CHAPTER 13

PRUNUS

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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 *Rosaceae* family as a subfamily, the *Prunoideae*. While some species do not yield edible fruits 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 (*Prunus persica* var. nectarina), European plum (*Prunus domestica*), Japanese plum (*Prunus salicina*), apricot (*Prunus armeniaca*), mume or Japanese apricot (*Prunus mume*), sweet cherry (*Prunus avium*), sour cherry (*Prunus cerasus*) and almond (*Prunus amygdalus*).

The fruit of these species is botanically defined 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 fruits 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 fruits 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 fruits within this group are by far peaches/nectarines and plums with an annual production around 17.5 and 9.7 million tons in 2007 (Table 13.1). Apricot production is around 3 million tons while almonds and cherries account for 2 million tons annually (Table 13.1). The main world producing countries of stone fruit include China, USA, Italy, Spain and Turkey. Serbia and Syria are also important producers for plums and almonds, respectively.

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

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 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 fruits 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, fruits 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 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 *et al*., 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 fruits and vegetables resulted in a significant increase of plasma antioxidants (Cao *et al*., 1998). The metabolites that have received more attention include ascorbic acid, tocopherols and tocotrienols, carotenoids and phenolics (Robards *et al*., 2003). It is currently recommended to have five to ten servings of fruits and vegetables daily (http://www.mypyramid.gov), and in the last several years there have been efforts to develop educational and promotional programs 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 last 20 years, the improvement in analytical techniques (mostly based on HPLC coupled with mass spectrometry detection) has allowed rapid progress in the identification and 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* fruits and seeds are commonly used for processing, including jam production, canning, drying or roasting and are regularly consumed year round. Black plum varieties are among fruits with the highest antioxidant capacity, and are very rich 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 due to their richness in antioxidants; *cf*. Chapter 14). Although peaches and nectarines do not rank top among fruits regarding 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 significantly contribute 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 fruits. Despite these general features, both the qualitative and quantitative profiles of these compounds vary markedly depending on the variety (Tomás Barberán *et al*., 2001; Dalla Valle *et al*., 2007; Díaz Mula *et al*., 2008, Vizzotto *et al*., 2007; 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 analyze the main factors affecting their levels.

13.2 Antioxidants

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

Figure 13.1. Total Oxygen Radical Absorbance Capacity (ORAC) of different fruits. The black columns highlight *Prunus* species (US Department of Agriculture, 2007a).

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 (Prior *et al*., 2003). 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 fruits. Plums are by far the stone fruits that rank on top, with ORAC values 6,239 μmol TE/100g. Black plum varieties have even higher ORAC than several fruits commonly recognized as antioxidant-rich fruits (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 cv. Blackamber showed highest antioxidant capacity followed relatively closely by cv. 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 (3,361 and 4,454 μmol TE/100g respectively). The ORAC reported for peach, nectarine and apricot are usually lower than that found in other *Prunus* species (Wu *et al*., 2004).

Table 13.2. Hydrophilic, lipophilic and total Oxygen Radical Absorbance Capacity (ORAC) of different *Prunus* species (adapted from US Department of Agriculture, 2007a and Wu *et al*., 2004).

In all cases it is clearly seen that the lipophilic contribution to total ORAC is low. This suggests that the relative relevance of phenolic compounds, ascorbic acid and possibly xantophylls in the antioxidant capacity of stone fruits 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 the real antioxidant contribution of these metabolites is underestimated is not known). Stone fruit 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 was always higher in the skin than in the flesh (Serrano *et al*., 2005; Díaz Mula *et al*., 2008). In peach, removal of 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 catalyzes the condensation of two molecules of geranyl-geranyl pyrophosphate to form phytoene, a colourless carotenoid (Gross, 1991). After that, double bonds are sequentially added 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 is usually 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 in 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 *et al*., 2001). Humans depend on dietary carotenoids for making their retinoids which are crucial for normal vision (Kopsel and Kopsel, 2006). Structurally, vitamin A (retinol) is basically a half molecule of β-carotene with an extra molecule of water and retinoids are generated upon 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 either 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 β-carotene/12 + μg other pro-vitamin A carotenoids/24) to account for differences in 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 upon consumption besides lycopene and β-carotein are 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, the nutritional status of the ingesting host as well as the presence of other components 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 xantophylls present in most *Prunus* fruits 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 (Rodríguez-Amaya, 2001; Tourjee *et al*., 1998; USDA 2009). Within this group, apricots show the highest levels of carotenoids and provitamin A followed by cherry (Table 13.3). Peaches, nectarines and plums have a lower concentration of carotenoids. However, the variation among varieties is large. For instance, studying eight plum varieties Díaz Mula *et al*. (2008) found fourfold variations in total carotenoid content. In apricot, Ruiz *et al*. (2005) found that total carotenoid content ranged from 1,500 to 16,500 μg /100 g depending on the variety being considered. Carotenoids are not evenly distributed in the food itself and various investigators found that they are 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).

