 1°C and suggested that storage at 25°C had a lesser negative effect on these metabolites.

17.5 Mango

17.5.1 Introduction

Mango (Mangifera indica L.) is known as the king of fruit and belongs to genus Mangifera, which consists of numerous species of tropical fruit in the family Anacardiaceae. Mango is considered indigenous to Eastern Asia, Myanmar and Assam state in India; however, it is widely cultivated as a fruit tree in frost-free tropical and warmer subtropical regions (Morton, 1987b). Despite most ripe mango fruit being consumed as a dessert, mature mangoes are also eaten as pickles, sliced or grated in fresh salads, soaked in water or sugar, salted and dried, sliced in vinegar or fish sauce, etc. Mango has special significance in Pakistan, India, Bangladesh, Sri Lanka and the Philippines, as its leaves are used spiritually for floral decorations at Hindu marriages and religious ceremonies (Kim et al., 2009). Total global mango production was forecast to reach 30.7 Mt/year by 2010 (FAO, 2003). Though mango fruit is well known for its characteristic aroma and taste, it is also an excellent source of carbohydrates and health-promoting dietary antioxidants (Kauer and Kapoor, 2001) such as ascorbic acid (Franke et al., 2004; de Oliveira et al., 2010), carotenoids (Godoy and Rodriguez-Amaya, 1989; de Oliveira et al., 2010), phenolic compounds (Berardini et al., 2004, 2005), vitamin E (α-tocopherol) (Xianli et al., 2004) and minerals (Ribeiro et al., 2007). Many of these compounds possess not only antioxidant (Berardini et al., 2004; Talcott et al., 2005; Mahattanatawee et al., 2006) but also immunomodulatory (Naved et al., 2005), antimutagenic (Botting et al., 1999) and/or anticancer activity (Percival et al., 2006).

17.5.2 Identity and role of bioactives

Phenolic compounds

Studies have demonstrated that polyphenolic compounds of mango fruit include various flavonoids, xanthones, phenolic acids and gallotannins (Schieber et al., 2003; Berardini et al., 2005). Among these compounds, gallic acid and hydrolysable tannins are the major antioxidant polyphenolics in mango. Mango peel has generally high TP content but the reported level varies widely with cultivar and assay method. Sri Lankan mango cv. Willard was found to have higher TP than cvs. Karutha Colomban and Malgova (Table 17.1) (Thanaraj, 2010).

About 12 flavonoids and xanthones were identified in Brazilian mango cvs. Haden (0.48 mg/g DW), Tommy Atkins, Palmer and Uba; however, cv. Uba (2.09 mg/g DW) contained a significantly higher concentration of these compounds than the other cultivars (Ribeiro et al., 2008). Phenolic compounds
Table 17.1. Total phenolic content in the peel of ripe mango fruit.

<table>
<thead>
<tr>
<th>Mango cultivars</th>
<th>Total phenolics (mg GAE/g DW)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haden</td>
<td>52.3</td>
<td>Larrauri et al. (1996)</td>
</tr>
<tr>
<td>Rasputi</td>
<td>46.3</td>
<td>Ajila et al. (2007)</td>
</tr>
<tr>
<td>Badami</td>
<td>33.3</td>
<td>Ajila et al. (2007)</td>
</tr>
<tr>
<td>Uba</td>
<td>57.2</td>
<td>Ribeiro et al. (2008)</td>
</tr>
<tr>
<td>Willard</td>
<td>84.6</td>
<td>Thanaraj (2010)</td>
</tr>
<tr>
<td>Karutha Colomban</td>
<td>74.3</td>
<td>Thanaraj (2010)</td>
</tr>
<tr>
<td>Malgova</td>
<td>31.9</td>
<td>Thanaraj (2010)</td>
</tr>
<tr>
<td>Anwar Rota le</td>
<td>48.9</td>
<td>Rajwana et al. (2010)</td>
</tr>
<tr>
<td>Faiz Kareem</td>
<td>29.8</td>
<td>Rajwana et al. (2010)</td>
</tr>
<tr>
<td>Chaunsa</td>
<td>51.2</td>
<td>Rajwana et al. (2010)</td>
</tr>
</tbody>
</table>

such as flavonol and xanthone glycosides, gallotannins and benzophenone derivatives (mangiferin, quercetin 3-O-galactoside, quercetin 3-O-glucoside, kaempferol-3-O-glucoside, quercetin-3-O-rhamnoside, quercetin 3-O-diglycoside, quercetin, quercetin 3-O-arabinofuranoside, quercetin-3-O-xyloside, isomangiferin and rhamnetin) are present mainly in mango peels and seeds. Mangiferin (xanthone-C-glycoside) is the prominent flavonoid in Sri Lankan mango cvs. Willard (5620 µg/g DW), Karutha Colomban (3920 µg/g DW) and Malgova (2350 µg/g DW), followed by quercetin-3-O-galactoside and quercetin-3-O-glucoside (Thanaraj, 2010). Mangiferin is also the main flavonoid in mango cvs. Uba (199 µg/g DW) and Tommy Atkins (1690 µg/g DW). Phenolic compounds generally help protect humans against some chronic degenerative diseases related to oxidative stress (Manach et al., 2005). Flavonols have strong antioxidant (Pannala et al., 2001), anticarcinogenic (Peng et al., 2006), antiatherogenic (Kim et al., 2006) and antitumour and antiviral (Guha et al., 1996) activities. The antioxidant activity of phenolic acids generally depends on their chemical structure, the more hydroxyl groups present in the phenol the higher the antioxidant capacity (Heo et al., 2007).

Vitamins

Both the peel and pulp of mango fruit are an excellent source of AsA. However, the concentration varies extensively with the cultivar, tissue, stage of maturity, postharvest ripening and storage, climatic conditions, cultural practices and pre- and postharvest factors (Lee and Kader, 2000). Vinci et al. (1995) reported that the AsA concentration of mango pulp ranges from 0.1 to 1.0 mg/g FW, although, again, reported values vary with cultivar and quantification method (Table 17.2). Sri Lankan mango cv. Willard (5.8 mg/g DW) contains exceptionally high levels of AsA, while cvs. Karutha Colomban (1.1 mg/g DW) and Malgova (0.6 mg/g DW) have moderate content (Thanaraj, 2010). Mango fruit (4.42 µg/g FW in cv. Ataulfo) is also a good source of vitamins A and E (α-tocopherol) (Corral-Aguayo et al., 2008).

Carotenoids

Mango is a rich source of carotenoids, which are responsible for the yellow-to-orange colour in ripe fruit, and contributes substantially to β-carotene supply in tropical countries. All-trans-β-carotene (> 50%) and violaxanthins and their isomers (xanthophylls) are the most common carotenoids in mango cultivars (Chen et al., 2004). The β-carotene provides higher provitamin A value and antioxidant capacity (Godoy et al., 1994; Pott et al., 2003). Ripe mango fruit generally contains a higher level of provitamin A than other tropical fruit; however, the level depends on cultivar, pulp colour and stage of ripening (West and Poortvliet, 1993;
Table 17.2. Concentration of ascorbic acid (AsA) in different mango cultivars.

<table>
<thead>
<tr>
<th>Mango cultivars</th>
<th>Plant tissue</th>
<th>AsA (mg/g FW)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tommy Atkins</td>
<td>Pulp</td>
<td>0.23</td>
<td>Mansour et al. (2006)</td>
</tr>
<tr>
<td>Keitt</td>
<td>Pulp</td>
<td>0.33</td>
<td>Mansour et al. (2006)</td>
</tr>
<tr>
<td>Kent</td>
<td>Pulp</td>
<td>0.32</td>
<td>Mansour et al. (2006)</td>
</tr>
<tr>
<td>Raspuri</td>
<td>Peel</td>
<td>0.35</td>
<td>Ajila et al. (2007)</td>
</tr>
<tr>
<td>Badami</td>
<td>Peel</td>
<td>0.39</td>
<td>Ajila et al. (2007)</td>
</tr>
<tr>
<td>Haden</td>
<td>Pulp</td>
<td>0.18</td>
<td>Ribeiro et al. (2007)</td>
</tr>
<tr>
<td>Tommy Atkins</td>
<td>Pulp</td>
<td>0.1</td>
<td>Ribeiro et al. (2007)</td>
</tr>
<tr>
<td>Palmer</td>
<td>Pulp</td>
<td>0.1</td>
<td>Ribeiro et al. (2007)</td>
</tr>
<tr>
<td>Uba</td>
<td>Pulp</td>
<td>0.72</td>
<td>Ribeiro et al. (2007)</td>
</tr>
<tr>
<td>Langra</td>
<td>Peel</td>
<td>5.2</td>
<td>Thomas and Oke (2007)</td>
</tr>
<tr>
<td>Langra</td>
<td>Pulp</td>
<td>0.98</td>
<td>Thomas and Oke (2007)</td>
</tr>
<tr>
<td>Ataulfo</td>
<td>Pulp</td>
<td>1.0</td>
<td>Corral-Aguayo et al. (2008)</td>
</tr>
<tr>
<td>Tainong</td>
<td>Pulp</td>
<td>0.45</td>
<td>Wang et al. (2009)</td>
</tr>
</tbody>
</table>

Englberger et al., 2003). Miller et al. (1996) reported that, among the various carotenoids present in mango fruit, lycopene has the higher antioxidant activity, followed by β-caryophyllene, β-carotene, lutein and zeaxanthin. Total carotenoid content usually varies from 9–92 μg/g FW in most of the mango cultivars; however, Indian mango cv. Alphonso (110 μg/g FW) (Padmini and Prabha, 1997) and Sri Lankan mango cvs. Willard (63 μg/g FW) and Karutha Colomban (76 μg/g FW) (Thanaraj, 2010) contain exceptionally high concentrations.

17.5.3 Chemopreventive activity and bioavailability

Percival et al. (2006) reported that whole mango fruit juice has the ability to inhibit cell proliferation in the leukaemic cell line HL-60 and also inhibits the neoplastic transformation of BALB/3T3 cells. The presence of antioxidant and antimutagenic (Botting et al., 1999) activities in mango and its antineoplastic effects detected using mammalian in vitro systems support the anticancer activity seen in vivo (García-Solís et al., 2008). Studies in humans show that accumulation of carotenoids by breast adipose tissue reduces the risk of breast cancer (Yeum et al., 1998). Carotenoids are also correlated with activities against different types of cancer and heart diseases and are a precursor of provitamin A (Yahia et al., 2006). However, the bioavailability of β-carotene depends on the ripeness of the fruit, although it can be improved by consuming the fruit with other, fat-containing food products (Ornelas-Paz et al., 2007). The mango triterpene (lupeol) is an effective inhibitor in laboratory models of prostate and skin cancers. Mango peel also contains the oil, urushiol, which can trigger a skin rash called urushiol-induced contact dermatitis (Nigam et al., 2007; Chaturvedi et al., 2008). Mango extract (Vimang) could be a useful new (natural) drug for preventing oxidative damage during hepatic injury associated with free radical generation (Sánchez et al., 2003). It is also believed that mango fruit may offer better protection against radical scavenger activity than many common food additives (Saleem et al., 2004).

17.5.4 Other beneficial effects

In India, mango fruit sap has been used to treat the pain of bee and scorpion stings. Many of the traditional Indian medicinal uses of mango involve eating unripe fruit. It should be noted that unripe fruit contains much toxic sap, which can cause throat irritation, indigestion, dysentery and colic. Unripe mango juice is also used as a remedy for exhaustion and heat stroke. Half-ripe fruit is usually eaten with salt and honey and is also used for the treatment of gastrointestinal,
biliious and blood disorders and scurvy in many tropical countries (Nigam et al., 2007; Prasad et al., 2008).

17.5.5 Effect of preharvest and postharvest continuum

The concentration of antioxidants varies greatly with genotype and pre- and postharvest factors such as climatic conditions, agricultural practices, harvest maturity, ripening stage, storage and processing (Lee and Kader, 2000). The total phenolic concentration of mango fruit decreases consistently during ripening and has positive correlation with antioxidant capacity (Kim et al., 2007). However, during low temperature storage, total phenolics decrease but this trend has no significant correlation with antioxidant capacity (Shivashankara et al., 2004). Therefore, low temperature (20°C) ripening of mango fruit is preferred over higher temperature (30°C) ripening as far as health-promoting properties are concerned (Thanaraj, 2010). The shelf life and external quality of mango fruit are improved greatly by low O₂ and/or high CO₂ storage conditions and hot water treatments. However, the colour, aroma and nutritional composition of mango fruit are reported to be reduced by controlled atmospheres (CAs). Despite this, hot water treatment plus CA storage is considered an effective treatment combination to extend the postharvest life of mangoes without greatly affecting the nutritional profile and overall quality adversely (Kim et al., 2007). Gallic acid and other hydrolysable tannins and their resultant antioxidant capacity were not affected by hot water treatment (Kim et al., 2007). It has been suggested, however, that major polyphenolic compounds in mango fruit may decrease during prolonged hot water treatment (Kim et al., 2009).

Hancock and Viola (2005) and Wang et al. (2009) reported that AsA content of mango decreases by 50% during ripening. However, Thanaraj (2010) found that there was no significant variation in AsA content during ripening in Sri Lankan mango cvs. Willard and Malgova, but that it decreased by 50% in cv. Karutha Colomban. He has also observed that high (30°C) temperature ripening enhanced the loss of AsA during ripening compared with a lower ripening temperature (20°C). Thomas and Oke (2007) made similar observations using mango cv. Alphonso. AsA losses are minimal during the storage of fresh-cut mangoes.

17.6 General Conclusion

Tropical fruit are eaten for their unique taste and aroma attributes; however, they are also a good source of dietary antioxidants and contribute significantly to daily dietary requirements, especially in developing countries. Given their importance, it is perhaps surprising that there is a paucity of information detailing the variation in health-promoting compounds between varieties and as affected by postharvest handling and storage. This may reflect the commoditization of many of these products and the concomitant narrowing of commercially available genotypes. Tropical fruit arguably have the greatest potential to improve the health of more people across the world than any other fresh produce category, given that they are dominant in many developing countries. A refocusing of research activity is thus required.

References


18 Methodologies for Extraction, Isolation, Characterization and Quantification of Bioactive Compounds

Katherine Cools, Ariel Vicente and Leon A. Terry

18.1 Introduction

Since it is widely recognized that the beneficial influence of fruit on human health is linked with the presence of specific phytochemicals, determining the nature of these compounds in different commodities, and the influence of preharvest, postharvest and processing treatments, have been major areas of research (Espín et al., 2007; Frankel, 2007). Antioxidants can be measured as individual compounds or as a total capacity, yet some methods used to quantify total antioxidant activity are thought to take into account compounds not classed as antioxidants, for instance, reducing sugars (Huang et al., 2005). This chapter summarizes the wide variety of extraction and characterization techniques used to quantify individual or total antioxidants, as well as other bioactive compounds.

