13 Physiology of Metabolites

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13.1 Introduction

The color, flavor, and nutritive value of pepper (*Capsicum* spp.) can be attributed to an array of different metabolites. Carotenoid pigments are responsible for generating color changes during fruit ripening; and a cocktail of organic acids and capsaicinoids (secondary metabolites found exclusively in *Capsicum*) yields the characteristic pungency of peppers. A significant component of the human diet in many regions of the world, peppers provide vitamins A and C and are a source of antioxidants. Among various plant metabolites that can help protect against free radical damage, phenolics (including flavonoids and capsaicinoids), vitamin C, and carotenoids are the major antioxidants produced in *Capsicum* species.

The study of pepper metabolites is rapidly expanding as interest in enhancing crop plant quality rises. Recent research elucidates how levels of these compounds depend upon the genotype fruit development, and stage of growing conditions.

13.2 Antioxidants

Antioxidant compounds protect macromolecules from free radicals, e.g. reactive oxygen species (ROS). Free radicals are unstable, highly reactive compounds produced as the result of normal aerobic metabolism and in response to stress. If not neutralized by endogenous or exogenous antioxidants, reactive species can damage nucleic acids, proteins, and lipids, which can hasten aging and the onset of diseases including cancer, heart disease, atherosclerosis, and cataracts. Although the human body can produce a variety of antioxidant compounds, these must be supplemented from the diet. Pepper is an excellent source of antioxidants including flavonoids, capsaicinoids, vitamin C, and carotenoids. While it is possible to measure contents of individual compounds with antioxidant activity, an assessment of the total antioxidant potential of fruits and vegetables is valuable because it takes into account synergistic interactions among different antioxidants. Assays to determine antioxidant activity of pepper extracts differ in mechanism and in the type of free radicals generated. Depending on the solvent used (water, methanol/water, or acetone/water), antioxidant activity values may be specific for hydrophilic or lipophilic compounds, or may represent total antioxidant capacity. Many studies do not make these distinctions. Because different antioxidants have distinct modes of action, it is nearly impossible to directly compare antioxidant activity values across measurement techniques.

When compared with other vegetables, pepper ranks high for antioxidant activity. Using the ferric reducing antioxidant power assay, researchers found that chilli and red bell pepper (both *C. annuum*) ranked in the top three when compared with other common vegetables (Halvorsen *et al.*, 2002; Ou *et al.*, 2002; Pellegrini *et al.*, 2003). Similar rankings were observed with trolox equivalent antioxidant capacity, total oxyradical scavenging capacity, and oxygen radical absorbance capacity assays (Chu *et al.*, 2002; Ou *et al.*, 2002; Pellegrini *et al.*, 2003; Chun *et al.*, 2005). When antioxidant activity was measured in terms of total radical-trapping antioxidants (Pellegrini *et al.*, 2003), chilli and red pepper were two of the top ten sources of antioxidant capacity.

Capsicum annuum is a morphologically diverse species with a variety of types which have different culinary uses. In a survey of 29 Turkish *C. annuum* cultivars, Frary *et al.* (2008) found that sivri (long, pointed) peppers had significantly higher antioxidant content than dolmalik (stuffing), çarliston (Charleston-type), and salçalik (paste) peppers. Although it has been suggested that capsaicinoids have antioxidant activity, there was no significant difference between pepper cultivars classified as hot or sweet. Antioxidant capacity variation is also apparent between genotypes (Deepa *et al.*, 2006; Guil-Guerrero *et al.*, 2006; Frary *et al.*, 2008). Pepper species including *C. frutescens* (Oboh and Ogunruku, 2010), *C. chinense* habanero type (Oboh *et al.*, 2007; Menichini *et al.*, 2009), *C. pubescens* (Oboh and Rocha, 2007, 2008), and *C. baccatum* var. *pendulum* (Kappel *et al.*, 2008) were assayed for antioxidant activity. However, comparisons for antioxidants among multiple species, and genotypes, of non-*C. annuum* species are limited. Such studies will be valuable as more "exotic" pepper types grow in popularity and are of increased interest to consumers.