Table 13.3. Carotenoid content in *Prunus* species. (US Department of Agriculture, 2009).

Though it has been suggested that breeding programmes might consider 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 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 and it is highly important for the *de novo* synthesis of bone, cartilage, and wound healing). The disorder associated with vitamin C deficiency is known as scurvy. Scurvy leads to the formation of liver spots on the skin, spongy gums, and bleeding from all mucous membranes. The compounds accounting for vitamin C activity present in foods include ascorbic acid and its initial oxidation product dehydroascorbic acid (which can be reduced back in the human body) (Lee and Kader, 2000). Besides its role in some enzymatic reactions, ascorbic acid is also a potent antioxidant, protecting the cell against reactive oxygen species (Halliwell and Gutteridge, 1995; Noctor and Foyer, 1998). Currently a dietary daily intake of 90 mg of vitamin C is recommended. Ascorbic acid is water soluble and highly available, but is also one of the bioactive compounds more susceptible to degradation (Rickman *et al*., 2007). Plants are able to synthesize vitamin C through sequence steps which convert L-galactose or galacturonic acid to ascorbic acid (Valpuesta and Botella, 2004; Smirnoff, 2000). Its concentration in fruits, vegetables and nuts varies from 1 to 150 mg/100 g (Lee and Kader, 2000).

Prunus species are not particularly rich in ascorbic acid. They range from negligible levels in almond to around 10-15 mg/100 g in most stone fruits (Table 13.4). In some products such as broccoli and pepper ascorbic acid seems to be one of the compounds contributing most to antioxidant capacity, but in peach it only accounts for 0.8% (Sun *et al*., 2002).

Table 13.4. Ascorbic acid content in *Prunus* species (US Department of Agriculture, 2009).

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 act 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 to other fruits and vegetables. α-tocopherol content in *Prunus* fruits varies from 0.07 to 26 mg/100g (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).

Table 13.5. Vitamin E content in *Prunus* species (US Department of Agriculture, 2009).

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 *et al*., 2003). Phenolics greatly contribute to the sensory qualities (colour, flavour, taste) of fresh fruits and their products (Kim *et al*., 2003a&b). Astringency is generally recognised 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 *et al*., 2007). Phenolic compounds have been associated with ecological functions. Some benefits associated with phenolic compounds 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 free radicals formation by chelating copper and iron. Finally, they can regenerate other antioxidants such as tocopherol (McAnlis *et al*., 1999). Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet (Balasundram *et al*., 2006). Some *Prunus* fruits such as red and black plum varieties, cherries and almonds are very good sources of phenolic compounds (Vinson *et al*., 2001; Wu *et al*., 2003) (Table 13.6). Sour cherries were reported to have higher level of total phenolics than sweet cherries (Kim *et al.*, 2005). Peach, nectarines and apricot have lower total phenolics as measured with the Folin-Ciocalteau reagent. However, wide inter-varietal differences exist (Tomás Barberán *et al*., 2001). Pinelo *et al*., (2004) suggested that relatively high levels of phenolic antioxidants may be recovered for further use from almond hulls which represent around 4.5% of total hull weight (Shahidi, 2002). The peels have also been found to contain higher amounts of phenolics than other fleshy parts (Balasundram *et al*., 2006).

Table 13.6. Total phenolics (TP) in *Prunus* species (adapted from US Department of Agriculture, 2007a and Wu *et al*., 2004).

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

Phenolic compounds might be divided in groups differently represented depending on the species considered. Here, we summarize the main characteristics of phenolic acids, flavonoids and 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 compared to 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 fruits, with the highest concentrations typically in the outer parts of ripe fruits. 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 (Möller and Herrmann, 1983). In cherries another isomeric form is the most abundant (3-CQA) (Herrmann, 1989). Relatively high amounts of 4-CQA is characteristic of prunes. Interestingly, besides its role as an antioxidant, it has been observed that chlorogenic and caffeic acids are two major phenolic acids in the epidermis and subtending cell layers of peach and that their concentrations 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, with 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 6,000 flavonoids have been identified. They are classified into at least 10 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

Their name 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 largely contribute to the visual quality of fruits (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, they are mainly associated 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.

Table 13.7. Flavonoids in *Prunus* species (US Department of Agriculture, 2007b).