18.2 Sample Preparation

Fruit and vegetables contain a complex profile of bioactive compounds, of which most are labile to heat, air and/or light (Brat, 2008). To extract bioactive compounds effectively from fresh tissue, samples are usually cut into small pieces and then snap-frozen immediately in liquid nitrogen, since chopped material is much more unstable. Failure to prepare samples in this way can result in enzymatic browning, as well as undesirable molecular, biochemical and physiological changes. Samples are often freeze-dried before extraction takes place, to remove water. Lyophilization aids sample grinding, which benefits extraction by increasing solvent penetration due to greater sample surface area. Puupponen-Pimiä et al. (2003) investigated the effects of freezer storage on fresh vegetables. After being frozen quickly to −40°C (method not stated), frozen vegetables were then stored at −20°C for 6, 12 and 18 months. The authors found that different bioactives were affected by long-term freezer storage in different vegetable groups. A decrease in α- and β-carotene was observed in processed carrots, yet not in peas, between 6 and 12 months at −20°C. Similarly, over twofold reductions in quercetin and kaempferol content were observed in cauliflower and broccoli over the same period. It is therefore important, especially when comparing different fruit and vegetable groups, that analyses are undertaken as soon as possible after sample preparation and, ideally, that samples are kept at or below −40°C. Differences among the literature may indeed be due to sample preparation and subsequent storage.
18.3 Bioactive Extraction

A factor that certainly differs among the literature is extraction procedure. An example of this is the extraction of fructans, which are thought to have prebiotic effects whereby they promote the beneficial bacteria of the gut; their health-benefiting properties are further described in Chapter 2 of this volume. Studies have shown that methanol is a superior extraction solvent to ethanol when extracting fructans from fresh produce (Davis et al., 2007; Downes and Terry, 2010). Downes and Terry (2010) found that extraction of fructans from onion using 62.5% (v/v) methanol (O'Donoghue et al., 2004) yielded approximately 40 mg/g DW more fructans than when extracted using 80% (v/v) ethanol (Vagen and Slimestad, 2008) (Fig. 18.1). Similarly, anthocyanins are another group of health-benefiting compounds that have been extracted using various solvent mixtures. Initial work by Giné Bordonaba and Terry (2008) identified acidified aqueous methanol as a superior extraction solvent to acidified aqueous ethanol when extracting anthocyanins from blackcurrant berries. However, acetone has also been used for the extraction of anthocyanins (Awika et al., 2004; Anttonnen and Karjalainen, 2006); therefore, recent work (Gine Bordonaba and Terry, in press) has compared the methanol-based method with extraction using acidified 70% (v/v) acetone. Results from this work revealed that acidified aqueous methanol extracted higher concentrations of anthocyanins, specifically pelargonadin derivatives, from strawberry.

18.4 Bioactive Isolation, Separation and Quantification

Various methods are available to measure ascorbic acid (AsA), carotenoids and phenolic compounds (Tsao and Deng, 2004) individually.

Fig. 18.1. Mean sugar and fructooligosaccharide concentrations (mg/g DW) in onion samples extracted with either methanol (MeOH) or ethanol (EtOH). Total sugars = sum of fructose, glucose and sucrose; total FOS = sum of DP3 – DP8 (Downes and Terry, 2010).
Bioactive Compounds

in foods. High performance liquid chromatographic (HPLC) techniques are now most widely used for both separation and quantification. For phenolic acids and flavonoid analysis, the chromatographic conditions of the HPLC methods may include the use of reverse phase C18 columns, a UV-visible diode array detector (DAD) and a binary solvent system containing acidified water and a polar organic solvent such as methanol or acetonitrile (for review, see Robards, 2003; Naczk and Shahidi, 2006). Separation of saponified carotenoids can be carried out on silica columns using gradient elution from 95% of light petroleum to 95% acetone (Almela et al., 1990). For AsA, samples must be prepared carefully to prevent oxidation. This usually involves extraction in citric or metaphosphoric acid at low temperature and in the dark, followed by rapid filtration and/or centrifugation. After that, the supernatant is injected into the HPLC. Most HPLC methods involve an isocratic method using a reversed phase column. AsA and dehydroascorbic acid (DHA) can be monitored with a UV detector. AsA can also be measured using test kits in which increase in absorbance at 578 nm, following the reduction of MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to a formazan compound by AsA, is measured (Megazyme, 2010).

Mass spectrometers are extremely useful for studying phytochemicals, either as highly selective detectors or as powerful tools for metabolite identification and profiling. When quantifying a bioactive compound using HPLC, peak identification and concentration are calculated according to a pure standard of known concentration. For compounds with high degrees of polymerization, for instance procyanidins and fructans (Fig. 18.2), standards are either scarce or expensive. Mass spectrometry can be used to identify unknown peaks according to the ion mass-to-charge ratio; a compound represented by such a peak can then be purified for future use as a standard.

Thin layer chromatography (TLC) can also be used to isolate bioactive compounds; extracts are spotted on to thin layer chromatography plates coated with silica gel, which can then be developed using an appropriate running solvent. This method is particularly useful for determining the antifungal activity of bioactive compounds. Once the compounds have been separated, the plates are dried and spores/conidia can then be sprayed on the surface. Compounds with antifungal activity can be identified as the area with reduced mycelial growth (Adikaram and Ratnayake Bandara, 1998; Terry et al., 2003, 2004). The identity of these antifungal compounds can be determined by developing spots of known standards.

18.5 Measurement of Total Antioxidants in Fruit

Single metabolite analysis might be the best choice for certain objectives but, given the variety of compounds present in fruit and the limited number of assays available in a given laboratory, such an approach might, in some cases, be tedious and impractical. In addition, the antioxidant properties of a pure compound might be different to those observed in most real samples, in which many other antioxidants are also present and/or in different matrices. Ideally, it might be desirable to have simple and accurate methods for the rapid quantification of food antioxidant capacity that could prevent diseases (Huang et al., 2005). Unfortunately, these methods are not yet available. Total antioxidant capacity (TAC; defined as the ability of a given sample to prevent the action of pro-oxidants) has been assumed to be related to the chemoprotective effect of foods. However, it has to be taken into account that studies comparing in vitro and in vivo testing of antioxidants generally have shown divergent results (Frankel, 2007; Frankel and Finley, 2008). The association between the TAC and the health effects of fruit and vegetables needs to be studied further. Multiple protocols have been used to evaluate the TAC of foods and these have employed a wide variety of free radical generating systems and different methods of inducing oxidation and final detection (Frankel and Meyer, 2000; Guiselli et al., 2000; Antolovich et al., 2002; Sánchez Moreno, 2002). There is evidence that in vitro assays might not reflect in vivo antioxidant capacity, especially if the compounds are metabolized to other derivatives (Cerdá et al., 2004, 2005). The results from such assays should not be considered a
Fig. 18.2. Chromatograms of fructans (a) and procyanidins (b) with degrees of polymerization ranging from one to eight and one to nine, respectively.
reflection of the effect on the human body, as sometimes has been the case. In vitro antioxidant assays might be used as indicators of relative capacity to quench specific radicals and might be of some value for general comparisons of different products or treatments. There seems to be no consensus of opinion about a single method that should be used as a reference (Prior and Cao, 1999; Huang et al., 2005; Roginsky and Lissi, 2005). The detailed analysis and comparison of the different assays are beyond the scope of this chapter (reviewed by Prior and Cao, 1999; Huang et al., 2005). Here, we summarize briefly the main characteristics of the most commonly used methods: oxygen radical absorbance capacity (ORAC), Trolox equivalence antioxidant capacity (TEAC), ferrous ion reducing antioxidant power (FRAP), the 2,2'-diphenyl-1-picyrilhydrazyl (DPPH) assay, and Folin–Ciocalteu reagent reducing substances (FC).

18.5.1 ORAC assay

The oxygen radical absorbance capacity (ORAC) was developed by Cao et al. (1995) in order to measure antioxidant scavenging activity. It was designed originally to determine the antioxidant status in biological systems. Later, the ORAC protocol was widely used to test antioxidants in food samples (Guo et al., 1997; Caldwell, 2001). Measurements are based on the quenching of phycoerytrin (PE) fluorescence in a phosphate buffer (excitation and emission at 540 nm and 565 nm, respectively), as caused by peroxyl radicals generated by thermolysis of 2,2’-azobis(2-amidinopropane) dihydrochloride (AAPH). In the presence of antioxidant-containing samples, loss of PE fluorescence would be reduced. For a given sample, fluorescence is measured over time and the area under the curve of the sample is compared to a blank. The ORAC is usually expressed in Trolox equivalents (Trolox being a water-soluble antioxidant analogue to vitamin E). Modifications to this protocol were proposed later by Ou et al. (2001). The latter authors showed that fluorescein (FL) (3’,6’-dihydroxyspiro[isobenzofuran-1[3H],9’ [9H]-xanthene]-3-one) (excitation at 493 nm and emission at 515 nm) might be a better probe than PE, which gave inconsistent behaviour between different lots, might be photo-bleached and could bind polyphenols. The value of some other assays used to assess food antioxidant capacity (e.g. ABTS+• (see below) and DPPH•) has been questioned because the radicals used are not present in biological systems. In contrast, the ORAC assay is based on quenching of peroxyl radicals. It is considered by some to be a preferable method because it directly estimates the chain-breaking antioxidant activity, while most other assays measure the specific reducing power.

18.5.2 TEAC assay

The Trolox equivalent antioxidant capacity (TEAC) assay was first reported by Miller et al. (1993). The assay should not be confused with other techniques that also use Trolox as a reference antioxidant to express the final results. TEAC measurements are based on antioxidant-mediated reduction of the absorbance of a radical cation (2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate)) (ABTS+•). The radical can be produced from the commercially available ammonium salt of ABTS using potassium persulfate (Alonso et al., 2002). ABTS+• has a strong absorption in the range of 600–750 nm and it is moderately stable. Conversion of ABTS+• into a non-coloured form, as caused by the addition of antioxidants, can be determined spectrophotometrically. Results are then expressed as Trolox equivalents. One advantage of this test is its simplicity, which makes it suitable for routine determinations of antioxidants.

18.5.3 FRAP assay

The FRAP (ferric reducing antioxidant power) assay was introduced by Benzie and Strain (1996) and was based on the ability of antioxidants to reduce iron from the ferric to the ferrous state. When this occurs in the presence of 2,4,6-trpyridyl-s-triazine, the reduction is accompanied by the formation of a blue
complex, with $\text{Fe}^{2+}$ increasing absorption at 593 nm. Other reagents able to form specific coloured complexes with $\text{Fe}^{2+}$, such as 1,10-fenantrolin (ferroin), may also be used for the determination. In this case, a red complex is formed and measured at 510 nm. In both cases, stronger absorption indicates a higher reducing power of the phytochemical, and thus a higher reducing capacity.

### 18.5.4 DPPH• assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) method was first reported by Blois (1958). DPPH• is one of a few stable and commercially available free radicals. The test is based on the reaction of DPPH• with antioxidants. DPPH• shows a very intensive absorption in the visible region and its concentration can be determined by visible spectroscopy (515 nm). Sample antioxidants cause a decrease in the initial DPPH• (Brand-Williams et al., 1995). Several aliquots of the sample to analyse are added to test tubes containing a DPPH• solution and left to react until the change in absorbance reaches a plateau. Then, the $EC_{50}$ (amount of sample required to reduce the initial concentration of DPPH• by 50%) is determined. The antioxidant capacity can then be expressed as $EC_{50}^{-1}$. This parameter does not take into account the time required for the antioxidants to consume 50% of the radical $(t_{50})$, which might be quite different depending on the compound measured. Ascorbic acid reacts rapidly with DPPH•, while in samples rich in phenolic compounds the time needed for the reaction to reach the end point is longer (30–60 min). Sánchez-Moreno et al. (1998) suggested using the combination of kinetic $(t_{50})$ and static parameters $(EC_{50})$ to characterize the antiradical efficiency $(AE)$ as $EC_{50}^{-1} \times t_{50}$. The DPPH• assay is simple and has the advantage that the reagent can be purchased directly. However, variations in initial DPPH• concentration would result in different $EC_{50}$ values for the same sample, making comparisons difficult between different lots. In addition, the radical has no similarity with peroxy radicals involved in lipid peroxidation and many antioxidants reacting quickly with peroxy radicals only react slowly with DPPH•.

### 18.5.5 The Folin–Ciocalteu assay (FC)

The FC assay was used initially to analyse proteins, taking advantage of the reagent’s reaction with aromatic amino acids (Folin and Ciocalteu, 1927). Singleton and Rossi (1965) proposed extending the use of this procedure in order to measure total phenolic compounds (Singleton et al., 1999). The reagent, containing phosphomolybdic and phosphotungstic heteropoly acids, is added to aliquots of the antioxidant-containing samples, and subsequently the pH is raised by addition of Na$_2$CO$_3$. To prevent potential precipitation of carbonates, a mixture of NaOH and more diluted Na$_2$CO$_3$ may be used as an alkalizing reagent. Under basic conditions, phenolics can form fenolate anions, which in turn can reduce molybdenum, yielding a blue oxide showing a high extinction coefficient near 750 nm. Results are commonly expressed in gallic acid equivalents. The FC assay actually measures a sample’s reducing capacity and not necessarily ‘total phenolics’, as is usually stated. Apart from flavonoids, groups also classed as polyphenols include stilbenes, lignans and tannins, many of which contain compounds not detected by the Folin–Ciocalteu method (Brat, 2008). AsA reacts significantly with the FC reagent. Depending on the fruit considered, the use of the term ‘total phenolics’ would be misleading to different extents. For instance, in blueberries the content of phenolic compounds is so far beyond the content of other reductants that calling results from FC assays as total phenolics might be reasonably accurate. One advantage of the FC assay over the ABTS and the DPPH• tests is that it is associated with increased absorbance rather than with a reduction. However, there is uncertainty over the compounds that are quantified by the FC assay since, in addition to phenolics, other reducing agents such as reducing sugars and possibly some metal chelators may be included (Prior et al., 2005). The assay is straightforward and reproducible, and has become a routine protocol for characterizing antioxidants in foods.
18.6 Conclusions

To prepare samples for quantification of bioactive compounds, care should be taken to avoid degradation or oxidation, by minimizing prolonged exposure to light, high or ambient temperatures and air. Extraction procedures should be chosen based on the literature, which has compared multiple techniques to identify the most effective method, as well as correct solvent choice. Methods used for the quantification of total bioactives, for instance the FC method, are less likely to differ among authors, since these methods are well documented and relatively easy to carry out. Yet many compounds exist that have reducing potential and are therefore quantified but may not necessarily be classed as antioxidants. The ORAC method is becoming the preferred assay for determination of antioxidant activity, as its mechanism is not through reducing power but an alternative mechanism. In addition, the ORAC method is favoured since antioxidant status can be determined in both lipophilic and hydrophilic fractions. Caution should be taken in comparing in vitro measurements and extrapolating these to prove a real effect in vivo.

The quantification of individual bioactive analytes using methods such as HPLC is more likely to differ between studies due to the multiple factors (mobile phases, stationary phases, column temperatures, etc.) that can differ between quantification techniques. Continued research comparing methodologies for the extraction, separation, isolation and quantification of bioactive compounds can only help to standardize methods further and reduce discrepancies among the literature.

References


hydroxy-6H-dibenzopyran-6-one derivatives by the colonic microflora of healthy humans. European Journal of Nutrition 43, 205–220.