Regardless of the method used, or the species studied, researchers found that antioxidant capacity of pepper fruit almost always increases with maturity (Howard *et al.*, 2000; Ou *et al.*, 2002; Wu *et al.*, 2004; Guil-Guerrero *et al.*, 2006; Navarro *et al.*, 2006; Conforti *et al.*, 2007; Deepa *et al.*, 2007; Kwon *et al.*, 2007; Matsufuji *et al.*, 2007; Sun *et al.*, 2007; Chuah *et al.*, 2008; Oboh and Rocha, 2008; Flores *et al.*, 2009a; Gorinstein *et al.*, 2009; Serrano *et al.*, 2010). Exceptions were observed when antioxidant content was analyzed with lipid peroxidation assays (Conforti *et al.*, 2007; Oboh *et al.*, 2007). The profile of antioxidants may alter during maturity such that compounds present in mature fruit are less efficient at preventing lipid peroxidation. Depending on variety, peppers may be one of several colors including red, orange, or yellow when fully mature. When the antioxidant capacity of different colored pepper fruit is compared, red peppers usually rank first (Ou *et al.*, 2002; Kwon *et al.*, 2007; Matsufuji *et al.*, 2007; Sun *et al.*, 2007). However, with some cultivars, orange peppers have higher antioxidant potential than red peppers (Wu *et al.*, 2004; Chuah *et al.*, 2008), probably reflecting genotypic differences among cultivars.

The literature suggests that conditions of growth have little or no effect on fruit antioxidant activity with no differences among organic, low-input, conventional, and soilless culture (Ren *et al.*, 2001; del Amor, 2007; del Amor *et al.*, 2009; Flores *et al.*, 2009b). However, Flores *et al.* (2004) found that calcium fertilization was associated with increased hydrophilic antioxidant content in pepper. Salinity level caused increased antioxidant activity in red peppers (Navarro *et al.*, 2006), but treatment with methyl jasmonate or ethylene and wounding did not (Heredia and Cisneros-Zevallos, 2009).

13.3 Carotenoids

The carotenoids are a family of yellow, orange, and red pigments synthesized in plants, fungi, bacteria, and algae. Carotenoids are produced in chloroplasts and chromoplasts and serve as accessory pigments in photosynthesis, attractants facilitating pollen and fruit dispersal, and as free radical scavengers protecting the photosynthetic apparatus against photo-oxidizing effects of light. In the human diet, carotenoids have beneficial antioxidant and provitamin A activities.

Over 30 different carotenoid pigments have been identified in pepper fruit (Deli *et al.*, 2001;

Gnayfeed *et al.*, 2001; Stommel and Griesbach, 2008). These include β-carotene and a range of xanthophylls, i.e. lutein, zeaxanthin, violaxanthin, cucurbitaxanthin A, and β-cryptoxanthin. Of these, only β-carotene and β-cryptoxanthin possess provitamin A activity. Oxidative cleavage of these compounds yields retinal. Levels of β-carotene in many varieties of fresh (100 g) and dried (10 g) peppers is sufficient to meet 100% of the recommended daily amount (RDA) for vitamin A in adults (Wall *et al.*, 2001). The main carotenoids responsible for the color of red fruits are three xanthophylls unique to *Capsicum*, i.e. capsanthin, capsorubin, and capsanthin 5,6-epoxide. While these oxygenated carotenoids lack provitamin A activity, they are effective antioxidants and a source of red pigments. Carotenoid extracts (oleoresins) are used as a coloring agent by the food, cosmetics, and pharmaceutical industries.

Carotenoid content is an indicator of nutritional value and quality in pepper, and the intensity of red pigmentation is a key trait in certain forms including jalapeño and paprika. Peppers high in β-carotene are especially desirable, for their provitamin A and antioxidant content. Significant variability exists among species and cultivars in levels of total and individual carotenoids (Wall *et al.*, 2001; Russo and Howard, 2002; Deepa *et al.*, 2006; Guil-Guerrero *et al.*, 2006; Suzuki *et al.*, 2007; Rodriguez-Burruezo *et al.*, 2009). However, comparisons between these results of different studies are difficult to make because carotenoid content is a function of genetic factors as well as physiological, developmental, and environmental conditions. Slight differences in methodology, harvest time, and growing conditions can significantly impact carotenoid values.

