Effects of Production and Processing Factors on Major Fruit and Vegetable Antioxidants

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ABSTRACT: Public awareness of the purported health benefits of dietary antioxidants has increased the demand for fruit and vegetable products with recognized and improved antioxidant quality and has created new opportunities for the horticulture and food industry to improve fruit and vegetable quality by enhancing antioxidant content. This review describes the production and processing factors that influence the content of the major fruit and vegetable antioxidants, namely vitamin C, carotenoids, and phenolics. There is substantial genetic variation in the content of each of these antioxidant types among fruit and vegetable cultivars. Compared with vitamin C and carotenoids, the levels of phenolic antioxidants appear to be more sensitive to environmental conditions both before and after harvest. Although vitamin C can be readily lost during fresh storage, the content of certain carotenoids and phenolics can actually increase during suitable conditions of fresh storage. Vitamin C and phenolics are more susceptible to loss during processing, especially by leaching from plant tissues into processing water. The combination of cultivar variation and responsiveness to specific environmental conditions can create opportunities for the production and processing of fruits and vegetables with improved antioxidant properties.

Keywords: antioxidant, phenolics, carotenoids, vitamin C, nutrition

Introduction

Today there is unprecedented interest by consumers, public lacksquare health organizations, and the medical community to improve health and wellness through dietary means. This interest arises in large part from the increased rates in Western society of adverse diet-related health conditions such as obesity, late-onset diabetes, and cardiovascular disease, and their associated social and economic costs (IFIC 2004). At the same time, our knowledge of the role that fruit and vegetable consumption can play in maintaining human health and reducing disease risk is growing. Spurred by epidemiological evidence of a positive association between fruit and vegetable consumption and a reduced risk of certain degenerative conditions (Ames and others 1993; Hertog and others 1995; Steinmetz and Potter 1996; Joshipura and others 1999), evidence is accumulating from in vitro, in vivo, and clinical studies that supports the notion that fruit and vegetable phytochemicals can affect specific physiological processes to benefit human health. In particular, phytochemicals that possess antioxidant characteristics are believed to contribute to the overall health-protective effects of fruits and vegetables because antioxidants may mitigate the consequences of oxidative stress in disease development and the aging process (Ames and others 1993; Joseph and others 2000; Meydani 2001).

Oxidative stress, which can lead to molecular damage to lipids, proteins, and DNA in the body, is an inevitable consequence of life in an oxygen environment. Mitochondrial respiration is a persistent and significant contributor to oxidative stress. During mitochondrial electron transport, 3% to 5% of the oxygen consumed may be released before it has undergone a complete 4 electron reduction to water. Incomplete reduction of oxygen, which is believed to

MS 20040208 Submitted 4/5/04, Revised 5/7/04, Accepted 8/17/04. The author is with Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre, 32 Main St., Kentville, Nova Scotia, Canada B4N 1J5. Direct inquiries to author Kalt (E-mail: <u>kaltw@agr.gc.ca</u>). occur at coenzyme Q, yields reactive oxygen species, such as superoxide anion and hydrogen peroxide (Jackson 2000). Oxidative damage to biological molecules is linked to the etiology and pathophysiology of many socially significant health conditions including cardiovascular disease (Steinberg 1997), certain cancers (Ames and others 1993), neurodegenerative disease (Joseph and others 2000), diabetes (Laaksonen and Sen 2000), rheumatoid arthritis (Jawed and others 2000), cataracts (Taylor 1992), and others. Lifestyle factors such as smoking (Duthie and others 2000), exposure to environmental pollutants (Furst 2002), exercise (Jackson 2000), and solar irradiation (Biesalski and Obermueller-Jevic 2001) can exacerbate the basal level of oxidative stress in the body. The accumulation of oxidative lesions in biological molecules is believed to underlie the degenerative processes that characterize aging (Meydani 2001). Against this backdrop, there is a substantial and growing body of evidence that fruit and vegetable antioxidants can bolster endogenous antioxidant defenses in the body, thus mitigating oxidative damage and its associated negative health impacts (Hertog and others 1995; Steinmetz and Potter 1996; Joshipura and others 1999; Joseph and others 2000; Aviram and others 2004).