19 Methodologies for Evaluating *In Vitro* and *In Vivo* Activities of Bioactive Compounds

Paul J. Thornalley, Mingzhan Xue and Nai la Rabbani

19.1 Introduction

The health-promoting effects of bioactive compounds found in fruit and vegetables are typically associated with decreasing risk of cancer, maintaining good vascular health and suppression of ageing-related disorders. Evaluations of related pharmacological activities of bioactive compounds are made in chemical and biochemical cell-free systems, cell systems (including use of inducible expression reporter systems), preclinical animal models and clinical dietary intervention and diet supplement trials. These methods are reviewed succinctly in this chapter. There is a continuing tendency to view the health effects of dietary bioactive compounds as being linked inextricably and sometimes only with antioxidant activity (Gorinstein *et al.*, 2009). This is probably a too narrow, blinkered approach (Stevenson and Hurst, 2007). Bioactive compounds are not essential nutrients for growth and development but rather are mainly non-nutrients improving health – particularly in adult and later life. They have been called ‘lifespan essentials’ (Holst and Williamson, 2008). The emerging consensus view on metabolic control for healthy ageing is a requirement to prevent not only oxidative damage in tissues but also to prevent and repair glycation damage, to regain or reset glycolytic and lipogenic control when lost in metabolic syndrome, diabetes and obesity (Kitano *et al.*, 2004), to remove damaged proteins and nucleotides and to maintain efficient metabolism and clearance of damaging metabolites and xenobiotic compounds (oxidizing and non-oxidizing) (Kwak *et al.*, 2003; Leahy, 2006; Xue *et al.*, 2008a). Indeed, bioactives may induce a weak damaging response to activate a stronger antistress gene response that is eventually protective. Hormesis is the beneficial effect of a treatment that, at a higher intensity, is harmful (Calabrese *et al.*, 1999). Agents inducing hormesis are called ‘hormetins’ and many bioactive compounds may be of this type. This might explain the observations, which currently are judged controversial, that bioactive compounds at high doses have adverse toxic effects, whereas at lower doses they are beneficial (Lambert *et al.*, 2007). For hormesis, this dose-dependent behaviour is expected – and will be accepted providing there are plausible mechanistic explanations for beneficial and adverse effects. Hormesis has been proposed to play a role in healthy ageing (Gems and Partridge, 2008). Improved evaluation of bioactive compounds in the future may have to take hormetic considerations into account.
19.2 Cell-free Assessment of Bioactivity

19.2.1 Antioxidant activity

Several protocols have been proposed to compare the antioxidant activity of bioactive compounds. These assays have been classified into two types, depending on the reactions involved: assays depending on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET). Most HAT-based assays use a competitive assay system in which the test antioxidant and a standard substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include: oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), crocin bleaching assays, and inhibition of induced low-density lipoprotein autoxidation. ET-based assays measure the propensity of an antioxidant to reduce an oxidant – the reaction typically monitored by chromophoric change in the oxidant accompanying reduction. ET-based assays include: total phenols assay based on the Folin-Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), 'total antioxidant potential' assay using a Cu(II) complex as an oxidant, and the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) assay. Other assays assess the scavenging capacity of test compounds of biologically relevant oxidants such as singlet oxygen, superoxide anion, peroxynitrite, and hydroxyl radical. It has been suggested that a HAT-based test of free radical scavenging activity and an ET-based test of reducing activity should be performed for bioactive compounds (Huang et al., 2005).

19.2.2 Oxygen radical absorbance capacity (ORAC) assay

This was developed by Cao et al. (1993) and employed as the standard substrate B-phycocerythrin (B-PE) – a protein product isolated from Porphyridium cruentum. B-PE is a fluorescence protein that loses fluorescence in reaction with peroxyl radicals. 2,2'-Azinobis(2-amidinopropane) dihydrochloride (AAPH) was used as the peroxyl radical generator. Use of B-PE suffered several disadvantages: (i) commercial sources of B-PE had marked variability in ORAC; (ii) B-PE was photobleached under microplate-reader conditions; and (iii) polyphenols and other bioactives bound to B-PE and lost fluorescence without peroxyl radical involvement. To avoid these problems, B-PE was replaced by fluorescein (FL) (Naguib, 2000). This improved the ORAC assay, provided a direct measure of the hydrophilic and lipophilic chain-breaking antioxidant capacity versus peroxyl radicals (Huang et al., 2002a) and was adapted for high-throughput application (Huang et al., 2002b). Further modification includes an organic solvent-based ORAC assay for lipophilic samples. The ORAC assay has been applied as a method of choice to quantify antioxidant capacity, and an antioxidant database has been generated applying the ORAC assay in combination with the total phenols assay (Wu et al., 2004b).

19.2.3 Crocin bleaching assay

This assay measures the ability of an antioxidant to protect bleaching of the carotenoid derivative crocin by peroxyl radicals generated by AAPH (Bors et al., 1984). The progress of the reaction is monitored spectrophotometrically at 443 nm. The bleaching rate becomes linear about 1 min after the addition of AAPH and is monitored for 10 min. By varying the antioxidant concentration, the ratio of the rate constants for reaction of the antioxidant and crocin with peroxyl radical is deduced and this is the measurement of antioxidant activity. Crocin is a mixture of natural pigments extracted from saffron, and preparations vary in composition, which limits the reproducibility of this procedure.

19.2.4 Total peroxyl radical-trapping antioxidant parameter (TRAP) assay

This assay uses R-phycoerythrin (R-PE) as a fluorescent substrate, with AAPH as peroxyl radical generator. The reaction progress of
R-PE with AAPH is monitored fluorometrically (at an excitation wavelength of 495 nm and an emission wavelength of 575 nm). The antioxidant capacity of test antioxidants is normalized to that of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) – a water-soluble derivative of vitamin E, and is therefore expressed in 'Trolox equivalents' (Ghiselli et al., 2000). A further modification has been to use dichlorodihydrofluorescein diacetate (DCFH-DA) as a fluorogenic reporter. In this case, peroxyl radical generation by AAPH oxidizes DCFH-DA to fluorescent dichlorofluorescein (DCF) (Valkonen and Kuusi, 1997).

19.2.5 Total phenols assay by Folin–Ciocalteu reagent

Folin–Ciocalteu reagent (FCR) was used initially in the Lowry protein assay. Singleton applied this reagent for the analysis of total phenols in wine (Singleton et al., 1999). The FCR-based assay is known commonly as the total phenols (or phenolic) assay. It measures the reducing activity of the sample. The exact chemical nature of the FCR is thought to be heteropolyphosphotungstates-molybdates, which undergo reversible one- or two-electron reduction reactions, leading to formation of a blue chromophore, possibly (PMoW_{11}O_{40})^{y+} (Huang et al., 2005). The FCR is not specific to phenolic compounds and it can be reduced by many non-phenolic compounds: vitamin C, Cu(I) and many others. Phenolic compounds react with FCR only under basic conditions (in sodium carbonate buffer, pH 10). The phenolate anion reduces FCR. The total phenols assay by FCR is convenient, simple and apparently reproducible. It has become a routine assay in studies of phenolic antioxidants (Jimenez-Alvarez et al., 2008).

19.2.6 Trolox equivalent antioxidant capacity assay (TEAC)

This assay was first reported by Miller et al. (1993), with later improvements (Re et al., 1999). In the improved version, persulfate oxidation of 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) forms a radical cation 2,2’-azino bis-(3-ethylbenzothiazoline-6-sulfonate), which is chromophoric and becomes colourless when reduced. The amount of antioxidant required to produce the same decrease in absorbance as Trolox is deduced. TEAC values of many compounds and food samples are reported (Seeram et al., 2008).

19.2.7 Ferric ion reducing antioxidant power (FRAP) assay

In the FRAP assay, a ferric complex with 2,4,6-tripyridyl-s-triazine (TPTZ), Fe(TPTZ)Cl_{3}, is reduced by the test antioxidant (Huang et al., 2002a). The FRAP assay is performed under acidic conditions (pH 3.6). Interferences in the assay arise from chelators in food extracts binding to Fe(III) and forming complexes. FRAP values of many compounds and food samples are reported (Seeram et al., 2008).

19.2.8 2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable, chromophoric and commercially available free radical. The chromophoric properties are decreased when DPPH is reduced by antioxidants. The time and concentration of antioxidant required to decrease the concentration of DPPH by 50% is recorded (Sharma and Bhat, 2009).

19.2.9 Problems of antioxidant capacity measurements of bioactive compounds

The major weaknesses and problems of the antioxidant assays considered above (and others) are: (i) assessment on the basis of scavenging a free radical or use of free radical generator that is not of physiological relevance; (ii) interference of non-antioxidant effects on the reporter chromophore or fluorophore; (iii) variability in composition and instability of
the reporter molecule; (iv) antioxidant capacity estimation under non-physiological conditions; and (v) sample matrix effects. The design of these assays has compromised quantitation and specificity for ease of procedure, accessibility to reagents and ease of understanding and comparison of outcomes. When comparing these methods in fruit juices and beverages, the TEAC assay gave lower estimates than the ORAC assay (Zulueta et al., 2009). Comparison of antioxidant capacity of 104 plant foods, beverages and oils consumed in Italy by the TEAC, TRAP and FRAP assays showed good correlations of estimates by these different methods (Pellegrini et al., 2003). Comparison of antioxidant capacity assays is intractable, however, in human serum. There was only a weak linear correlation between serum ORAC and serum FRAP activities and no correlation between serum ORAC and TEAC or serum FRAP and serum TEAC outcomes (Cao and Prior, 1998). Confounding interferences, masking effects or other antioxidant activities appear to be operating in vivo, such that the simple assays listed above appear unable to provide a coherent report on antioxidant capacity.

19.2.10 Oxidation of low-density lipoprotein

A potential target of bioactive compounds to decrease atherosclerosis and cardiovascular disease is low-density lipoprotein (LDL). A decrease in oxidation of LDL is linked to concomitant decrease in atherogenicity (Fraley and Tsimikas, 2006). The ability of isolated bioactive compounds to delay the oxidation of LDL initiated by copper sulfate (10 μM) in physiological saline can be assessed. Oxidation is followed by spectrophotometric detection of conjugated dienes at 234 nm. The protective effect is assessed from the increase in time required before the oxidation enters the rapid propagation phase (Souza et al., 2008). The weakness of this approach is that the kinetics of LDL oxidation employed therein are far higher than those found in vivo, where even small, dense atherogenic LDL has low or minimal modification (Navab et al., 2004).

19.3 In Vitro Assessments: Cell Culture Systems

The pharmacological activities of bioactive compounds and fruit and vegetable extracts are achieved mostly within the body, where exposure of cells to the proinflammatory effects of bacterial endotoxins is suppressed by endotoxin-binding proteins (Munford, 2005). It is important, therefore, in the evaluation of pharmacological activities of bioactive compounds and extracts in cell culture cells to avoid inadvertent introduction of endotoxin (or lipopolysaccharide) into the test system with the added bioactive compound or extract. The normal concentration of endotoxin in human plasma is c.0.1 endotoxin unit/ml (Hiki et al., 1999), and endotoxin levels should not be increased significantly beyond this by addition of the test compound. Endotoxin testing is performed conveniently by the chromogenic limulus assay (Piotrowicz et al., 1985). Endotoxin may be removed from test solutions by ultrafiltration, elution through columns of Detoxigel™ (agarose-immobilized polymyxin B) or neutralized by addition of polymyxin B. Care is required if Detoxigel™ or the convenient addition of polymyxin B is the chosen strategy. Polymyxin B does not inactivate all types of endotoxin (Kluger et al., 1985). Polycationic polymyxin B binds to polyanionic endotoxin. If the test compound or extract contains polyanionic substances, they may displace endotoxin from polymyxin B and activate an endotoxin-mediated response.

19.3.1 Cellular antioxidant activity assay

Bioactive compounds absorbed from dietary foodstuffs may derive part of their antioxidant activity by interaction with the metabolic capacity of cells. An attempt to quantify antioxidant activity of bioactive compounds in a cellular matrix is made in the cellular antioxidant assay (CAA) (Wolfe and Liu, 2007). The CAA of bioactive food extracts and dietary supplements uses DCFH-DA as a fluorogenic reporter probe. This probe enters the human hepatoma HepG2 cells used in the assay and is de-esterified by non-specific esterase and
trapped therein as the fluorogenic reporter dichlorodihydrofluorescein. The assay measures the ability of compounds to prevent the formation of DCF by the free radical generator AAPH in HepG2 cells. The results have been expressed in micromoles of quercetin equivalents/100 µmol of phytochemical or in micromoles of quercetin equivalents/100 g of fresh fruit; quercetin had the highest CAA value (Wolfe and Liu, 2007). Of the selected fruit tested by the CAA assay, blueberry had the highest CAA value, followed by cranberry > apple = red grape > green grape (Wolfe and Liu, 2007). The CAA assay has been applied to flavonoids, common fruit and other bioactive compounds and foodstuffs (Wolfe and Liu, 2007, 2008; Wolfe et al., 2008).

The CAA assay has greater biological relevance than cell-free antioxidant capacity activity assays, as potentially it is influenced by cellular uptake, metabolism and location of antioxidant compounds within the HepG2 cells. As bioactive compounds influence the antioxidant capacity of cells by indirect methods – activation of antioxidant-linked gene expression, for example (see below) – there are often potential confounding and masking effects. To explore these, the CAA assay could be performed after varied periods of pre-incubation of HepG2 cells with bioactive compounds or food extract. The assay retains the weakness of using a non-physiological stressor, AAPH, to produce oxidizing free radicals. Use of cultured cells also requires that activators of cellular oxidative stress are not introduced into the assay inadvertently – such as endotoxin, for example. It is not clear from current use of the CAA assay that endotoxin contamination has been controlled and excluded.

19.3.2 Activation of NF-E2-related factor-2 and the antistress gene response

One of the key transcriptional systems in which dietary bioactive compounds are thought to have influence – leading to beneficial health effects – is transcription factor NF-E2-related factor-2 (Nrf2) and its interaction with promoter antioxidant response elements (AREs) (Fig. 19.1). This factor coordinates the antistress gene response. ARE-linked genes code for a battery of protective and metabolic enzymes: γ-glutamylcysteine ligase (GCL), glutathione reductase (GR), aldo-keto reductase (AKR), glutathione transferases (GSTs), quinone reductase (NQO1), Nrf2 itself (Kwak et al., 2002) and others (Thimmulappa et al., 2002). Nrf2-linked gene expression has a key role in the protection of cells against oxidative stress, carbonyl compounds and electrophilic agents. Key enzymes of the pentose phosphate pathway – the source of reducing equivalents for several protective enzymes, transketolase (TK) and transaldolase (TALDO) – are also coded by ARE-linked genes (Thimmulappa et al., 2002). Recent studies have shown that the expression of TK is particularly important in resisting mechanisms underlying vascular disease induced by the hyperglycaemia associated with diabetes (Xue et al., 2008b). Components of the ubiquitin-independent 20S proteasome that degrade damaged proteins also have ARE-linked expression (Woods et al., 1995). All of these genes have expression increased by activation of Nrf2, tempered by inhibitory or activatory effects of small Maf proteins, F, G and K. Some other genes linked to lipogenesis – those coding for sterol regulatory element binding protein 1 (SREBP1) and lipoprotein lipase (LPL) – have expression repressed through the ARE promoters (Thimmulappa et al., 2002). Repression of SREBP1 decreased expression and activities of diacylglycerol acyltransferase-1 and -2 activity and fatty acid synthase (FASN), leading to decreased synthesis of triglycerides (TG) and cholesterol esters (CE) and secretion of apolipoprotein B100 (apoB) in very low-density lipoprotein (VLDL) (Maiyoh et al., 2007). Activation of Nrf2, therefore, has the potential to protect against proteome and lipidome damage, enhance removal of residual damage and decrease lipogenesis – all important for maintaining good vascular health (Fig. 19.2).