Wall *et al.* (2001) assessed 57 varieties and accessions of peppers across six species (*C. annuum*, *C. baccatum*, *C. chacoense*, *C. chinense*, *C. eximium*, and *C. frutescens*) and found a direct correlation between β-carotene concentration and total carotenoids. Fresh fruit of cvs Greenleaf Tabasco, Pulla, Guajillo, NuMex Conquistador, Ring-O-Fire, and Thai Dragon contained levels of β-carotene in excess of the RDA for vitamin A (per 100 g FW). Among dried types, fruit of New Mexican, aji, pasilla, ancho, and guajillo had the greatest amount of β-carotene. Twenty-three aji (*C. baccatum*), eight rocoto (*C. pubescens*), and three control (*C. annuum*) accessions were analyzed for total red (capsanthin and capsorubin) and total yellow (β-carotene, β-cryptoxanthin, and zeaxanthin) carotenoids (Rodriguez-Burruezo *et al.*, 2009). The greatest concentration of red carotenoids was found in the *C. annuum* accessions; *C. baccatum* had noteworthy levels and could be valuable breeding material. A survey of β-carotene content in 63 accessions of *C. chinense* revealed significant variability and helped to identify accessions that could be a useful source of antioxidants (Antonious *et al.*, 2009b).

Total carotenoid levels in fruit of a given cultivar can vary depending on developmental stage/fruit maturity and growing conditions. Fruit maturity is perhaps the most important of these factors. Carotenoid biosynthesis is upregulated during ripening and in red-fruited genotypes, a 6- to 90-fold increase in fruit total carotenoid concentration can result (Deli *et al.*, 2001; Ha *et al.*, 2007; Menichini *et al.*, 2009). A large shift in the fruit carotenoid profile and/or composition occurs as green chloroplasts in immature fruit differentiate into red chromoplasts of ripe fruit. The main carotenoids in unripe fruit are lutein, β-carotene, and violaxanthin. During maturation, lutein levels decline and β-carotene, β-cryptoxanthin, and zeaxanthin increase. As fruit reach the breaking stage of color development, the red carotenoids, capsanthin and capsorubin, are produced (Deli *et al.*, 2001; Gnayfeed *et al.*, 2001; Marin *et al.*, 2004; Niizu and Rodriguez-Amaya, 2005; Ha *et al.*, 2007). Capsanthin levels continue to increase as fruit ripen; concentrations increase six-fold during the transition from orange to red (Huh *et al.*, 2001). β-carotene is another component of ripe fruit, and provitamin A activity may differ 3- to 15-fold between green and red fruit (Wall *et al.*, 2001). However, capsanthin is the predominant pigment of red ripe fruit, comprising between 37 and 80% of total carotenoid content (Deli *et al.*, 2001; Maoka *et al.*, 2001; Ha *et al.*, 2007; Topuz and Ozdemir, 2007).

Carotenoid content is also a function of fruit color. Depending on variety and stage of ripeness, pepper fruit show a range of coloration, including green, white, yellow, orange, red, purple, and brown. A survey of red, orange, yellow, and green *C. annuum* varieties revealed over 200-fold variation in total carotenoid levels (Guil-Guerrero *et al.*, 2006), with the lowest levels in green cultivars and the highest in red. Rodriguez-Burruezo *et al.* (2009) found a complete absence of red carotenoids (capsanthin and capsorubin) in *C. pubescens* and *C. baccatum* pepper accessions with yellow or orange mature fruit. In 12 varieties representing four species (*C. annuum*, *C. baccatum*, *C. chinense*, and *C. frutescens*) and a range of ripe fruit colors, Ha *et al.* (2007) found a direct correlation between capsanthin content and color value in fully mature fruit, such that varieties could be ranked: deep red>red>light red>orange>pale yellow orange. No increases in total carotenoids were detected during maturation of non-red pepper varieties (Ha *et al.*, 2007), illustrating the role genotype plays in carotenoid production.

Growing conditions and crop production practices can have substantial effects on carotenoid content. While greenhouse-grown peppers typically have higher levels than field-grown plants (Russo and Howard, 2002; Lee *et al.*, 2005), this is not always the case (Rodriguez-Burruezo *et al.*, 2009). Variability can be attributed to the influence of light on pigment biosynthesis; it is hypothesized that elevated light levels in the field (Rodriguez-Burruezo *et al.*, 2009) or greenhouse (Russo and Howard, 2002) actively stimulate carotenoid synthesis as a defense against photo-oxidation. Low irrigation frequency increased total carotenoids by 30% and provitamin A carotenoids by 15% in red fruit (Marin *et al.*, 2009). Salinity, however, seems to have no effect on pepper carotenoid content (Navarro *et al.*, 2006; Marin *et al.*, 2009). Mineral nutrients have variable effects, depending on genotype and production system. Addition of Ca^{2+} and NO_3^- to hydroponically grown pepper plants caused increases in carotene concentration, whereas K^+ treatments had no effect (Flores *et al.*, 2004). In an experiment using doses of Ca^{2+} and K^+ at comparable levels, only Ca^{2+} at low doses (2 and 4 mmol l^{-1}) enhanced carotenoid production (Marin *et al.*, 2009). In contrast, mineral fertilization of peppers grown in soil did not affect carotenoid content (Flores *et al.*, 2009a).