The most abundant types of antioxidants contained in fruits and vegetables include vitamin C, carotenoids, and phenolics. Tocopherols and tocotrienols are also important phytochemical antioxidants, but they are present in relatively low levels in fruits and vegetables as compared with nuts and grains. Intrinsic to their nature, phytochemical antioxidants are preferentially oxidized by reactive oxygen species, thereby protecting the structural and functional integrity of biological molecules and systems against oxidative damage. The antioxidant properties of vitamin C, carotenoids, and phenolics result from their electron-rich structure in the form of oxidizable double bonds and hydroxyl groups (Table 1). Vicinal hydroxyl groups on certain phenolic antioxidants can chelate free metal cations, such as copper and iron, which are potent pro-oxidants in their unchelated forms. Fruit and vegetable antioxidants are chemically diverse and are found in different locations and

Antioxidant group	Representative structure	Antioxidant mechanism	Key features
Vitamin C	HOH JC HOH OH	Direct electron donation Enzymatic reduction ROS ^a quenching	Vicinal OH groups
Carotenoids	grapping and a start and	Electron donation ROS quenching	Conjugated double bonds
Phenolics	- yest	Electron donation Metal ion chelation Ascorbic acid sparing ROS quenching	Vicinal OH groups Conjugated double bonds

aROS = reactive oxygen species.

Table 2-Properties of	ph	vtochemical	antioxidants	in	plant tissues
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Feature	Vitamin C	Carotenoids	Phenolics
Subgroups	Ascorbic acid and dehydroascorbic acid	Numerous, for example, lutein, lycopene, α-carotene, β-carotene, zeaxanthin; cryptoxanthin	Numerous, for example, phenolic acids, hydroxycinnamates, flavonoids inc. flavonols, catechins
Solubility	Water	Lipid	Water
Cellular localization	Dissolved in apoplast, cytosol, chloroplast, mitochondria, and vacuole	Associated with membrane protein complexes in chloroplast or chromoplast	Dissolved in vacuole and apoplast
Structural localization	Uniformly distributed?	Some types (for example, tomato lycopene) preferentially in surface tissues like peel and outer pericarp	Anthocyanins preferentially in peel; proanthocyanidins in peel and seed; hydroxycinnamates in flesh
Changes with ripening	Differs with species	Pigmented forms change	Pigmented forms increase

forms in plant tissues and cells; they also differ in size, water solubility, and susceptibility to oxidation (Table 1 and 2). The major antioxidants are absorbed and metabolized in the body in a variety of ways, and some antioxidants are more bioavailable than others (Schwedhelm and others 2003). All of these factors influence the functionality of the antioxidant in the plant itself, in foods, and in the body. Because of the diversity in their chemical properties, antioxidant mechanisms, and physiological effects, it is generally considered, but not well substantiated, that antioxidants provide the most efficacious protection when several types are present at sites where antioxidant protection is needed.

Phytochemicals possessing antioxidant properties are part of a sophisticated array of secondary compounds that have evolved to help plants survive. As sessile organisms, the ability of plants to survive and multiply under changing environmental conditions depends on how well they use their phytochemical machinery. Thus, the constitutive levels of phytochemicals, including those with antioxidant properties, depends on their specific role in the plant, on the plant's age and reproductive status, and on various environmental factors. Phytochemicals serve a variety of purposes in the plant, which often involve roles in protection and signaling. For example, the potent phenolic antioxidant resveratrol is a protective phytoalexin in grapes, playing a role in defense against fungal pathogens. In signaling, the carotenoid antioxidant lycopene increases dramatically as tomato fruit and seeds ripen; this provides a visual cue of fruit palatability and ultimately aids in seed dispersal. The rapid increase in anthocyanin content during the ripening of many berries crops is an important cue to signal that fruit are ripe and ready for consumption. Certain antioxidants contribute to the plant's defense against oxidative stress. Plants are subjected to substantial oxidative stress in the light because of oxygen evolution in chloroplasts during photosynthesis. In addition to the oxidative stress imposed by photosynthesis, many of the abiotic and biotic stresses encountered by plants before and after harvest have oxidative stress as their mechanistic basis (Hodges 2001).

Fruits and vegetables are harvested, transported, and stored in ways that can impose physiological stresses that lead to adverse changes in their visual quality and chemical profile. Domestic and commercial food processing typically has drastic effects on the structural integrity of fruits and vegetables. Because essentially all fruits and vegetables in the typical North American diet are subjected to processes that can alter their composition, it is important to know how specific antioxidant components are affected.

The aim of this review is to show how the health functionality of the diet may be improved by increasing and retaining the antioxidant content of fruits and vegetables through varietal selection and by controlling conditions of production, harvest, storage, and processing. Plant factors, such as genetics and maturity, environmental factors, and the effects of postharvest handling, storage, and processing will be reviewed with respect to how they impact the content of major groups of fruit and vegetable antioxidants, namely, vitamin C, carotenoids, and phenolics.

Genetic Factors

Without question, the most important factor that determines the antioxidant content of the diet is food choice. Consumers have access to various information sources related to the nutrient content of foods; the United States Dept. of Agriculture (USDA) Food Nutrient Database (<u>http://www.nal.usda.gov/fnic/foodcomp/</u>) is one of the most comprehensive and authoritative sources of data on the antioxidant content of fresh and processed fruit and vegetables. For example, the vitamin C content of more than 1000 foods is listed. A partial list of the carotenoid content of various foods is also included, and the USDA is presently expanding the database to include the phenolic flavonoid content of foods.