Under basal conditions, Nrf2 is complexed with Kelch-like ECH-associated protein 1 (Keap1) – a BTB-Kelch protein. Keap1 is a substrate adaptor protein for Cullin-3 (Cul3)-dependent E2 ubiquitin ligase complex, directing Nrf2 for proteasomal degradation (Kobayashi et al., 2004). In oxidative stress, lipid peroxidation products, 4-hydroxynonenal and
Activation of Nrf2

Endogenous activators:
- 4-Hydroxynonenal,
- J-Isoprostanes

Exogenous activators:
- Dietary bioactive compounds (isothiocyanates, polyphenols, allyl sulfides, oxidized omega-3 fatty acids)

Increased expression of protective proteins (GST, NQO1, GSHRd, AKRd, TK, TA, GCL, Nrf2, and others) and decreased expression of lipogenic proteins (SREBP-1, lipoprotein lipase and others)

Fig. 19.1. Activation of Nrf2 and dynamic nuclear-cytoplasmic shuttling of Nrf2 for expression of antioxidant response element-linked genes.
Antistress gene response:
Transcription factor Nrf2 / antagonist Keap1 (iNrf2)

Antioxidant response element (ARE)-linked genes

Protection against oxidative damage
Glutathione synthesis and GSH-dependent enzymes GCL, GSHPd, GSHPx and GSTs
Superoxide dismutase-1 (SOD1) and catalase (CAT)
Peroxiredoxins (PRDXs)
Thioredoxin (TXN) and thioredoxin reductase (TXNRd1) ... and others

Protection against glycation
Aldoketo reductase (AKRd)
Aldehyde dehydrogenase (AldDH)

Protection against metabolic stress
Transketolase (TKT)
Transaldolase (TALDO)

Protection against lipogenic stress
SREBP-1 (down regulation)
Lipoprotein lipase and others (downregulation)

Removal of damaged proteins
20S Proteasome induction (PSMA1, PSMA4, PSMB3, PSMB5, and PSMB6)

Fig. 19.2. Antistress gene response: a multi-layered protective response.

J3-isoprostanes, may disrupt the Nrf2–Keap1 or Keap1–Cul3 interactions. These are current candidate physiological activators of Nrf2 signalling; there may be others. The Nrf2 thereby liberated or stabilized from degradation translocates to the nucleus and, combining with small Maf protein (Motohashi et al., 1997), in Maf–Nrf2 heterodimer or (Maf)2–Nrf2 homodimer complexes (Kimura et al., 2007), induces or represses ARE-linked gene expression (Zhang et al., 2005) (Fig. 19.1). MafF, MafG and MafK isoforms have differential potency and specificity for subsets of ARE-linked genes (Katsuoka et al., 2005). Keap1 has concurrent increased susceptibility to degradation, but also has ARE-linked gene expression and may be induced by Nrf2 activation providing an autoregulatory feedback loop for post-stimulation return to Nrf2 homeostasis (Lee et al., 2007). There is an active Nrf2–Keap1 system in human vascular endothelial cells (Chen et al., 2003), hepatocytes (Keum et al., 2006), related cell lines (Qian et al., 2006; Lee et al., 2007) and many other cell types.

Activation of Nrf2 has been implicated in the prevention of cancer (Surh et al., 2008), cardiovascular disease (Zhu et al., 2005), vascular complications of diabetes (Xue et al., 2008b) and renal failure (Thornalley and Rabbani, 2009), dementia (Singh et al., 2008), arthritis (Mahajan et al., 1994), other diseases and ageing. In this regard, health benefit may
be achieved by dietary bioactive compounds enhancing the endogenous activation of the Keap1–Nrf2 system and provide a supranormal defence pathogenic mechanism. Key dietary bioactive activators of this system are: glucosinolate-derived dietary isothiocyanates (Thornalley and IARC Workgroup, 2004) and indoles (Maiyoh et al., 2007), thioethers and disulfides (Chen et al., 2004), polyphenols (Tanigawa et al., 2007), flavonoids (Mann et al., 2007) and carotenoids (Ben Dor et al., 2005), as well as omega-3 fatty acids via formation of J3-isoprostanes (Gao et al., 2007). The activation is thought to occur by different mechanisms: isothiocyanates such as sulforaphane (SFN) release Nrf2 from Keap1 by modification of critical cysteine thiol residues (Dinkova-Kostova et al., 2002); some polyphenols induce downregulation of Keap1 expression (Tanigawa et al., 2007) and/or induce mild oxidative/nitrosative stress (Mann et al., 2007), and J3-isoprostanes disrupt the Keap1–Cul3 complex, preventing Keap1–Nrf2 targeting to the proteasome (Gao et al., 2007). Increased levels of ARE-linked gene products provide for increased protection against reactive oxidizing, nitrating and glycat-
ing species – preserving the integrity of the vascular cell proteome and lipidome and enhancing the activity of enzymes of detoxification (Thimmulappa et al., 2002). Activation of Nrf2 also suppresses the expression of key regulators of lipogenesis (Kwak et al., 2003).

The ability of bioactive compounds to increase inducible expression of a particular protective enzyme via the ARE promoter may be assessed by the design of reporter constructs in expression vectors containing the ARE nucleotide sequence of interest linked to a luciferase or similar reporter system (Table 19.1). Transfection efficiency is controlled by incorporation of a different constitutive reporter either in the same vector or in a similar vector included in co-transfection. Additional controls include transfection with empty vector and with vector containing a mutant non-functional ARE promoter.

Alternatively, the ability of bioactive compounds to induce ARE-linked gene expression may be assessed by gene microarray analysis, real time RT-PCR array analysis, immunoblotting of ARE-linked gene products and measurement of the activities of related enzymes. Typical marker enzymes of the anti-stress gene response are quinone reductase (NQO1) and isozymes of glutathione transferase (GST). Other ARE-linked gene expression may be assessed for particular applications; for example, TK involved in countering metabolic dysfunction in hyperglycaemia (Xue et al., 2008b) and SREBP1 in reversal of dyslipidaemia (Maiyoh et al., 2007) (Table 19.2).

The effects of bioactive compounds on the Nrf2 system may increase the cellular concentration of GSH by increased expression of the GCL involved in GSH synthesis and GSHRd. The effect may be time dependent; for example, dietary isothiocyanates tend to induce initially a decrease in GSH after 3 h exposure, with a rebound to increased

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reporter system</th>
<th>Expression vector/construct</th>
<th>Bioactives</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinone reductase</td>
<td>Luciferase</td>
<td>NQO1hARE-tk-luc</td>
<td>Lycopene, flavonoids</td>
<td>Mulcahy et al. (1997); Ben Dor et al. (2003); Lee-Hilz et al. (2006)</td>
</tr>
<tr>
<td>γ-Glutamylcysteine ligase – heavy chain (GCSh)</td>
<td>Luciferase</td>
<td>GCShARE4-tk-luc</td>
<td>Lycopene</td>
<td>Mulcahy et al. (1997); Ben Dor et al. (2003)</td>
</tr>
<tr>
<td>Human GSTP1</td>
<td>Luciferase</td>
<td>–336-GSTP1-luc</td>
<td>Curcumin</td>
<td>Nishinaka et al. (2007)</td>
</tr>
<tr>
<td>Thioredoxin reductase</td>
<td>Luciferase</td>
<td>pARE-CAT</td>
<td>SFN</td>
<td>Hintze et al. (2003)</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>Chloramphenicol amino transferase</td>
<td></td>
<td>SFN</td>
<td>Yu et al. (1999)</td>
</tr>
</tbody>
</table>
Table 19.2. Evaluation of antioxidant response element linked gene expression in cell systems in vitro.

<table>
<thead>
<tr>
<th>Target disease/abnormal physiological state</th>
<th>Cell type</th>
<th>Gene expression and metabolites</th>
<th>Bioactives</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td>Human mammary cancer MCF-7 cells and hepatoma HepG2 cells</td>
<td>γ-Glutamylcysteine ligase – heavy chain (GCL₇)</td>
<td>Lycopene</td>
<td>Ben Dor et al. (2005)</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Rat hepatocytes</td>
<td>CAT, SOD, GSHPx, GSR, GST, NQO1 activities</td>
<td>Resveratrol (25–75 μM)</td>
<td>Rubio lo et al. (2008)</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Human HepG2 cells</td>
<td>Nrf2</td>
<td>SFN, AITC, I3C</td>
<td>Jeong et al. (2005)</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Human leukaemia HL60 and ML-1 cells; HepG2 cells</td>
<td>NQO1, GST and cellular GSH</td>
<td>SFN</td>
<td>Xu and Thornalley (2000a); Ye and Zhang (2001)</td>
</tr>
<tr>
<td>Hyperglycaemia (diabetes)</td>
<td>Microvascular endothelial cells</td>
<td>TK, GSRd, NQO1</td>
<td>SFN</td>
<td>Xue et al. (2008b)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>Human hepatoma HepG2 cells</td>
<td>Apolipoprotein B100 secretion, LDL uptake, triglycerides, cholesterol, cholesterol esters and lipogenic gene expression</td>
<td>Naringenin (citrus flavonoid); I3C; red grape juice</td>
<td>Boradaile et al. (2003); Davalos et al. (2006); Mayoh et al. (2007)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Human chondrocytes</td>
<td>NQO1, GST, Nrf2 and others</td>
<td>SFN</td>
<td>Healy et al. (2005)</td>
</tr>
</tbody>
</table>
cellular GSH concentrations thereafter (Xu and Thornalley, 2000a; Ye and Zhang, 2001).

Activation of the Nrf2 system by vegetable and fruit extracts in cell culture has also been studied. Broccoli sprout extract processed with myrosinase to convert glucosinolates to related isothiocyanates induced both GST and NQO1 significantly in cultured bladder cells (Zhang et al., 2006). Aqueous extracts of black radish (Raphanus sativus L. var. niger) increased the activity of NQO1 in HepG2 cells in vitro (Hanlon et al., 2007). Anthocyanin-enriched bilberry extracts modulated pre- or post-translational levels of oxidative stress defence enzymes heme-oxygenase-1 and GST in cultured human retinal pigment epithelial cells (Milbury et al., 2007).

Innovative high-throughput screening methods are in development; for example, an ARE sequence-specific, fluorescence-quenching hybridization probe (Wang et al., 2008). However, the Nrf2 system has complex time course activation characteristics influenced by multiple positive and negative feedback mechanisms. All of these influences are potential factors affecting bioactive compound stimulatory activity.

### 19.3.3 Inhibition of malignant and non-malignant cell growth

Bioactive compounds have been assessed for their ability to inhibit the growth of malignant and non-malignant cells in vitro; for example, inhibition of human leukaemia cells and human lymphocyte growth (Xu and Thornalley, 2000b). Inhibition of malignant tumour cell growth has been linked to the cancer preventive activity of many bioactive compounds via inhibition of growth and induction of cell death of preclinical tumours. This remains controversial and it is often unclear how concentrations can be achieved and maintained in vivo to produce this response. Glucosinolates derived isothiocyanates (ITCs) inhibited the growth of human tumour cells in vitro. The median growth inhibitory concentration (CC50) values were in the range 0.7–40 μM (Table 19.3). Apoptotic death was a characteristic of dose-limiting toxicity; at much higher concentrations, ITCs induced necrotic cell death. ITCs may also be toxic to non-malignant cells in vitro (Table 19.4). Mixtures of dietary ITCs induced similar effects (Fimognari et al., 2005). There is relatively low toxicity of ITCs to colonic, prostate, bronchial and oral mucosal epithelial cells, but significant toxicity to keratinocytes and renal tubular, cervical and mammary epithelial cells (reviewed by Thornalley and IARC Workgroup, 2004).

Assessment of inhibition of cell growth is made with greatest security by counting viable cell numbers in a haemocytometer or by automated low-cost image analysis. Viability is assessed by exclusion of the dye, Trypan blue (Maruhashi et al., 1994). Other indirect methods have been used: reduction of the redox dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium (MTS) or related compounds (Wang et al., 1996), uptake of neutral red (Repetto et al., 2008) and sulforhodamine B detection of protein content (Vichai and Kirtikara, 2006). The colorimetric methods relate metabolic reduction activity or protein dye binding to cell number. If treatment of cells with bioactive compounds interferes in the metabolic reduction activity or cell protein content without change in cell number, then false positive outcomes will be registered. Viable cell number counts are the most secure method for assessing effect on cell growth and viability.

### 19.3.4 Assessment of cytotoxicity

Toxicity of bioactive compounds is usually undesirable unless it is selective to malignant cells or invading microorganisms. In pharmacological studies of bioactive compounds and fruit and vegetable extracts in cell culture models, initial study of the effect of concentration dependence on cell viability, at least up to the peak plasma concentration, is advisable to ensure that other pharmacological effects are not compromised by decreased cell viability. As for all cell culture studies, bioactive compounds and fruit and vegetable extracts should be endotoxin-free before
Table 19.3. Inhibition of human tumour cell growth and induction of cytotoxicity by dietary isothiocyanates in vitro.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell</th>
<th>GC(_{50}) (pM)</th>
<th>TC(_{50}) (pM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Methylsulfinylbutyl isothiocyanate (SFN)</td>
<td>Jurkat</td>
<td>4.9</td>
<td>–</td>
<td>Fimognari et al. (2002b)</td>
</tr>
<tr>
<td></td>
<td>HT29</td>
<td>15</td>
<td>–</td>
<td>Gamet-Payrastre et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>LS-174</td>
<td>55</td>
<td>–</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Caco-2</td>
<td>40</td>
<td>–</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>P-3</td>
<td>20</td>
<td>–</td>
<td>Nasruzzi et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>K562</td>
<td>11</td>
<td>–</td>
<td>Tang et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>UM-UC-3</td>
<td>6.8</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Allyl isothiocyanate</td>
<td>HL60</td>
<td>2.6</td>
<td>11</td>
<td>Xu and Thornalley (2000b)</td>
</tr>
<tr>
<td></td>
<td>ML-1</td>
<td>2.6</td>
<td>7.7</td>
<td>Xu and Thornalley (2000b)</td>
</tr>
<tr>
<td></td>
<td>HeLa</td>
<td>20</td>
<td>–</td>
<td>Hasegawa et al. (1993)</td>
</tr>
<tr>
<td>Benzyl isothiocyanate</td>
<td>K562</td>
<td>1.5</td>
<td>–</td>
<td>Nastruzzi et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>LS-174</td>
<td>15</td>
<td>–</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Caco-2</td>
<td>2</td>
<td>–</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>HT29</td>
<td>3</td>
<td>–</td>
<td>Musk et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>Jurkat</td>
<td>2</td>
<td>–</td>
<td>Chen et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>HeLa</td>
<td>2</td>
<td>–</td>
<td>Hasegawa et al. (1993)</td>
</tr>
<tr>
<td>Phenethyl isothiocyanate</td>
<td>HL60</td>
<td>1.5</td>
<td>5.0</td>
<td>Xu and Thornalley (2000b)</td>
</tr>
<tr>
<td></td>
<td>ML-1</td>
<td>2.7</td>
<td>3.3</td>
<td>Xu and Thornalley (2000b)</td>
</tr>
<tr>
<td></td>
<td>LS-174</td>
<td>20</td>
<td>–</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Caco-2</td>
<td>12</td>
<td>–</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>HeLa</td>
<td>2</td>
<td>–</td>
<td>Hasegawa et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Jurkat</td>
<td>3</td>
<td>–</td>
<td>Chen et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>PC-3</td>
<td>6</td>
<td>–</td>
<td>Xiao and Singh (2002)</td>
</tr>
<tr>
<td>Broccoli isothiocyanates (extract)</td>
<td>UM-UC-3</td>
<td>6.6</td>
<td>–</td>
<td>Tang et al. (2006)</td>
</tr>
</tbody>
</table>

Notes: Cell lines: Caco-2, human colonic adenocarcinomas; HCEC, SV40 T-antigen immortalized human colonic epithelial cells; HeLa, human cervical carcinoma; HL60, human acute myeloblastic leukaemia; HT29, human colonic adenocarcinoma cells; Jurkat, human T-cell leukaemia; K562, human erythroleukaemia; LS-174, human colorectal adenocarcinomas; ML-1, human myeloblastic leukaemia; PC-3, human prostate cancer; UM-UC-3, bladder carcinoma.