For peppers grown under organic, integrated (low-input), and conventional greenhouse conditions comparisons are similarly variable. Under organic greenhouse conditions, Perez-Lopez *et al.* (2007a, b) found higher levels of total carotenoids, but del Amor (2007) found decreased β-carotene concentrations. Soilless (hydroponic) production systems yielded red fruit with significantly higher total carotenoid content than organic or low-input practices, leading to speculation that lower abiotic stress levels facilitate carotenoid accumulation over phytoalexin synthesis (Marin *et al.*, 2008). Application of nitrophenolates (compounds suspected of promoting auxin synthesis) (Serrano *et al.*, 2010) and salicylic acid (a plant hormone involved in stress responses) (Elwan and El-Hamahmy, 2009) produced elevated carotenoid levels.

13.4 Phenolic Compounds

Phenolic compounds are widespread in plants and interest in these metabolites is increasing because of their antioxidant properties. Plant phenolics include simple phenols, phenolic acids, hydroxycinnamic acid derivatives, and flavonoids. Flavonoids are the largest class of phenolic compounds and are treated separately in this chapter. In plants, phenolics are important for growth, signaling, defense, and reproduction (Dennis *et al.*, 1997). Phenolics include pigment and flavor compounds which act as attractants for flower pollination and seed dispersal. Although it is possible to extract, quantify, and identify individual phenolic compounds in plants, the process is expensive and somewhat laborious. In contrast, relatively simple assays have been devised for determining total phenolic content of samples.

Pepper usually ranks first or second in phenolic content with greater levels than found in spinach, broccoli, and garlic (Gunduc and El, 2003; Wu *et al.*, 2004; Chun *et al.*, 2005; Kevers *et al.*, 2007). Green bell peppers had 1.6-fold more total phenolics than spinach and two-fold more than broccoli (Chun *et al.*, 2005). A sample of Turkish pepper germplasm varied for phenolic content, with süs (literally "fancy," used for pickling) and sivri types having the highest levels (Frary *et al.*, 2008). Pungent types had more phenolic compounds than sweet types, an expected result given that pungency is due to capsaicinoids, which are phenolic compounds. The greatest diversity for phenolics was in sivri (e.g. "Ayaş," "Sera Demre") and dolmalik (e.g. "Domat Biberi," "Kale") types. In a study of Mexican peppers, green poblano and habanero types had the highest and lowest phenolic contents, respectively (Ornelas-Paz *et al.*, 2010), despite the fact that habanero types are usually more pungent than poblano peppers. Deepa *et al.* (2007) found six-fold variation for phenolic content in ten genotypes of sweet pepper when measured on a dry weight basis. Materska and Perucka (2005) found variability for individual phenolic compounds in two hot and two semi-hot *C. annuum* cultivars. When pepper species were compared, *C. chinense* and *C. baccatum* had significantly more phenolics than *C. annuum*, *C. frutescens*, and *C. pubescens* accessions (Antonious *et al.*, 2006; Rodriguez-Burruezo *et al.*, 2009). Oboh *et al.* (2007) and Ornelas-Paz *et al.* (2010) noted that habanero peppers (*C. chinense*) ranked below *C. annuum* types like "Tepin" and poblano for total phenolic content. However, Antonious *et al.* (2009b) noted considerable variation within *C. chinense* for phenolic content, with accessions from Mexico and the USA ranking highest. Surveys of genotypic variation are useful for identification of breeding materials for improved antioxidant content in pepper. Rodriguez-Burruezo *et al.* (2009) studied 23 cultivars of *C. baccatum* and found that some cultivars had high nutritive value because of their high phenolic content.