Flavones, flavonoles, flavanones and flavan-3ols

The main flavones in the diet are apigenin and luteolin. In *Prunus* species they are found at low concentration in red plum varieties. Among flavonols, quercetin is the compound most commonly found 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. They are almost exclusively located in the peel (Tomás Barberán *et al*., 2001). Flavanones are typical of citrus but eriodictyol and naringenin are also present in almond nuts. Finally flavan-3ols including (+)-catechin and (⁻)-epicatechin and their gallic acid esters show six times higher levels in black plums than in most other *Prunus* fruits which have comparable 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 *Prunus armeniaca* roots were suggested to limit germination and growth of surrounding species (Rawat *et al*., 1999). Polymers of catechin and epicatechin are the most common PAs in foods. Colonic flora could metabolize these compounds producing phenolic acids (Depréz *et al*., 2000). PAs have received increasing attention due to evidence associating their action with some health benefits (Lunder, 1992; 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 fruits as opposed to PAs from other *Prunus* species which show lower mean molecular mass (Table 13.8).

Table 13.8. Proanthocyanidins in *Prunus* species (US Department of Agriculture, 2004).

Ellagitannins (ETs) are a subgroup of tannins (Hemmer, 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 which exert a medicinal effect on human health through the prevention and progression of chronic diseases. Extracts from fruits 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, Alzheimer, metabolic syndrome and others (Sun *et al.*, 2002; Jiang and Dusting, 2003; Kottova *et al.*, 2004; Heo and Lee, 2005; Chen *et al.*, 2007; Chen and Blumberg, 2008b). 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, studies conducted with 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 hyperlipidemic 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, anti-hepatotoxicity effects and immunity boosting properties (Puri, 2002) and a review on almond nutraceutical properties has been recently published (Shahidi *et al.*, 2008).

Studies with peach and plums have been conducted to determine their effects against cancer, mitigation of cognitive deficits and anti-hepatotoxicity effects. On the other hand, nectarines and apricots still await to be characterized for their nutraceutical properties.

Peach and plum phenolic compounds have been shown to inhibit growth and induction of differentiation of colon cancer cells (Lea *et al.*, 2008). Furthermore, studies with plum extracts (dried plums) have shown to alter several risk factors related to colon carcinogenesis in rats, including a reduction of fecal total and secondary bile acids concentrations, decrease of colonic β-glucoronidase and 7α-dehydroxylase activities and an increase in antioxidant activity. The effect was associated with the phytochemical content (Yang and Gallaher, 2005). More recently, chlorogenic acid and neo-chlorogenic acid in plum and peach fruit have been identified as bioactive compounds with potential chemopreventive properties against an oestrogen independent breast cancer cell line whilst having little effect on normal cells (Noratto *et al.*, 2009). On the other hand, a study conducted with plum juice (*Prunus domestica* L.) was effective in mitigating cognitive deficits in aged rats and this effect was associated with the amount of phenolics supplemented (Shukitt-Hale *et al.*, 2009). In addition, studies with *Prunus persica* pericarp extracts were conducted against cisplatin-induced acute toxicity in mice. Results showed a significant protection against the acute nephrotoxicity and hepatotoxicity likely by reducing cisplatin-induced oxidative stress in mice (Lee *et al.*, 2008). Similar results were observed with *Prunus persica* flesh (Lee *et al.*, 2007). Likewise, studies with methanolic extracts of immature plum fruit (*Prunus 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 which is the primary cytochrome P450 involved in the metabolism and bioactivation of B(α)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 ~ 6.3). Using cholestyramine (bile acid binding, cholesterol lowering drug) as a reference indicated that binding potential follows the order peach > apricots > nectarines. 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 have been reported in the areas of metabolic syndrome, cancer, inflammation, and studies with neuronal cells. For example, studies have shown that cherry enriched diets reduce metabolic syndrome and oxidative stress in rats (Seymour *et al.*, 2007, 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 risk factors that are associated with risk for metabolic syndrome and Type 2 diabetes in rats, by altering hyperlipidemia, hepatic steatosis and hepatic peroxisome proliferator-activated receptors in rats (Seymour *et al.*, 2008). In clinical studies, it was shown that consumption of cherries lowers plasma urate in healthy women (Jacob *et al.*, 2003). This has important implications, since high levels of plasma urate have been associated to cardiovascular disease, diabetes and the metabolic syndrome.