Table 19.4. Toxicity of dietary isothiocyanates to non-malignant cells in vitro.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species origin</th>
<th>Cell type</th>
<th>TC(_{50}) (\mu M)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFN</td>
<td>Human</td>
<td>Lymphocytes</td>
<td>30</td>
<td>Fimognari et al. (2002a)</td>
</tr>
<tr>
<td>AITC</td>
<td>Rat</td>
<td>RL-4 liver epithelial</td>
<td>175</td>
<td>Bruggeman et al. (1986)</td>
</tr>
<tr>
<td>BITC</td>
<td>Human</td>
<td>Colon epithelial</td>
<td>22</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>RL-4 liver epithelial</td>
<td>25</td>
<td>Bruggeman et al. (1986)</td>
</tr>
<tr>
<td>PEITC</td>
<td>Human</td>
<td>Colon epithelial</td>
<td>20</td>
<td>Bonnesen et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Lymphocytes</td>
<td>53</td>
<td>Xu and Thornalley (2000b)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Keratinocytes</td>
<td>0.5</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Renal tubular epithelial</td>
<td>4.5</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Mammary epithelial</td>
<td>1.3</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Cervical epithelial</td>
<td>3.2</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Prostate epithelial</td>
<td>12</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Bronchial epithelial</td>
<td>15</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Oral mucosal epithelial</td>
<td>19</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>Liver epithelial (Chang)</td>
<td>20</td>
<td>Elmore et al. (2001)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Bovine</td>
<td>Vascular smooth muscle cells</td>
<td>c.10</td>
<td>Poussier et al. (2005)</td>
</tr>
</tbody>
</table>
being added to cultured cells for evaluation of cell viability. Cell death may be induced by several mechanisms: apoptosis, autophagy, necrosis, anoikis (detachment stimulated apoptosis) and other mechanisms. The generally agreed indications of cell death are when one or more of the following molecular or morphological criteria are met: (i) the cell has lost the integrity of the plasma membrane, as defined by vital dyes in vitro; (ii) the cell including its nucleus has undergone complete fragmentation into discrete bodies (which are frequently referred to as ‘apoptotic bodies’); and/or (iii) its corpse (or its fragments) have been engulfed by an adjacent cell in vivo (Kroemer et al., 2005).

One of the most commonly used methods to assess cell death is exclusion of the dye, Trypan blue. This has been automated recently for low-cost image analysis (Maruhashi et al., 1994). In a cytotoxic response, some cells may die and fragment prior to visualization and hence the total cell number seen (viable plus non-viable cells) may be less than in control cultures. The commitment to cell death or point-of-no-return may have been reached many hours before dying cells become permeable to Trypan blue. This can be investigated by examining the minimum period of exposure to bioactive compound (washing it away and replacing with fresh medium thereafter) required for later development of decreased cell viability.

Surrogate markers of cell death relate to biochemical detection of changes thought to be characteristic of commitment to cell death. In this regard, a profound increase in caspase activity, complete permeabilization of the outer mitochondrial membrane, or exposure of phosphatidylserine residues are markers of commitment to cell death by apoptosis. There are some examples, however, where these phenomena are found without cell death, and hence evidence of the harsher end points of cell death should be sought (Kroemer et al., 2005). Other methods used for assessment of apoptosis are: the terminal deoxynucleotidyl transferase (TDT)-mediated 2’-deoxyuridine-5’-triphosphate (dUTP) nick end labelling (TUNEL) assay (which utilizes TDT to add a poly-U tail on to DNA strand breaks, which are then detected using immunocytochemistry) (Heatwole, 1999), and propidium iodide staining of cellular DNA and DNA fragments (Riccardi and Nicoletti, 2006). Dual staining with Hoechst 33342 and propidium iodide can discriminate between cells dying by necrosis and apoptosis: necrotic cells show red fluorescence due to the uptake of propidium iodide, apoptotic cells show bright blue fluorescence and normal cells show low blue, low red fluorescence (Ormerod et al., 1993). Anoikis is detected by analysis for apoptosis in cells floating free of the extracellular matrix to which they are normally adherent. All methods require care in interpretation and the reader is directed to the expert references cited herein for further guidance.

### 19.3.5 Anti-inflammatory activity

The anti-inflammatory properties of some bioactive compound ITCs have been investigated in cellular systems. The inhibition of bioactive compounds on formation of superoxide in the respiratory burst of neutrophils stimulated by phorbol ester – an activator of protein kinase C – has been studied. The median inhibitory concentration (EC₅₀) value was 3.5 μM for phenethyl isothiocyanate (PEITC) and 0.26 μM for hydroxytyrosol (Visioli et al., 1998; Gerhauser et al., 2003). The inhibition of lipopolysaccharide (LPS)-induced inflammatory responses by bioactive compounds has also been investigated via the formation of nitric oxide in a macrophage cell line – an inducible nitric oxide synthase response, the secretion of tumour necrosis factor (TNF) and the expression of COX-2 and associated formation of prostaglandin E₂ (Heiss et al., 2001; Ippoushi et al., 2002; Gerhauser et al., 2003; Allen and Walker, 2003).

### 19.3.6 Antibacterial activity

Antibacterial activity of bioactive compounds has been investigated in relation to countering gastrointestinal infection by Helicobacter pylori, which has been linked to gastritis, peptic ulcer disease and gastric cancer. Bioactive compounds are also potentially health
beneficial in countering dermatological infections and foodborne bacterial pathogens. Both glucosinolate-derived ITCs, the flavonoids quercetin and cranberry juice extract rich in polyphenols have been found to have antibacterial activity (Fahey et al., 2002; Ibrahim et al., 2007; Wu et al., 2008).

19.3.7 Other effects: effect on endogenous conversion of cis- to trans-fatty acids

Consumption of unsaturated fatty acids with at least one double bond in the trans configuration has been linked to increased cardiovascular disease. This is thought to be mediated by effects on membrane structure, function and cell signalling (Mozaffarian et al., 2006). It has emerged recently that trans-fatty acid content of cells can also be increased by in situ geometric isomerization of cis-fatty acids of membrane lipids. This process is mediated by thyl free radicals formed in oxidative stress (Ferreri et al., 2005). It will be of future interest to study the effects of dietary bioactive compounds for their ability to prevent the cis-/trans-isomerization of fatty acids in membrane lipids and assess the link to risk of cardiovascular disease.

19.4 Preclinical Assessment of Bioactive Compounds

The pharmacological activities of bioactive compounds are evaluated in preclinical studies typically in laboratory animals (mice, rats, hamsters and others). Initially, bioactive compounds are administered to healthy animals and pharmacological effects assessed. Any adverse effects are also recorded. Traditionally, the effects of bioactives compounds on target enzyme activities are assessed. With modern transcriptomic, proteomic and metabolomic techniques, a different approach may be taken. Firstly, gene microarray analysis can give a qualitative assessment of global changes in expression of c.30,000 genes. This is followed by quantitative analysis of expression of a selection of genes of particular interest, by real-time RT-PCR array. Changes in gene product levels may then be investigated by quantitative Western blotting and also assay of relative enzyme activities. Finally, changes in metabolites of interest may also be measured. Changes in gene expression will indicate the type of the most potent pharmacological activity: cancer chemopreventive activity, antioxidant activity, antilipogenic activity or other. The bioactive compounds may then be taken forward for evaluation in specific disease models in further preclinical studies. Examples are given in Table 19.5.

19.4.1 Chemoprevention of cancer

Experimental models used in preclinical assessment of chemoprevention of cancer are typified by those employed in assessment of cancer preventive activity of dietary-derived isothiocyanates and Brassica vegetable consumption – reviewed in the International Agency for Research on Cancer (IARC) handbook (Thornalley and IARC Workgroup, 2004). The standard protocol involves administration of a bioactive compound of dietary intervention before, during and after administration of a chemical carcinogen and assessing tumour incidence, tumour mass or related biomarkers of neoplasma (e.g. aberrant crypt foci). The IARC report concluded there was sufficient evidence to indicate that consumption of cruciferous vegetables and intake of aromatic dietary isothiocyanates and indole-3-carbinol (I3C) decreased the risk of cancers of the colon, mammary gland and liver in experimental animal models; there was insufficient evidence of similar effects of glucosinolates or SFN.

19.4.2 Prevention of cardiovascular disease

Prevention of cardiovascular disease in experimental animals commonly targets suppression of atherosclerotic plaque formation in the atherosclerosis-prone apolipoprotein E knockout mouse or hamster (Zadelaar et al., 2007). Prevention of atherosclerosis by quercetin and catechin has been studied by Hayek et al. (1997). The effect of extracts has also
<table>
<thead>
<tr>
<th>Target disease</th>
<th>Animal model</th>
<th>Specimen dosing</th>
<th>Primary end point</th>
<th>Other</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Mouse, Balb/c</td>
<td>SFN, c.160 mg/kg SFN, c.60 mg/kg</td>
<td>Microarray gene expression</td>
<td>Tumour mass, Biomarkers – aberrant crypt foci, etc.</td>
<td>Thimmulappa et al. (2002)</td>
</tr>
<tr>
<td>None</td>
<td>Rat, Fisher F344</td>
<td></td>
<td>Microarray gene expression</td>
<td></td>
<td>Hu et al. (2004)</td>
</tr>
<tr>
<td>Cancer</td>
<td>Mouse, rat – various strains</td>
<td>0.075–1.25 μmol/g PEITC</td>
<td>Tumour incidence</td>
<td></td>
<td>Thornalley and IARC Workgroup (2004)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Apolipoprotein E deficient mice</td>
<td>Quercetin and catechin (50 μg/day and red wine (0.5 ml/day)</td>
<td>Atherosclerotic plaque area</td>
<td>Serum paraoxonase and LDL aggregation</td>
<td>Hayek et al. (1997)</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>Spontaneously hypertensive stroke-prone rat</td>
<td>Broccoli sprouts, dried (200 mg/day)</td>
<td>Blood pressure – decreased by 20 mmHg</td>
<td>Improved endothelial-dependent relaxation, increased heart and kidney GSH, GSRd and GSHPx</td>
<td>Wu et al. (2004a)</td>
</tr>
<tr>
<td>Longevity</td>
<td><em>Caenorhabditis elegans</em></td>
<td>Resveratrol, 100 μM in food</td>
<td>Median lifespan</td>
<td></td>
<td>Viswanathan et al. (2005); Bass et al. (2007)</td>
</tr>
<tr>
<td></td>
<td><em>Drosophila melanogaster</em></td>
<td>Resveratrol, 1 μM–1 mM in food</td>
<td>Median lifespan</td>
<td>Fecundity</td>
<td>Baur and Sinclair (2006); Bass et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Mice, male C57BL/6NIA</td>
<td>Resveratrol, 0.01 and 0.04% (w/w) diet</td>
<td>Median lifespan</td>
<td>Vascular function, histopathology, other Inflammatory mediators concentrations</td>
<td>Fraley and Tsimikas (2006)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Rat, adjuvant-induced arthritis</td>
<td>5 x 30 mg/kg quercetin (swelling, immobility)</td>
<td>Clinical score</td>
<td></td>
<td>Mamani-Matsuda et al. (2006)</td>
</tr>
</tbody>
</table>
been studied: green and black tea, pomegranate juice, grape extracts, red wine, de-alcoholized wine and wine polyphenols. The effects observed may be due to the antioxidant and anti-inflammatory activities of the bioactives. The doses of bioactive compound applied for significant beneficial effect are, however, often much higher than typically seen in dietary exposure of human subjects (reviewed in Manach et al., 2005).

19.4.3 Renal disease

Renal disease is a major health concern as it is associated with markedly decreased life expectancy and increased risk of cardiovascular disease. One of the major causes of renal disease is diabetes. Bioactive compounds may be evaluated for ability to prevent diabetic nephropathy in a streptozotocin-induced diabetic rat model (McNeil, 1999). Early stage nephropathy develops over 12-24 weeks, as indicated by increased urinary albumin excretion rate. Tea catechins and other bioactive compounds suppress the development of diabetic nephropathy (Hase et al., 2006). A model of chronic renal failure can be produced in rats by partial nephrectomy, where one whole kidney and part of the other kidney is removed surgically. Bioactive compounds were found to improve residual renal function in this model (Yokozawa et al., 1996).

19.4.4 Survival and longevity studies

Bioactive compounds are often evaluated for effects on survival and lifespan in experimental models of ageing – such as the nematode Caenorhabditis elegans, the fruitfly Drosophila melanogaster and laboratory mice. The compounds are often thought to act as antioxidants or as dietary restriction mimetics – in the latter case, seeking to mimic the life extension effect achieved by caloric restriction regimes (Lane et al., 2007). Many claims for life-extending effects have not been confirmed by later studies. For example, resveratrol was claimed to increase the lifespan of C. elegans (Viswanathan et al., 2005) and Drosophila (Baur and Sinclair, 2006), but these claims could not be verified in later studies (Bass et al., 2007). Recent translation of these findings to mice found some beneficial effects of resveratrol on general health but no significant increase in lifespan (Fraley and Tsimikas, 2006). Longevity studies have often been poorly designed – particularly underpowered and susceptible to false positive outcomes. In response to these and other concerns, the US National Institute on Ageing has formulated standard operating procedures for conducting such studies (Nadon et al., 2008).

19.4.5 Other effects

The anti-inflammatory activity of bioactive compounds is evaluated in preclinical studies against experimental models of arthritis and endotoxaemia. Quercetin decreased inflammation in rat adjuvant-induced arthritis (Mamani-Matsuda et al., 2006).

SFN (c.2 mg/kg, daily) inhibited angiogenesis – as judged by decrease of blood content of a subcutaneous implant in mice of an extracellular matrix Matrigel™ plug impregnated with recombinant murine vascular endothelial growth factor (VEGF) and heparin (Jackson et al., 2007). This dose, however, is c.20-fold higher than achieved maximally by consumption of 100 g broccoli (Song and Thornalley, 2007).

19.5 Clinical Assessment of Bioactive Compounds

Clinical assessments of the health benefits of bioactive compounds are often judged by dietary interventions – change in the dietary content of compounds known to be rich in the bioactive compound of interest. The effects on clinical end points or biomarkers indicative of health benefit are often predicted to be small and therefore studies must be designed, randomized and powered for significant effect. Where possible, environmental factors should be controlled for and participant and investigator awareness of the study group allocation of each subject should be prevented (by
In Vitro and In Vivo Activity

Participant and investigator blinding). Increased statistical power may be achieved by expanding subject or patient numbers in the study, by crossover study design in which participant groups are switched between periods of normal and intervention dietary regime, and by repeated observations over time or ‘repeated measures’ analysis. A simple and effective way to control for environmental factors and provide for participant and investigator blinding is to identify two varieties of dietary component (fruit or vegetable, for example), one relatively poor and one relatively rich in the bioactive compound of interest, and produce a dietary intervention with these in a randomized, participant- and investigator-blinded design. In addition, periods of abstaining from the dietary component of interest, or washout period before and after the dietary intervention – providing it is ethical to do so (with no significant and long-term health impairment likely) – will assist in assessing the health benefit of the dietary bioactive compound and whether the benefits achieved are maintained or reversed with change in diet.

Given an appropriate study design and protocol, there is also assessment and monitoring of compliance to the dietary regime to be considered. Dietary compliance may be assessed by questionnaire, interaction with the subject by calls and visits by the research clinic, and by urinary metabolite analysis. Steps to improve compliance have been: diet counselling (at the research clinic and remotely by calls and electronic communication media), direct supply of dietary supplement, written goals and instructions, portion size guides, study cookbook containing appropriate recipes, skills training in a teaching kitchen, subject motivation assessment in pre-screening, diet diaries with periodic review with a researcher, and dietician-led group sessions (Fowke et al., 2006).