Phenolic content of pepper fruit increases with maturity (Howard *et al.*, 2000; Wu *et al.*, 2004; Materska and Perucka, 2005; Deepa *et al.*, 2007; Kwon *et al.*, 2007; Oboh and Rocha, 2007, 2008; Perez-Lopez *et al.*, 2007a; Sun *et al.*, 2007; Chuah *et al.*, 2008; Flores *et al.*, 2009a; Gorinstein *et al.*, 2009; Serrano *et al.*, 2010). However, when ripe peppers are compared, there is some disagreement among results. Studies found that phenolic content of red peppers is greatest, followed by orange and yellow peppers (Sun *et al.*, 2007; Chuah *et al.*, 2008). Other work indicates yellow peppers are richest in phenolics, followed by orange and red peppers (Wu *et al.*, 2004; Kwon *et al.*, 2007), suggesting that genotypic variation is an important determinant of phenolic content. A few studies suggest there is no change in phenolic content during maturation (Fox *et al.*, 2005; Navarro *et al.*, 2006) or that immature green fruit have more phenolics than ripe fruit (Marin *et al.*, 2004; Conforti *et al.*, 2007; Oboh and Rocha, 2007; Kappel *et al.*, 2008; Menichini *et al.*, 2009). These seemingly contradictory findings may reflect genotypic differences. Moreover, as many compounds contribute to total phenolic content, it is not surprising that the results of these studies may disagree.

In general, growth conditions do not have significant effects on levels of phenolics in pepper fruit. For organic, low-input, conventional, and soilless culture, no differences in total phenolic content were observed (Flores *et al.*, 2004, 2009a, b; Chassy *et al.*, 2006; Marin *et al.*, 2008, 2009). However, in a few studies phenolic content increased when plants were grown organically (Perez-Lopez *et al.*, 2007b; del Amor *et al.*, 2008b) or with low nitrogen (del Amor *et al.*, 2008a) or high calcium (Marin *et al.*, 2009). Del Amor *et al.* (2009) attributed higher levels of phenolic compounds to a possible defense response of organically grown plants to pathogens. However, other types of stress including salinity (Navarro *et al.*, 2006; Marin *et al.*, 2009), wounding, and wounding plus ethylene or methyl jasmonate treatment (Heredia and Cisneros-Zevallos, 2009) did not affect phenolic content.

13.5 Capsaicinoids

Capsaicinoids are a group of phenolic alkaloids specific to the genus *Capsicum* and comprised of a vanillyamine head and a fatty acid tail. Synthesized in the fruit placenta, capsaicinoids are responsible for the unique pungency of hot peppers. However, not all members of the genus accumulate capsaicinoids, suggesting that it is a derived trait (Tewksbury *et al.*, 2006). Capsaicinoids bind to a thermoreceptor to cause a sensation of heat and pain in mammals and serve as a natural defense against herbivory. Birds, the natural dispersal agents of wild-grown peppers, are unaffected by these compounds (Tewksbury and Nabhan, 2001).

More than 20 different capsaicinoids have been identified in hot peppers (Schweiggert *et al.*, 2006). Capsaicin and dihydrocapsaicin are the most pungent, and together these two compounds typically contribute more than 80% of the total capsaicinoid content (Topuz and Ozdemir, 2007). While domestication and variety improvement have reduced pungency in some peppers (especially bell types) (Paran and van der Knapp, 2007), capsaicinoid content is a major quality factor in spice (chilli, tabasco, and paprika) peppers. In addition to their use as seasonings, peppers containing capsaicinoids have been employed medicinally for their antioxidant and antimicrobial properties; and capsaicin's ability to desensitize pain receptors has led to its application as a topical analgesic (Materska and Perucka, 2005).

Considerable variability for capsaicinoids exists among pepper fruit depending on genotype, developmental stage, and environmental conditions (Estrada *et al.*, 2000; Gnayfeed *et al.*, 2001; Zewdie and Bosland, 2001; Antonious *et al.*, 2006; Deepa *et al.*, 2007; Barrera *et al.*, 2008). More than three-fold variability in capsaicinoid content was detected in individual fruit harvested from a single plant (Kirschbaum-Titze *et al.*, 2002), and in fruit of the same age and position on a given plant (Mueller-Seitz *et al.*, 2008). It can be difficult to make meaningful comparisons among results obtained by different researchers; nevertheless, trends exist. Capsaicin levels are generally higher than those of dihydrocapsaicin (Antonious *et al.*, 2009a) and there is a direct correlation between total capsaicinoid levels and pungency (as measured in Scoville heat units). Capsaicin and dihydrocapsaicin are not detectable in sweet pepper varieties but significant amounts of these compounds are present in chilli and paprika types (Kozokue *et al.*, 2005; Garces-Claver *et al.*, 2006). Cultivars of *C. chinense* habanero type, considered the hottest of peppers, contain the highest levels of capsaicin, with a 1.6-fold range of variability in capsaicin content (Pino *et al.*, 2007).