Studies with anthocyanin-rich tart cherry extracts have shown inhibition of intestinal tumorogenesis in mice (Bobe *et al.*, 2006) and anti-proliferation activity against human colon cancer cells (Kang *et al.*, 2003). Tart, sweet and sour cherries have shown cyclooxygenase enzyme inhibitory activities (Mulabagal *et al.*, 2009; Seeram *et al.*, 2001; Wang *et al.*, 1999) and suppress inflammation pain behaviour in rat has been shown by tart cherry anthocyanin intake (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 nutraceutical properties of *Prunus* species as well as the different genotypes involved in each type of fruit since the phytochemical makeup may vary as mentioned earlier in this chapter. In addition, novel approaches may be used as tools for bioactive compound discovery and mechanisms of action such as 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 in 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 genetics factors, cultural practices, environmental conditions and storage-processing treatments.

13.5.1 Genetic factors

As previously shown, large differences in total antioxidants, phenolics and carotenoids 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 10-fold differences 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 (Rupashige *et al*., 2006). The plum cvs. Black Beauty and Angeleno were especially rich in phenolics (Tomás Barberán *et al*., 2001). In peach, fruit showing higher 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 and 1997). In contrast, the influence on fruit bioactive compounds is quite variable. In some cases, no pronounced differences were observed (Drogoudi *et al*., 2007). On the other hand, some works reported that the levels of phytochemicals might be significantly influenced by rootstock (Giorgi *et al*., 2005; Remorini *et al*., 2008).

13.5.2 Cultural practices and environmental conditions

The accumulation of bioactive compounds is greatly affected 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 proceeds, ascorbic acid levels drop and then progressively increase to reach maximum content at full maturity (Serrano *et al*., 2005).

Table 13.9 Change in ascorbic acid content in some *Prunus* species during ripening (adapted from Lee and Kader, 2000).

Carotenoid concentration also increases steadily during ripening in most stone fruit (Katayma *et al.*, 2006; Díaz Mula *et al*., 2008). Besides its contribution for full colour development, enzymatic carotenoid degradation of nectarines was shown to play a role in C-13 norisoprenoid aroma compounds 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*., 2008a). Plum colour development is also directly related to increased anthocyanidin content (Díaz Mula *et al*., 2008; Usenik *et al*., 2008b) 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 *et al*., 1993). Phenylalanine ammonia lyase, chalcone synthase, and dihydroflavinol reductase enzymes involved in phenylpropanoid metabolism are induced by UV radiation (Tomás-Barberán and Espin, 2001). Temperature 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 on fruits and vegetables are variable. Organic peaches showed higher content of phenolic and ascorbic acid than conventionally produced fruits (Carbonaro *et al*., 2002; Carbonaro and Mattera, 2001). Ascorbic acid, vitamin E and β-carotene were higher in organic plums grown on soil covered with natural meadow, while total phenolics content was higher in conventional plums (Lombardi-Boccia *et al*., 2004). Bourn and Prescott (2002) concluded that further studies are required to fully understand the influence of inorganic and organic fertilizers on the nutritional value of crops. Winter and Davis (2006) affirmed that it is not possible to ensure that organically grown products are nutritionally superior to those obtained by conventional agricultural techniques.

13.5.3 Storage-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 fruits is minimal compared to that of phenolic compounds. Several reports showed no reductions or even increased levels of phenolic compounds were observed during 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 even increases in total phenolics were found during storage (Díaz 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 fruits susceptible to enzymatic browning, and this is a major problem encountered during handling and processing (Brandelli and Lopes, 2005). Improper storage conditions in many cases can lead 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 the nutritional quality of fruits 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 compounds 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), with 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 other processing operations (Rickman *et al*., 2007).

Preservation methods are usually 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, almonds industrial drying (oven drying) and roasting almond skins increased (two-fold) the contents of phenolic compounds. In prunes, although drying results in marked losses of ascorbic acid and anthocyanins, total antioxidant capacity might 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 rapidly increasing. Accumulating evidence suggests that having 5-10 daily servings of fruits 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 is lacking.

Fresh *Prunus* species are significant contributors of bioactive compounds to the diet during spring and summer season. 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 better with the level of phenolics suggesting that this is the predominant group. Plums rank on top among *Prunus* species in antioxidant capacity and black varieties have ORAC values close to those observed in fruits richest in antioxidants such as black currants 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 with dietary 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 showing usually 2-40 fold higher content of these substances. 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 formation of new metabolites and a concentration affect. Consequently, intake of all forms of fruits 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 within fruits 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 compounds protection;
- study bioactive compounds bioavailability, metabolism, dose/response and toxicity;
- start evaluating the interactions among bioactive compounds, since they are almost exclusively consumed in combinations;
- generate guidelines and protocols to bring some order related to the analytical determination of antioxidant capacity on foods (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 programs, 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, positive attributes associated with bioactive compounds in fruits in general, and in *Prunus* species in particular, provide an opportunity that might be capitalized upon.

13.8 References

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