19.5.1 Biomarkers

The use of biomarkers in the surrogate assessment of clinical end points is advantageous because: (i) they often indicate early, preclinical stages or risk of chronic disease and can be accessed in subjects in otherwise good health; (ii) they are present in a larger population, which decreases subject recruitment problems; (iii) they may be measured with non-invasive or minimally invasive procedures (sampling blood and urine); (iv) they are often more responsive to interventions than established disease; and (v) they may be relatively inexpensive to measure. Clinical studies using biomarkers depend on the validity of the biomarker employed. The biomarker should be a validated surrogate end point for the disease or abnormal metabolic state of interest.

One of the most common biomarker assessments used is that of oxidative stress. The Biomarkers of Oxidative Stress Study (BOSS) was designed to validate markers of oxidative stress in specific animal models of oxidative stress: (i) carbon tetrachloride poisoning; (ii) environmental exposure to ozone; (iii) systemic exposure to endotoxin; and (iv) continuous skin exposure to cumene hydroperoxide. It remains unclear how these models translate to oxidative stress in the clinical setting; initial conclusions indicate that measurements of malondialdehyde (MDA) and isoprostanes in plasma and urine and 8-hydroxydeoxyguanosine in urine are potential biomarkers of oxidative stress (Kadiiska et al., 2005).

A further area of bioactive compound evaluation where biomarkers offer critical insights is in the chemoprevention of cancer. The IARC Working Party assessing chemoprevention of cancer by cruciferous vegetables accepted evidence of the following biomarkers: (i) detectable precancerous changes in tissue assessed by histology; (ii) change in gene expression thought to play a causal role; (iii) DNA damage; (iv) exposure to known carcinogen; and (v) effects on metabolic factors thought to be involved in cancer aetiology. Intervention studies with cruciferous vegetables were reviewed (Thornalley and IARC Workgroup, 2004).

19.5.2 Clinical evaluation of bioactive compounds

A selection of clinical studies on the effect of bioactive compounds derived from fruit and vegetable is presented in Table 19.6. The reader is also directed to several detailed
<table>
<thead>
<tr>
<th>Target disease/abnormal physiological state</th>
<th>Study design (participant number)</th>
<th>Dietary intervention</th>
<th>Primary end point</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon adenoma patients</td>
<td>Randomized, crossover (n = 20)</td>
<td>Brassica vegetables (&gt;160 g/day) for 4 weeks</td>
<td>Urinary isoprostanes</td>
<td>20% decrease with intervention</td>
<td>Fowke et al. (2006)</td>
</tr>
<tr>
<td>Normal healthy subjects</td>
<td>Three-phase crossover (n = 16)</td>
<td>Broccoli soup (normal and high glucosinolate)</td>
<td>Gastric mucosa gene expression</td>
<td>Induction of expression of GCLm TrRd</td>
<td>Gasper et al. (2007)</td>
</tr>
<tr>
<td>Normal healthy subjects</td>
<td>Open trial (n = 12)</td>
<td>Vegetable soup, ‘gazpacho’, 500 ml/day</td>
<td>Biomarkers of oxidative stress and vascular inflammation</td>
<td>28% decrease in plasma isoprostanes</td>
<td>Sanchez-Moreno et al. (2004)</td>
</tr>
<tr>
<td>Normal healthy subjects</td>
<td>Three-phase crossover (n = 59)</td>
<td>Baseline diet, then sunflower oil diet for 25 days, followed by rapeseed oil for 25 days</td>
<td>Markers of lipid metabolism</td>
<td>Decreased total cholesterol and LDL</td>
<td>Sanchez-Moreno et al. (2004)</td>
</tr>
<tr>
<td>Normal healthy subjects</td>
<td>Double-blind, randomized, placebo controlled study (n = 173)</td>
<td>Oil supplements for 8 months: placebo, sunflower oil, evening primrose oil, soyabean oil, tuna fish oil and tuna/evening primrose oil mix</td>
<td>Endothelium-dependent and -independent vascular responses</td>
<td>Acetylcholine responses were improved significantly after tuna oil supplementation</td>
<td>Khan et al. (2003)</td>
</tr>
<tr>
<td>Normal healthy subjects</td>
<td>Randomized open trial (n = 175)</td>
<td>β-Carotene, lutein, lycopene (15 mg/day) or placebo/day for 12 weeks</td>
<td>LDL oxidation, blood GSH, plasma protein thiols and antioxidant enzyme activities</td>
<td>No significant effects</td>
<td>Hininger et al. (2001)</td>
</tr>
</tbody>
</table>
reviews of these studies – including analysis of combinations of studies: cruciferous vegetables (Thornalley and IARC Workgroup, 2004), lycopene and tomato paste (Basu and Imrhan, 2007), flavonoids (Erlund, 2004), cranberries (Neto, 2007), blackcurrants (Lister et al., 2002), n-3 polyunsaturated fatty acids (Hartweg et al., 2007), tea polyphenols (Sajilata et al., 2008) and other chapters in this book. There is often insufficient and inadequate clinical evidence of the health benefit of bioactive compounds derived from fruit and vegetables, and a tendency to claim clinical benefits of bioactive compounds before sufficient evidence has been obtained to sustain such claims.

19.6 Conclusions

The wealth and diversity of bioactive compounds present in and extracted from fruit, vegetables and other plants provide a rich palate on which to draw to produce valuable pharmacology for health benefit. Many compounds appear to exert their strongest effects by enhancing the antistress gene response, strengthening the body’s innate response to stress and tissue damage. Working by this transcriptional and enzymatic gene product mechanism, the potential potency of action of bioactive compounds is much greater than small molecule antioxidants with stoichiometric action. Non-antioxidant effects – such as antilipogenic activities – will also likely provide future valuable therapeutics for cardiovascular disease. There remains much further preclinical and clinical evaluation to be done to reap the potential of dietary bioactive compounds.

19.7 Acknowledgements

The authors thank the Biotechnology and Biological Sciences Research Council (UK), the Wellcome Trust, British Heart Foundation (BHF) and Diabetes UK for support for their bioactive compound research. NR is a BHF Intermediate Research Fellow.

References


Bonnescn, C., Eggleston, I.M. and Hayes, J.D. (2001) Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. Cancer Research 61, 6120–6130.


398
P.J. Thornalley et al.


phenomenon with important implications for risk assessment. Risk Analysis 19, 261–281.

al Biology and Medicine 14, 303–311.

detoxifying enzymes by garlic organosulfur compounds through transcription factor Nrf2: effect of
chemical structure and stress signals. Free Radical Biology and Medicine 37, 1578–1590.

genes in endothelial cells. Journal of Biological Chemistry 278, 703–711.


Davalos, A., Fernandez-Hernando, C., Cerrato, E., Martinez-Botas, J., Gomez-Coronado, D., Gomez-Cordoves,
C. and Lasuncion, M.A. (2006) Red grape juice polyphenols alter cholesterol homeostasis and increase

and Talalay, P. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating in-
duction of phase 2 enzymes that protect against carcinogens and oxidants. Proceedings of the National

Elmore, E., Luc, T.-T., Steele, A.E. and Redpath, J.L. (2001) Comparative tissue-specific toxicities of 20 can-
cer preventive agents using cultured cells from 8 different normal human epithelia. In Vitro and Mo-
lecular Toxicology 14, 191–207.


Fahey, J.W., Haristoy, X., Dolan, P.M., Kensler, T.W., Scholtus, I., Stephenson, S.S., Talalay, P. and Lozniews-
ki, A. (2002) Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helico-
bacter pylori and prevents benzo[a]pyrene-induced stomach tumours. Proceedings of the National
Academy of Sciences of the United States of America 99, 7610–7615.

Ferrari, C., Kratzsch, S., Brede, O., Marciniak, B. and Chatgilialoglu, C. (2005) Trans lipid formation in-

sulforaphane-induced cell cycle delay and apoptosis in non-transformed human T lymphocytes.

Fimognari, C., Nusse, M., Cesari, R., Iori, R., Cantelli-Forti, G. and Hrelia, P. (2002b) Growth inhibition,
cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane. Carci-

Fimognari, C., Berti, F., Iori, R., Cantelli-Forti, G. and Hrelia, P. (2005) Micronucleus formation and induc-
tion of apoptosis by different isothiocyanates and a mixture of isothiocyanates in human lymphocyte
cultures. Mutation Research-Genetic Toxicology and Environmental Mutagenesis 582, 1–10.

tion reduces urinary F2-isoprostane levels independent of micronutrient intake. Carcinogenesis 27,
2096–2102.


phane on a prostate cancer cell line. Australian and New Zealand Journal of Surgery 73, 154–156.

toxic and cytotoxic effects of glucosinolates hydrolysis products on human colon cancer cells in vitro.
Anti-Cancer Drugs 9, 141–148.

M.E., Chan, J.Y., Morrow, J.D. and Freeman, M.L. (2007) Novel n-3 fatty acid oxidation products
activate Nrf2 by destabilizing the association between Keap1 and Cullin3. Journal of Biological Chemis-
try 282, 2529–2537.


Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A. and Prior, R.L. (2002b) High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled...
with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry* 50, 4437-4444.


Index

abortifacients 359
ageing, protection against
   animal studies 309–310, 393, 394
   blackcurrant 281
   blueberry 57–59
   strawberry 309–310
AIDS/HIV 120, 164
alkaloids
   peppers 331–332, 332–333, 335
   potato 222–224, 232
   yam 227
allergic reactions
   latex-associated 32, 339
   to kiwifruit 143
allicin 15, 17, 20
Allium genus (garlic, leek, onion, shallot) 5
   bioactive compounds 5–12
   health benefits 12–18
   pre- and postharvest period 18–21
   almond (Prunus amygdalus) 239
      bioactive compounds 240, 242, 244, 245, 247, 249
      health benefits 248
Alzheimer’s disease see cognitive decline, protection against
amaranth species 174
amino acids
   avocado 36–37
   banana 354
   cucurbits 125
   grape 163
   see also peptides
analytical methods 377
   animal testing 392–394
antioxidant assays
   cell culture 383–384
      cell-free 186, 240, 373–376, 381–383
   cell culture systems
      antibacterial activity 391–392
      anti-inflammatory activity 391
      antioxidant activity 383–384
      antistress gene response 384–389
      cis- to trans-isomerization of fatty acids 392
      cytotoxicity 389–391
      endotoxin and 383
      inhibition of cell growth 389
clinical studies 394–397
   extraction methods 136, 372
   in vitro versus in vivo testing 56–57, 63, 304, 373
   polyphenol content 263, 296
   preparation of samples 371
   separation and quantification 136, 372–373
angiotensin I converting enzyme (ACE) inhibition 225
animal testing (preclinical studies) 392–394
anthocyanins and anthocyanidins 246
   analytical methods 296, 372
   apple 198, 205
   aubergine 337
   avocado 33
   banana 353
   bioavailability 228, 268–269, 299, 300
   blueberry and cranberry 53, 59–60, 64–67
   carrot 227
   citrus fruit 93
anthocyanins and anthocyanidins continued

- grape 159–160
  - health benefits 59–60, 282, 310
- onion 7, 19
- potato 221, 231
- Prunus spp. 246, 251
- Ribes and Rubus spp. 264–268, 282
- strawberry 296–9, 300, 310, 313–314

antiasthmatic activity 15, 203
antibiotic activity see antimicrobial activity
anticancer activity
- Allium spp. 12, 13, 15, 16, 17–18
- analytical methods 389, 392, 395
- apple 201–202
- aubergine 338
- avocado 30–31, 39–40
- blueberry and cranberry 63
- Brassica spp. 78–79, 392
- carrot 229–230
- citrus fruit 98, 100–101, 102, 103–104
- cucumber 128
- grape 162
- leafy vegetables 175, 181
- mango 362
- peppers 333, 334
- pomegranate 141
- Prunus spp. 248
- Ribes and Rubus spp. 272–274, 278–279
- Sapindaceae 139
- strawberry 304–308
- tomato 325–326

antifungal activity 6, 14, 18, 125, 373
antihelmintic activity 359–360
anti-inflammatory activity
- Allium spp. 14–15
- analytical methods 391, 394
- blackcurrant 275
- blueberry 58
- cherry 250
- citrus fruit 101
- Ribes and Rubus spp. 275, 276
- strawberry 307, 309

see also arthritis
antimicrobial activity 391–392
- Allium spp. 14, 17
- apple and other pome fruit 201
- cranberry 60–62
- grape 164
- peppers 333–335
- Ribes and Rubus spp. 276, 277, 280–281, 282
- Sapindaceae 139

antinutrients 79

see also detrimental effects
antioxidant activity 2, 3, 241
analytical methods
- antistress gene response 384–389
- cell culture assay 383–384

see also ascorbic acid; phenolic and polyphenolic compounds
- apple and other pome fruit 199–200, 204, 207, 208
- avocado 31, 38–39
- blueberry and cranberry 56
- carambola 146
- citrus fruit 101
- grape 165
- kiwifruit 143
- papaya 358–359
- peppers 334
- persimmon 145
- pomegranate 141
- potato 221–222
- Prunus spp. 240–242, 253

antithrombotic/antiplatelet activity
- Allium spp. 13, 14
- blueberry and cranberry 62
- citrus fruit 101
- grape 164
- strawberry 309