A comparison of 17 accessions representing four species of *Capsicum* revealed a 650-fold range in the total capsaicinoids (capsaicin plus dihydrocapsaicin) concentration; however, no correlation was found between capsaicinoid content and fruit weight, or levels of other antioxidant compounds measured (Antonious *et al.*, 2006). A similar level of variability was seen in a comprehensive survey of *C. chinense* accessions from eight countries. In that study, fruit from plants originally collected in Mexico, the USA, and Brazil were the most pungent among those examined (Antonious *et al.*, 2009a). Capsaicinoid profiles, based on the measurement of seven different compounds, were generated for species of *C. annuum*, *C. baccatum*, *C. chinense*, *C. chacoense*, *C. frutescens*, and *C. pubescens* in order to assess taxonomic relationships (Zewdie and Bosland, 2001). Unfortunately, the extent of variability among accessions precluded use of this information to identify species. These researchers found a number of accessions (particularly in *C. pubescens*) in which capsaicin and dihydrocapsaicin were not the two principal capsaicinoids.

The accumulation of capsaicinoids during maturation of pepper fruit has been examined. While variability exists between genotypes, capsaicinoid synthesis generally begins within 1 week after fruit set and continues as placenta tissues develop. The compounds reach maximum levels approximately 40–50 days after fruit set (Barrera *et al.*, 2008; Mueller-Seitz *et al.*, 2008). Mueller-Seitz *et al.* (2008) observed that the profile of the three main capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) remained fairly uniform throughout fruit development. Upon maturation, capsaicin levels in most cultivars show a noticeable though variable rate of decline associated with an increase in the activity of peroxidase enzymes (which oxidize the capsaicinoids) in ripe fruit (Estrada *et al.*, 2000; Deepa *et al.*, 2007).

While developmental stage and genotype play large roles in determining capsaicinoid levels in pepper fruit, growing conditions may also affect pungency. Anecdotal accounts suggest that conditions of abiotic stress (poor soil, hot temperatures, and water deficit) enhance pungency, but data are limited. Water stress induced a four-fold increase in capsaicin concentration in some pepper varieties (Sung *et al.*, 2005). Studies exploring effects of nitrogen and potassium on capsaicinoid content suggested some validity to the notion that excess fertilizer can negatively impact pungency (Medina-Lara *et al.*, 2008).

13.6 Flavonoids

Flavonoids are the largest category of phenolic compounds in plants and have diverse roles. Among these are: determination of flower and fruit color; protection from pathogens, herbivores, insects, and UV radiation; filtration of light for photosynthesis; maintenance of symbiotic interactions with microorganisms; and determination of flavor (Dennis *et al.*, 1997). Flavonoids are also believed to have antioxidant, anticancer, anti-inflammatory, anti-allergenic, and antimicrobial activities (Chun *et al.*, 2005). The pericarp and seed of pepper contain similar amounts of flavonoids as the fruit flesh (Sim and Sil, 2008); however, pepper is not considered to be a rich source of flavonoids when compared to asparagus, sweetcorn, and potatoes (Chun *et al.*, 2005).

Flavones and flavonol aglycones are the most prevalent flavonoid classes in pepper (Materska *et al.*, 2003; Bahorun *et al.*, 2004; Marin *et al.*, 2004; Materska and Perucka, 2005). Luteolin and derivatives are the primary flavones while quercetin and derivatives are the primary flavonol aglycones (Materska *et al.*, 2003; Arabbi *et al.*, 2004; Bahorun *et al.*, 2004; Materska and Perucka, 2005; Sun *et al.*, 2007). In chilli pepper, quercetin is the main flavonoid with a level 7.5-fold higher than luteolin. Quercetin derivatives also dominate in green pepper (Materska and Perucka, 2005). Additional flavones identified in pepper include apigenin, O-glycosyl flavones and C-glycosyl flavones (Bahorun *et al.*, 2004; Marin *et al.*, 2004, 2009). Flavonols in pepper also include sinapoyl and feruloyl glycosides (Materska *et al.*, 2003).

Lee *et al.* (2005) found that wax-type peppers had the highest total flavonoid content followed by bell and chilli peppers, while jalapeños had the least flavonoids. Among species, *C. annuum* and *C. frutescens* had higher flavonoid contents than *C. chinense* (Howard *et al.*, 2000). This might be because the *C. chinense* cultivar tested was pungent, and flavonoid precursors may have been diverted to capsaicinoid rather than flavonoid production.

Several studies found that total flavonoid content and the levels of individual flavonoids decrease as pepper fruit mature (Arabbi *et al.*, 2004; Marin *et al.*, 2004, 2008; Materska and Perucka, 2005; Menichini *et al.*, 2009). Immature habanero peppers had three times more flavonoids than mature fruit (Menichini *et al.*, 2009). Immature green "Vergasa" peppers had four- to five-fold more flavonoids than mature green to mature red fruit (Marin *et al.*, 2004). Materska and Perucka (2005) hypothesized that reduction in total and individual flavonoids was related to flavonoids being photoprotectors and no longer necessary in red fruit which lack chlorophyll and do not photosynthesize. In contrast, Gorinstein *et al.* (2009) measured higher levels of flavonoids and flavonols in red compared to green peppers, and Sun *et al.* (2007) observed that colored peppers generally had higher quercetin and luteolin contents (red peppers had higher contents than yellow and orange peppers). Conflicting results among studies may be due to genotypic differences.

Research indicates that conditions of growth have little effect on flavonoid content with no significant differences observed between field- and greenhouse-grown peppers (Lee *et al.*, 2005) or with organic, soilless, integrated, and conventional growth cropping systems (Chassy *et al.*, 2006; Marin *et al.*, 2008).

13.7 Vitamin C

Vitamin C, also known as L-ascorbic acid, is an organic acid which acts as a reducing agent, and is a potent antioxidant. Because it is water soluble, vitamin C can rapidly neutralize reactive oxygen species and thereby protect macromolecules such as DNA, proteins, and lipids. Humans do not synthesize L-ascorbic acid; this vitamin must be obtained from the diet. Fruits and vegetables are the best sources as many plants produce abundant amounts of vitamin C in their leaves and fruit. Pepper is an excellent source of vitamin C; a 100 g serving of raw sweet green peppers provides more than the RDA (60 mg) of this vitamin (International Food Information Council, 2002; USDA Nutrient Laboratory, 2010). It is estimated that 23% of total antioxidant activity of pepper extracts is attributable to vitamin C content (Chu *et al.*, 2002).

The vitamin C contents of *C. annuum* cultivars vary widely. Concentrations of vitamin C depend on genotype, fruit color, and fruit maturity stage. Topuz and Ozdemir (2007) observed low values (15–65 mg $100\,g^{-1}$ fresh weight) for five cultivars. Frary *et al.* (2008) studied Turkish and non-Turkish cultivars and reported significantly higher levels (up to 163 mg $100\,g^{-1}$ fresh weight). Deepa *et al.* (2006) also reported a similar range of diversity in vitamin C content with significant interactions between genotype and year. In *C. chinense* accessions, significant variation was observed, with Brazilian and Ecuadorean accessions having the highest vitamin C content (Antonious *et al.*, 2009b). In an even wider survey of *C. chinense* germplasm from the Americas, Jarret *et al.* (2009) noted a mean concentration of 391 mg $100\,g^{-1}$ fresh weight. Fruit of *C. chinense* appear to be a richer source of vitamin C than fruit of *C. annuum*. A possible exception is tepin pepper, *C. annuum* var. *glabriusculum* (also referred to as var. *aviculare*), which has twice as much vitamin C as a *C. chinense* habanero type (Oboh *et al.*, 2007). Rodriguez-Burruezo *et al.* (2009) found that greenhouse-grown *C. pubescens* fruit had five- to ten-fold lower vitamin C content than *C. annuum* while *C. baccatum* had intermediate levels.