antiviral activity 164, 276, 282
anxiolytic activity 144
apoptosis 15, 39, 391
apple (Malus domestica) 196
  - bioactive compounds 196–201
  - cultivars 203–204
  - health benefits 201–203, 210
  - pre- and postharvest period 205–210
apricot (Prunus armeniaca) 242, 243, 244, 245, 247, 249, 250
arthritis
- animal studies 393
- avocado 32, 40
- blackcurrant 275, 281
- citrus fruit 100
- leafy vegetables 175
ascorbic acid (vitamin C)
- analytical methods 373
- apple and pear 199, 205, 207, 208, 209
- aubergine 336, 337
- avocado 36
- banana 353
- citrus fruit 103, 106–108
- cucumber 121, 126, 127, 128
- dietary fibre and 15
- function 1, 121, 199, 243–244
- grape 162, 164
- leafy vegetables 179, 184
- mango 361, 363
- onion 6, 15
- papaya 358, 360
- peppers 330–331
- Prunus spp. 244, 251, 252
- Ribes and Rubus spp. 270–271, 282–283
Sapindaceae family 138
strawberry 304, 313
tomatillo 338
tomato 323–324, 327–328, 329
Asian leafy vegetables 174, 182, 183
asthma, protection against 15, 203
atherosclerosis, prevention of 162–164, 276, 309, 392
see also lipid-lowering activity
aubergine (eggplant, Solanum melongena) 336–337, 338–339, 339
avocado (Persea americana) 27–29
bioactive compounds 27, 29–38
cultivars 28
health benefits 38–42, 43
pre- and postharvest period 34, 35–36, 37–38, 42–43, 44
bacteriocidal/bacteriostatic activity see antimicrobial activity
banana (Musa acuminate/Musa balbisiana) 352–355
beetroot (Beta vulgaris)
leaves 174, 178	
tuber 227
berries see Ribes genus; Rubus genus; strawberry; Vaccinium genus
betalains 227
bilberry (Vaccinium myrtillus) 51, 59
see also blueberry (Vaccinium spp.)
bile acids, binding of 63, 250, 354
bioavailability of compounds
Allium spp. 12
anthocyanins 228, 268–269, 299, 300
avocado 33
carotenoids 39, 175, 228–229, 243, 359
citrus fruit 99
flavonoids 200–201, 228, 268–269, 299, 300
leafy vegetables 175, 187, 188
papain 359
peppers 332
peptides 229
phenolic compounds 33, 63, 200–201, 228, 268–269, 269–270, 299, 300
Ribes and Rubus spp. 268–269
strawberry 299, 300
tomato 325
biomarkers 395
blackberry (Rubus spp.) 261, 262, 265, 269, 272, 281, 283
see also Rubus genus
blackcurrant (Ribes nigrum) 261
see also Ribes genus
blanching 85
blood orange (Citrus sinensis) 93
see also sweet orange
blueberry (Vaccinium spp.) 51–52, 67–68
bioactive compounds 53–56
genotype 51, 64–65
health benefits 52–53, 57–60, 62–63
pre- and postharvest period 65–66, 67
boysenberry (Ribes ursinus X idaeus) 272, 280
brain
neuroprotective activity
banana 354
blueberry 57–59, 63
citrus fruit 104–105
Ribes and Rubus spp. 277, 281
strawberry 309–310
neurotoxins 146
Brassica genus 74–75
Asian leafy vegetables 174, 182, 183
bioactive compounds 75–80, 83, 84
cultivars 81, 182
health benefits 76, 78–79, 392
pre- and postharvest period 81–85, 183
breast cancer 202
broccoli (Brassica oleracea) 74, 75
bioactive compounds 76, 77, 79, 80, 83, 84
cultivars 81
health benefits 76, 78–79
pre- and postharvest period 81–85
bromelain 355, 356
browning of fruit and vegetables
apple 206
aubergine 337
avocado 29
lettuce 182, 184
peach 252
Brussels sprouts (Brassica oleracea) 77, 82, 83
see also Brassica genus
CA storage see controlled atmosphere (CA) storage
cabbage (Brassica oleracea) 75, 83
see also Brassica genus
caffeic acid 246, 267
calcium
applied to apple trees/fruit 205–206, 209
in Brassica spp. 76
cancer see anticancer activity
canned peaches 252
cantaloupe melon (Cucumis melo) 120, 121, 122, 126–128
see also cucurbits
cape gooseberry (uchuva, Physalis peruviana) 321, 337–338, 339
capsaicin 331–332, 332–333, 335
capsaicinoids 332, 335
Capsicum species see peppers
carambola (starfruit, Averrhoa carambola) 145-146
carbohydrates
avocado 37–38, 40, 42–43
exotic fruit 140, 142, 144, 146
grape 161, 163, 165
potato 225
carcinogens 332–333
cardioprotective activity
Allium spp. 13, 14, 16, 17
almond 248
animal studies 392–394
apple 202–203
aubergine 338–339
avocado 30, 40–41
blueberry and cranberry 62–63
carrot 230
citrus fruit 102, 104
grape 162–164
peppers 333
pomegranate 141
potato 225, 230
Ribes and Rubus spp. 275, 276, 279–280
strawberry 307, 308–309
tomato 356–357
β-carotene (provitamin A)
avocado 36
banana 354
cucurbits 121, 123–124, 125–126, 127, 128
leafy vegetables 176
mango 362
papaya 358, 360
pineapple 356
Prunus spp. 242–243
tomato 323
carotenoids
avocado 34, 36, 38, 39
banana 353–354
bioavailability 39, 175, 228–229, 243, 359
carambola 145–146
carrot 226, 232
citrus fruit 92–93
cucurbits 121–124, 125–126, 127, 128
health benefits 123–124, 175, 229, 243, 325–326
leafy vegetables 175, 176
mango 361–362
papaya 358, 359, 360
peppers 330, 335–336
pineapple 356
potato 225
Prunus spp. 242–243, 251, 252
Sapindaceae family 138
structure 226, 242–243
tomato 323–324, 325–326, 327, 328
carrot (Daucus carota) 233
bioactive compounds 226–227, 228–229, 232
health benefits 219, 226, 229–230, 230
pre- and postharvest condition 232–233
cassava (Manihot esculenta) 227–228
catechins (flavan-3-ols) 158–159
apple 198, 199, 202, 203
avocado 33
grape 159
health benefits 202, 203
strawberry 292–293, 299
see also proanthocyanidins
cauliflower (Brassica oleracea) 80, 83
see also Brassica genus
cell culture assays 383–392
see also Brassica genus
cell death 15, 39, 391
cell growth, inhibition of 389
cellular antioxidant assay (CAA) 383–384
cerebrovascular disease 59, 63, 354
chard (Beta vulgaris) 174, 182
cherry (Prunus spp.) 238
bioactive compounds 242, 244, 245, 246, 247, 249, 251
health benefits 250
see also Prunus genus
chicory (Cichorium endivia) 174, 182
chilli pepper (Capsicum frutescens) 331–332, 333–333, 335
Chinese gooseberry (kiwifruit, Actinidia deliciosa) 143
Chinese leafy vegetables 174, 182, 183
Chinese quince 200
chitinases 14, 18
chlorogenic acid 267
apple 199, 207, 208
aubergine 337
blueberry 55
potato 220
Prunus spp. 246
chlorophyll 91
cholecystokinin (CCK) 230–231
cholesterol-lowering activity see lipid-lowering activity
cinnamic acid 160
citric acid (in onion) 5–6
[l]-citrulline 125
citrus fruit 90–91, 108
bioactive compounds 91–98
cultivars 106
health benefits and disbenefits 98, 99–105
pre- and postharvest period 105–108
clinical studies 394–397
cognitive decline, protection against 57–59, 281, 309–310
colon cancer 175, 229, 248
colonic bacteria 10, 280–281
commercial importance
Allium spp. 5
avocado 28
banana 353
blueberry and cranberry 51-52
Brassica spp. 74-75
citrus fruit 90-91
cucurbits 118-119
leafy vegetables and salads 171-173
mango 360
papaya 357
pineapple 355
potato 218
Prunus spp. 238
Ribes and Rubus spp. 261, 262
strawberry 292
tomato and other Solanaceous fruit 321, 322
controlled atmosphere (CA) storage
Allium spp. 19, 21
apple 208-209
avocado 43
blueberry 66
Brassica spp. 82
citrus fruit 107
mango 363
Ribes and Rubus spp. 282, 283
strawberry 313
cooking
Brassica spp. 83-85
carrot 226
cucurbits 128-129
leafy vegetables 171, 174
onion 7
potato 221
coriander (Coriandrum sativum) 174
cosmetics 31
cowpea (Vigna sesquipedalis) 174-175
cranberry (Vaccinium macrocarpon) 52, 67-68
bioactive compounds 53-56
cultivars 65
health benefits 52-53, 60-62, 63
pre- and postharvest period 66, 67
cresses (Lepidium sativum and Nasturtium officinale) 172, 174
Crocin bleaching assay 381
cucumber (Cucumis sativa) 120, 122, 126
see also cucurbits
cucurbits (cucumber, melon, pumpkin, squash) 118-121, 130
bioactive compounds 121-125, 129
cultivars 127, 128, 129-130
health benefits 125-126
pre- and postharvest changes 126-129
cultivars 2-3
apple 203-204
avocado 28
blueberry 64-65
Brassica spp. 81, 182
citrus fruit 106
cranberry 65
cucurbits 127, 128, 129-130
grape 155
leafy vegetables and salads 181-182
onion 18
peppers 335
potato 231
Prunus spp. 250-251
strawberry 305
tomato 322, 327
see also taxonomy
currants see Ribes genus
cutting, leafy vegetables 184
cytotoxicity, assessment of 389-391
dental health 61-62, 164
dermatitis 277, 282, 362
detrimental effects
Allium spp. (organosulfur compounds) 14, 17
aubergine 339
avocado 32
Brassica spp. (glucosinolates) 79
carambola (neurotoxins) 146
of β-carotene 124
cassava (cyanogenic compounds) 227-228
cytotoxicity assessment 389-391
drug interactions 17, 105
kiwifruit 143
papaya 359
peppers (capsaicin) 332-333, 335
potato (glycoalkaloids) 223
diabetes mellitus
animal studies of diabetic nephropathy 394
protective effects
Allium spp. 14, 16, 17, 18
avocado 41-42
grape 164
Ribes and Rubus spp. 276, 280
sour cherry 250
strawberry 307, 309
dietary fibre see fibre
digestive disorders 335, 355
2,2-diphenyl-1-picrylhydrazyl (DPPH) assay 376, 382
diphenylamine (DPA) treatment 209
diuretic effects 165
dopamine 59
dopaminergic neurons 59
DPPH assay (2,2-diphenyl-1-picrylhydrazyl) 376, 382
dried fruit
blueberry 67
garlic 20
grape 165
plum 252
drug interactions
  garlic 17
grapefruit 105
drumstick tree (Moringa oleifera) 181, 183
druses see Prunus genus
dyspepsia 335, 355
economic importance see commercial importance
eggplant (aubergine, Solanum melongena) 336–337, 338–339, 339
ellagic acid 267
  raspberry and blackberry 269
  strawberry 300–301, 313
ellagitannins 248, 270, 301
endotoxin, in cell culture assays 383
ethylene
  as cause of bitterness in carrots 233
  inhibition with 1-MCP 43, 209
  postharvest ripening of tomatoes 329
  scavengers of 43
  as suppressant of postharvest sprouting in potatoes 232
extraction methods for bioactive compounds 136, 372
falcarinol 226, 229–230
fatty acids
  avocado 31, 35–36, 38, 40–41, 42
  cis- to trans-isomerization 392
  exotic fruit 143, 144
  pumpkin 129
  Ribes and Rubus spp. 271–272, 280
ferric ion reducing antioxidant power (FRAP) assay 375–376, 382
fertilizer use
  citrus fruit 106–107
  leafy vegetables 182–183
  onions 18–19
  pineapple 356
  Sapindaceae 139–140
  tomato 328
fibre
  ascorbic acid and 15
  banana 353
  citrus fruit 98
  potato 225
  Sapindaceae 139
fisetin 303
  ‘5-a-day’ campaign 2
flavan-3-ols (catechins) 158–159
  apple 198, 199, 202, 203
  avocado 33
  grape 159
  health benefits 202, 203
  strawberry 292–293, 299
  see also proanthocyanidins
flavanones 158, 198, 247
flavones 159, 198, 246
flavonoids (including anthocyanins)
  Allium spp. 7, 14, 18–19
  apple 198–199, 205
  aubergine 337
  avocado 33
  banana 353
  bioavailability 200–201, 228, 268–269, 299, 300
  blueberry and cranberry 53–55, 59–60, 64–67, 68
  Brassica spp. 76
  carrot 226–227
  citrus fruit 93, 94–97, 103–104
  extraction 372
  grape 156–160
  health benefits
    anticancer activity 18, 103–104, 201–202
    antimicrobial activity 14
    antiobesity activity 310
    cardioprotective activity 104, 202–203
    eye conditions 59–60, 282
  mango 361
  peppers 331
  potato 221, 231
  Prunus spp. 246, 251
  Ribes and Rubus spp. 263–269, 282
  strawberry 296–300, 303, 310, 313–314
  structure 156, 197–198
  sweet potato 227
flavonols 156–157, 267
  Allium spp. 7, 14, 15, 18, 19
  apple 198, 199
  bioavailability 200–201, 300
  blueberry and cranberry 55
  grape 157–158
  Prunus spp. 246
  Ribes and Rubus spp. 263–264
  strawberry 299–300
flavour compounds
  Allium spp. 7–8
  Brassica spp. 79
  carrot 226, 233
  citrus fruit 97
  peppers 331–332
  folate/folic acid 76, 104
  cucurbits 124, 127, 128
  leafy vegetables 180–181
  strawberry 304
Foulin–Ciocalteu assay 263, 376, 382
FRAP assay (ferric ion reducing antioxidant power) 375–376, 382
freezing, postharvest 85, 371
fructans 10–12, 372
fungi
  contamination of grape 166
see also antifungal activity
furocoumarins 98, 105

garlic (Allium sativum) 5, 8, 12, 15–17, 20
see also Allium genus
gastric cancer 322–323
genetics
fruit and vegetables see cultivars
human 78
genomic studies 384–389, 392
glucosinolates
Brassica spp. 76–78, 79, 80, 81, 82, 83, 84, 85
leafy vegetables 181, 182
glycoalkaloids 222–224, 232
glycoprotein 224–225
goitrogenic compounds 79
gooseberry (Ribes uva-crispa) 261
see also Ribes genus
grape (Vitis spp.) 154, 166
bioactive compounds 154–162
cultivars 155
health benefits 162–165, 166
pre- and post harvest changes 159, 165–166
grapefruit (Citrus paradisi) 93, 94, 97, 102, 105
see also citrus fruit

harvesting see postharvest changes; preharvest conditions; ripening

hawthorn fruit 200
heart disease see cardioprotective activity
heat treatment 85, 184
heptose sugars 37–38, 40, 42–43
highbush blueberry (Vaccinium corymbosum) 51, 64
see also blueberry
history 1, 261, 291
HIV (human immunodeficiency virus) 120, 164
honeydew melon (Cucumis melo) 120, 122, 126–128
see also cucurbits

diagnostic activity assessment 391
antioxidant activity
cell culture 383–384
cell-free methods 304
compared with in vivo testing 56–57, 63, 304
cytotoxicity assessment 389–391

in vitro testing
animal (preclinical) studies 392–394
clinical studies 394–397
inflammation see anti-inflammatory activity
inositol hexakisphosphate (IP6) 139
intestinal nematodes 359–360
intestinal pathogens 280–281
iron 37
irradiation, postharvest treatment
ionizing
citrus fruit 107
garlic 20
leafy vegetables 185–186
ultraviolet
apple 209–210
blueberry 66
Prunus spp. 251
tomato 328
isothiocyanates 78, 79, 85, 181, 390
kaempferol 7, 156–157, 267
kale (Brassica oleracea) 77
see also Brassica genus
Kelch-like ECH-associated protein 1 (Keap1) 384, 386
kidney disorders 354
calculi 275, 281–282
diabetic nephropathy 394
kiwifruit (Actinidia deliciosa) 143

leaky vegetables and salads 171–172
bioactive compounds 176–181, 182, 186, 187–188

cultivars 181–182
health benefits 175–176, 186–187, 188
pre- and post harvest period 182–186
species 172–175
leek (Allium ampeloprasum leek group) 6, 7, 12, 17–18, 20–21
see also Allium genus
lemon (Citrus limon) 97, 101–102
see also citrus fruit
lettuce (Lactuca sativa) 172, 173–174
bioactive compounds 175, 180