Researchers have examined vitamin C variability among *C. annuum* types at different maturity stages. Chuah *et al.* (2008) observed that paprika peppers had higher vitamin C levels than bell peppers. Vitamin C content in paste types (such as "Salcalik Biber") was significantly lower than in stuffing and other commonly used types of Turkish peppers (Frary *et al.*, 2008). No difference was observed in Vitamin C content for pungent and nonpungent types in that study. On a fresh weight basis, vitamin C content has been shown to increase as fruit mature (Howard *et al.*, 2000; Marin *et al.*, 2004; Martinez *et al.*, 2005, 2007; Perez-Lopez *et al.*, 2007a; Bernardo *et al.*, 2008; Serrano *et al.*, 2010). The net increase in vitamin C content during ripening varies with genotype but was in the range of 1.5- to 2.2-fold for *C. annuum* (Marin *et al.*, 2004; Martinez *et al.*, 2005; Perez-Lopez *et al.*, 2007a). Howard *et al.* (2000) recorded a five-fold increase in vitamin C content in immature (green) versus mature (red) tabasco (*C. frutescens*) peppers. The increase in vitamin C content during maturation was attributed to increased production of this photoprotector in response to greater amounts and intensities of light (Marin *et al.*, 2004; Chuah *et al.*, 2008). When peppers are over-ripened, as is necessary for production of paprika, vitamin C content significantly decreases (Gnayfeed *et al.*, 2001; Martinez *et al.*, 2007).

Differences in vitamin C content between green and other colored peppers are analogous to those seen between fruit at different developmental stages. Red (mature) peppers usually have higher vitamin C content than green (immature) peppers (Jimenez *et al.*, 2003; Guil-Guerrero *et al.*, 2006; Matsufuji *et al.*, 2007; Oboh *et al.*, 2007; Chuah *et al.*, 2008; Marin *et al.*, 2008). When different colored bell peppers were compared, orange ranked first for vitamin C content followed by red and yellow (Matsufuji *et al.*, 2007). White peppers, eaten at the immature stage, had among the lowest amounts of vitamin C; levels were similar to those in immature green peppers (Matsufuji *et al.*, 2007). Guil-Guerrero *et al.* (2006) and Chuah *et al.* (2008) saw similar rankings among colored bell peppers; red paprika peppers were found to have higher vitamin C content than orange paprika peppers (Chuah *et al.*, 2008).

Vitamin C content may also be influenced by conditions of growth and cropping system. Field-grown peppers had higher vitamin C content than those grown in the greenhouse, a difference attributed to the greater amount and intensity of light in the field (Rodriguez-Burruezo *et al.*, 2009). When compared with conventional and integrated (limited chemical treatment) growth systems, the highest vitamin C levels in fruit were measured in plants grown under organic or soilless systems (Perez-Lopez *et al.*, 2007b; Marin *et al.*, 2008; Flores *et al.*, 2009b). Marin *et al.* (2008) found that *C. annuum* cv. Quito peppers grown under organic and soilless conditions had four-fold more vitamin C than those grown in an integrated system. Others reported no effect of cropping system on vitamin C content (Flores *et al.*, 2004, 2009a; Chassy *et al.*, 2006). However, when plants are stressed, significant changes in vitamin C are seen. Low irrigation frequency (Marin *et al.*, 2009) and salicylic acid treatment, which is known to elicit defense responses (Elwan and El-Hamahmy, 2009), resulted in increased vitamin C content up to two-fold. Salinity (up to 30 mM NaCl) resulted in a decrease of 1.2-fold (Navarro *et al.*, 2006). Heavy metal stress appeared to have an additive effect on vitamin C level as cadmium or lead alone reduced the vitamin C content by approximately 25 and 37%, respectively, while presence of both metals reduced vitamin C levels by 50% (Fu *et al.*, 2009).

13.8 Future Prospects

The study of pepper metabolites is a rapidly expanding field, a result of pepper's importance as a vegetable crop and staple ingredient in many cuisines. *Capsicum* fruit contain a rich diversity of metabolites that are reported to have antioxidant, hypoglycemic, immunogenic, antihypertensive, anticholesterol, anti-inflammatory, and antimutagenic effects (Kwon *et al.*, 2007; Menichini *et al.*, 2009). Given the wide variation for individual compounds in different species and genotypes, pepper lends itself to the enhancement of its qualities through manipulation of metabolite levels using conventional breeding and non-conventional approaches. It is expected that the next 10 years of research in this area will prove very productive.

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