leek (Allium ampeloprasum leek group) 172, 173, 174
bioactive compounds 175, 180
lettuce (*Lactuca sativa*) continued
  pre- and postharvest period 182, 183, 184, 185, 186
lime (*Citrus spp.*) 91, 97, 101
  see also citrus fruit
limonoids 97–98, 104
lipid-lowering activity
  almond 248
  avocado 30, 40–41
  blueberry and cranberry 62
  citrus fruit 102, 104
  root crops 230
  strawberry 309
  tomato 326
litchi (*Litchi chinensis*) 135–140
liver damage, protection against 231, 249
longan (*Dimocarpus longan* or *Euphoria longana*) 135–140
longevity see ageing, protection against
low-density lipoprotein (LDL) see lipid-lowering activity
low-density lipoprotein (LDL) oxidation assay 383
lowbush blueberry (*Vaccinium angustifolium*) 51, 64
  see also blueberry (*Vaccinium spp.*)
lung disorders, protection against
  asthma 15, 203
  cancer 201–202
  lutein 34, 39, 323
  lycopene
    papaya 358, 359
    tomato 323, 325–326, 327, 328
    watermelon 124, 125–126, 129
malic acid (in onion) 5–6
manganese 355–356
mango (*Mangifera indica*) 360–363
mangosteen (*Garcinia mangostana*) 141–142
mannoheptulose 37–38, 40, 42–43
MAP see modified atmosphere packaging
mass spectrometry 373
melon see sweet melon; watermelon
metabolic syndrome 250, 309
  see also diabetes mellitus
1-methylcyclopentene (1-MCP) (ethylene inhibitor) 43, 209
3-methylthiopropionic acid ethyl ester (MTPE) 128
microwave cooking 7, 83
minerals
  avocado 37
  banana 354
  *Brassica* spp. 76
  cucurbits 124–125, 127, 128
  grape 162, 163
  papaya 358
  pineapple 355–356
modified atmosphere packaging (MAP)
  *Brassica* spp. 82–83
  leafy vegetables 184–185
monounsaturated fatty acids (MUFA) 35–36, 40–41, 42
mouth, oral health 61–62, 164
nectarine (*Prunus persica* var. nectarina) 240, 242, 244, 245, 247, 249
negative effects see detrimental effects
nematodes, activity against 359–360
neuroprotective activity
  blueberry 57–59, 63
  citrus fruit 104–105
  *Ribes* and *Rubus* spp. 277, 281
  strawberry 309–310
neurotoxins 146
NF-E2-related factor-2 (nrf2) activation 384–389
night vision 59–60
nitrates, health effects of 21
nitrogen fertilizer
  *Allium* spp. 18–19, 20–21
  leafy vegetables 182–183
obesity, control of 230–231, 277, 307, 310
oesophageal cancer 308
oils
  avocado 31, 35–36, 40–41
  exotic fruit 143, 144
  pumpkin seeds 129
  *Ribes* and *Rubus* seeds 271–272, 280
oilseed rape (*Brassica* seeds) 79
onion (*Allium cepa*) 5
  bioactive compounds 5–12
  cultivars 18
  health benefits 12–15
  pre- and postharvest treatment 18–19
ORAC assay (oxygen radical absorbance capacity) 375, 381
oral health 61–62, 164
orange (sweet orange, *Citrus sinensis*) 91
  bioactive compounds 93, 94, 97, 106, 107–108
  health benefits 101
  juice 107–108
  see also citrus fruit
organic acids
  *Allium* spp. 5–6
  exotic fruits 141, 144, 145, 146
  grape 161, 164, 165
  see also phenolic acids
organic farming
  apple 206
  blueberry 65
  *Prunus* spp. 251–252
  strawberry 312
organoselenium compounds 9–10, 76
organosulfur compounds
   *Allium* spp. 7–9, 12, 14, 15, 17, 19, 20
   *Brassica* spp. 76–78, 79, 80, 81, 82, 83, 84, 85
   flavour 7–8, 79
   health benefits and disbenefits 12, 14, 15, 17,
   76–78, 390
   leafy vegetables 181
osteoarthritis
   avocado 32, 40
   citrus fruit 100
   leafy vegetables 175
oxidative stress
   biomarkers of 395
   genomic response to 384–389
   see also antioxidative activity
oxygen radical absorbance capacity (ORAC) assay 375, 381
packaging see modified atmosphere packaging (MAP)
PACs see proanthocyanidins
pak choi (*Brassica* spp.) 174, 182, 183
PAL (phenylalanine ammonia lyase) 184, 205
papain 357, 358
papaya (*Carica papaya*) 357–360
Parkinson’s disease 59
passion fruit (*Passiflora edulis*) 143–144
patatin 224–225
peach (*Prunus persica*) 238
   bioactive compounds 240, 242, 243, 244, 245, 246, 247, 249
   health benefits 248–250
   pre- and postharvest period 251, 252
pear (*Pyrus* spp.) 197, 199, 200, 205, 206, 207, 209
pectin 15, 98, 225, 353
peppers (*Capsicum* spp.) 321, 329–330
   bioactive compounds 330–332
   cultivars 335
   health benefits and disbenefits 331, 332–336
   pre- and postharvest period 330–331, 335–336
peptides 225, 229
persimmon (*Diospyros* spp.) 144–145
phenolic acids 157, 160–161
   apple 198–199
   bioavailability 269–270
   potato 220–221
   *Prunus* spp. 246
   *Ribes* and *Rubus* spp. 268–269
phenolic and polyphenolic compounds
   *Allium* spp. 6–7, 12, 14, 18–19
   analytical methods 372, 373, 376, 382
   apple (and other pome fruit) 197–199, 204–206, 207–209
   aubergine 336, 337
   avocado 29–34, 39, 42
   banana 353
   bioavailability 33, 63, 200–201, 228, 268–269, 269–270, 299, 300
   blueberry and cranberry 53–55, 56, 59–60, 62, 64–67, 68
   *Brassica* spp. 76
   carambola 146
   carrot 226–227
   citrus fruit 93–97, 103–104
   cucurbits 125
   grape 154–161, 162, 164, 165, 166
   health benefits 245
      anticancer activity 12, 18, 39, 103–104, 162, 201–202
      antimicrobial activity 14, 60, 61, 139, 164
      antiobesity activity 310
      cardioprotective activity 62, 104, 162–164, 202–203, 230
      eye conditions 59–60, 282
   kiwi fruit 143
   leafy vegetables 175, 179–180, 182, 186–187
   mango 360–361
   mangosteen 142
   papaya 358
   passion fruit 144
   peppers 331
   persimmon 145
   pineapple 356
   pomegranate 141
   potato 220–222, 228, 231–232
   *Prunus* spp. 245–248, 251, 252
   *Ribes* and *Rubus* spp. 262–270, 282
   Sapindaceae family 138–139
   strawberry 292–303, 310, 313–314
   structure 157, 196, 197–198, 267
   sweet potato 227
   tomato 323
   phenylalanine ammonia lyase (PAL) 184, 205
   phylloquinone (vitamin K) 175, 180
   phytosterols 34–35, 40
   pineapple (*Ananas comosus*) 355–357
   plantain 353, 355
   see also banana
   platelets see antithrombotic/antiplatelet activity
   plum (*Prunus* spp.) 238
      bioactive compounds 240, 244, 245, 247, 249, 250, 251
      health benefits 248–250
   polyacetylenes 226, 229–230
   polyphenols see phenolic and polyphenolic compounds
   polyunsaturated fatty acids (PUFA) 271–272, 280
   pome fruit see apple; pear
   pomegranate (*Punica granatum*) 140–141
   pomelo fruit (*Citrus grandis*) 93, 105
   see also citrus fruit
Index

postharvest changes 3
- Allium spp. 19, 21
- apple 206–210
- aubergine 337
- avocado 42–43
- banana 355
- blueberry and cranberry 66–67
- Brassica spp. 82–85
- cape gooseberry 338
- citrus fruit 107–108
- cucurbits 126, 127, 128, 129
- grape 165–166
- leafy vegetables and salads 184–186
- mango 363
- papaya 360
- peppers 336
- pineapple 357
- potato 232
- Prunus spp. 252
- Ribes and Rubus spp. 282–283
- Sapindaceae 140
- strawberry 311–312, 313–314
- tomato 328–329
- see also cooking

potassium (K)
- content of
  - banana 354
  - cucurbits 124–125, 127, 128
  - grape 162
- fertilizer requirements
  - litchi 139–140
  - pineapple 356
  - tomato 328
- potato (Solanum tuberosum) 1, 218, 233
- bioactive compounds 220–225, 228, 231, 232
- cultivars 231
- health benefits 219, 225, 230, 231
- pre- and postharvest period 221, 231–232
- prebiotic agents 10–12
- preclinical studies 392–394

preharvest conditions
- Allium spp. 18–19, 20–21
- apple 205–206
- aubergine 337
- avocado 42
- blueberry and cranberry 65–66
- Brassica spp. 81–82
- citrus fruit 106–107
- cucurbits 127
- grape 159
- leafy vegetables and salads 182–184
- papaya 360
- peppers 330–331, 335–336
- pineapple 356
- potato 231–232
- Prunus spp. 251–2

Sapindaceae 139–140
- strawberry 298, 310–313
- tomato 327–328
- see also cultivars

pressure, high-pressure treatment 107, 314
proanthocyanidins (PACs) (condensed tannins)
- blackcurrant 270
- blueberry and cranberry 53–55, 59, 60, 61
- grape 158–159, 162
- Prunus spp. 247–248
- strawberry 293–296

processing activities
- avocado 43
- blueberry and cranberry 67
- Brassica spp. 83–85
- carrot 226
- citrus fruit 107–108
- cucurbits 128–129
- garlic 20
- grape 165
- leafy vegetables 184–186, 185–186
- potato 221
- Prunus spp. 252, 253
- Ribes and Rubus spp. 283
- see also cooking

prostate cancer 15, 325, 326
protease inhibitors 231
protein
- avocado 36–37
- potato 224–225, 231, 232
- see also amino acids; peptides

proteolytic enzymes
- bromelain 355, 356
- papain 357, 358

provitamin A see β-carotene
Prunus genus 238–240, 253
- bioactive compounds 239–248, 253
- cultivars 250–251
- health benefits 248–250
- pre- and postharvest period 239, 251–252

pulmonary disorders, protection against
- asthma 15, 203
- cancer 201–202

pummelo (Citrus grandis) 93, 105
- see also citrus fruit

pumpkin (Cucurbita spp.) 120, 122, 126, 128–129
- see also cucurbits

quercetin 267
- Allium spp. 7, 14, 15, 18, 19
- apple 198, 199
- bioavailability 200–201
- blueberry and cranberry 55
- grape 157, 158
- Prunus spp. 246
Index

Ribes and Rubus spp. 263–264
strawberry 299–300
quince 200

rabbiteye blueberry (Vaccinium virgatum) 52
see also blueberry (Vaccinium spp.)
radiation treatments see irradiation, postharvest treatment
radish (Raphanus sativus) 227
rambutan (Nephelium lappaceum) 135–140
raspberry (Rubus spp.) (see also Rubus genus) 261, 262
bioactive compounds 265, 269, 283
health benefits 278, 279
red beet (Beta vulgaris)
leaves 174, 178
tuber 227
redcurrant (Ribes rubrum) 261
see also Ribes genus
renal disorders 354
calculi 275, 281–282
diabetic nephropathy 394
resveratrol 56, 161, 162, 301–303, 394
retinol (vitamin A) 36, 138, 242–243, 353
see also β-carotene (provitamin A)
rheumatoid arthritis 275, 281
Ribes genus 260–261, 283–284
bioactive compounds 262–272
health benefits 272–282
pre- and postharvest period 282–283
ripening
apple and pear 205
aubergine 337
avocado 34, 35–36, 37–38, 42, 43
blueberry and cranberry 65–66
citrus fruit 91, 105
grape 155–156
peppers 330, 331, 336
pineapple 356–357
Prunus spp. 251
tomato 327, 328–329
rootstock effects
citrus fruit 106
Prunus spp. 251
Rubus genus (blackberry and raspberry) 260, 262, 283–284
bioactive compounds 262–272
health benefits 272–282
pre- and postharvest period 282–283
salad vegetables see leafy vegetables and salads
salicylic acid, citrus trees sprayed with 107
Sapindaceae (lichii, longan and rambutan) 135–140
saponins 12, 18, 239
scurvy 1, 244
selenium compounds 9–10, 76
separation methods 136, 373
serotonin 354
shallot (Allium cepa Aggregatum group) 5, 8, 12, 18, 20
see also Allium genus
sight see vision
skin disorders 277, 282, 327, 362
Solanaceae 321, 336–339
see also peppers; potato; tomato
sour cherry (Prunus cerasus) 250
sour orange (Citrus aurantium) 97
see also citrus fruit
spinach (Spinacia oleracea) 174
bioactive compounds 180, 181, 182, 183, 185
health benefits 126, 175
squash (Cucurbita spp.) 120, 122, 128–129
see also cucurbits
starfruit (carambola, Averrhoa carambola) 145–146
steroids see glycoalkaloids; saponins; withanolides
sterols 34–35, 40
stilbenes
blueberry and cranberry 56, 62
grape 161, 162, 166
longevity studies (resveratrol) 394
strawberry 301–303
stomach cancer 322–323
storage 1–2, 371
Allium spp. 19, 20, 21
apple 207–209
aubergine 337
avocado 42–43
blueberry and cranberry 66–67
Brassica spp. 82–3
cape gooseberry 338
carrot 232–233
citrus fruit 107
cucurbits 126, 127, 128, 129
grape 166
leafy vegetables 185
mango 363
papaya 360
peppers 336
pineapple 357
potato 232
Prunus spp. 252
Ribes and Rubus spp. 282–283
strawberry 313–314
tomato 328–329
strawberry (Fragaria x ananassa) 291
bioactive compounds 292–304
genotype 291–292, 305
health benefits 57, 304–310, 314
pre- and postharvest period 298, 310–314
stroke 59, 63, 354
<table>
<thead>
<tr>
<th>Sugars</th>
<th>avocado 37–38, 40, 42-43</th>
<th>Exotic fruit 140, 142, 144, 146</th>
<th>Grape 161, 165</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur compounds</td>
<td>see organosulfur compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet cherry</td>
<td>(Prunus avium) see cherry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet melon</td>
<td>(Cucumis melo) 120, 121, 122, 126–128</td>
<td>see also cucurbits</td>
<td></td>
</tr>
<tr>
<td>Sweet orange</td>
<td>(Citrus sinensis) see orange</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet potato</td>
<td>(Ipomoea batalas) leaves 175, 182, 185</td>
<td>tubers 219, 227</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tannins</th>
<th>blueberry and cranberry 53–55, 59, 60, 61</th>
<th>Grape 155–156, 158–159, 162</th>
<th>Persimmon 145</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prunus spp. 247–248</td>
<td>Ribes and Rubus spp. 270</td>
<td>Strawberry 293–296, 301</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>Allium 5</th>
<th>Blueberry 51</th>
<th>Brassica 74</th>
<th>Citrus 90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cucurbits 118</td>
<td>Grape 154</td>
<td>Leafy vegetables and salads 172–173</td>
<td>Prunus 238</td>
</tr>
<tr>
<td></td>
<td>Ribes and Rubus 260, 262</td>
<td>Solanaceae 321, 329</td>
<td>Strawberry 291–292</td>
<td></td>
</tr>
<tr>
<td></td>
<td>see also cultivars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td>202, 203</td>
<td>TEAC assay (Trolox equivalence antioxidant capacity) 375, 382</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Teeth, prevention of caries 61–62, 164</td>
<td>Thrombosis see antithrombotic/antiplatelet activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thyroid deficiency 79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLC (thin layer chromatography)</td>
<td>373</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol (vitamin E)</td>
<td>36, 129, 176, 244–245</td>
<td>Tomato</td>
<td>321, 338</td>
<td></td>
</tr>
<tr>
<td>Tomato (Solanum lycopersicum)</td>
<td>321–322</td>
<td>Bioactive compounds 322–324</td>
<td>Cultivars 322, 327</td>
<td>Health benefits 324–327</td>
</tr>
</tbody>
</table>
passion fruit 143
peppers 330-331
persimmon 145
pineapple 355
pomegranate 140
Prunus spp. 243-245
Ribes and Rubus spp. 270-271
Sapindaceae family 138
strawberry 304
tomatillo 338
tomato 323-324, 327-328, 329

washing of leafy vegetables 184
water convolvulus (Ipomoea aquatica) 175, 178

watermelon (Citrullus lanatus) 120-121, 122, 125, 125-126, 129
see also cucurbits
weight management 230-231, 277, 307, 310
white currant (Ribes glandulosum) 261
see also Ribes genus
wine 156, 160
see also grape
withanolides 338

xanthones 142

yam (Dioscorea villosa) 227