Vitamin C

Plant foods vary greatly in their vitamin C content. For example, some cruciferous vegetables such as broccoli have a high vitamin C (ascorbic acid + dehydroascorbic acid) content (http://www.nal.usda.gov/fnic/foodcomp/). Among common fruit crops, citrus, strawberries, and raspberries are noted for their high vitamin C content. However, of greatest relevance to this review is the substantial variation in the content of vitamin C and other antioxidants among different varieties of fruits and vegetables. In a survey of 50 broccoli varieties, ascorbic acid content ranged from 56 to 120 mg/100 g fresh weight (Kurilich and others 1999). Ascorbic acid content ranged from 32 to 99 mg/100 g FW among 308 varieties of strawberries (Maas and others 1995). Two apple varieties differed more than 2-fold in their ascorbic acid content (Lachman and others 2000).

Carotenoids

Similar to vitamin C, the content of various carotenoids varies substantially among fruits and vegetables. Kale is especially rich in carotenoids, particularly those with pro-vitamin A activity (β-cryptoxanthin, α-carotene, and β-carotene). Spinach, carrots, and tomatoes also have a relatively high carotenoid content, although their content is lower than kale. However, because spinach, carrots, and tomatoes are consumed more frequently than kale, they are more important sources of dietary carotenoids. The total carotenoid content of 6 varieties of spinach ranged by 1.3-fold (Kidmose and others 2001). In carrots, β -carotene makes up about 45% to 80% of the total carotenoids present. In a survey of 19 carrot cultivars, β-carotene varied over a 2.2-fold range in content, with a mean content of 6300 µg/100 g FW. Selected carrot hybrids have been produced with β -carotene content as high as 20000 μ g/100 g FW (Alasalvar and others 2001). In sweet corn, zeaxanthin and lutein are the major carotenoids. Among 44 lines of sweet and dent corn, total carotenoid content varied over a greater than 200-fold range. Total carotenoids ranged from 0.40 to 33.1 μ g/g DW. In the same survey, 700-fold differences in zeaxanthin content were reported, whereas lutein content ranged from 0 to 20 μ g/g DW (Kurilich and Juvik 1999).

Because of their high frequency in the diet, tomatoes are an important source of carotenoids, particularly lycopene. Among different phenological types of tomatoes, carotenoid content differed over a 20-fold range. Salad varieties were lowest and cherry-type were highest, whereas elongated and cluster-type tomatoes were intermediate in carotenoid content (Leonardi and others 2000). In a comparison of 12 salad tomato varieties, total carotenoid content varied by 1.5 times. Among the 12 varieties, lycopene, which accounts for more than 75% of the carotenoid content of ripe tomatoes, varied by 1.6-fold. The next predominant carotenoid, β -carotene, varied by 2-fold, and lutein varied by more than 4-fold (Abushita and others 2000). In general, processing varieties had higher carotenoid levels

than domestic varieties. Carotenoid content has been associated with specific genes in tomatoes. In 3 varieties possessing the crimson gene, lycopene content ranged from 5086 to 5786 μ g/100 g FW, whereas the lycopene content of 5 varieties that did not have the gene ranged from 2622 to 4319 μ g/100 g FW (Thompson and others 2000). In tomatoes, the BET gene is related to high β -carotene content, and DEL is related to high δ -carotene content (Nguyen and others 2001). A USDA breeding program to develop tomato varieties with higher levels of provitamin A carotenoids (β -cryptoxanthin, α -carotene, and β -carotene) has developed a phenotype that contains 55 to 58 μ g/100 g FW β -carotene, compared with the 2 to 3.5 μ g/100 g FW in conventional varieties (Stommel 2001).

Phenolics

Common fruits and vegetables that are abundant in phenolic antioxidants include most berry crops, many tree fruit crops, and onions. It has been reported that apples, onions, and tea make the greatest contribution of antioxidant flavonoids to the Western European diet by virtue of their content and their frequency of consumption (Hertog and others 1993).

Numerous surveys report the range in both phenolic content and antioxidant capacity among berry crop species (Kalt and others 1999; Wang and Lin 2000; Moyer and others 2002; García-Alonso and others 2004). Despite the high vitamin C content in certain berry crops, it is typically the phenolic content, not the vitamin C content, that is positively correlated with antioxidant capacity (Kalt and others 1999). As expected, phenolics differ quantitatively and qualitatively among berry crop species and among phenotypes within species.

Highly colored fruit that have a high level of anthocyanin, such as black currants, elderberry, and blueberry, typically possess a high antioxidant capacity. To improve the color quality of certain fruit crops, such as cranberries and red-skinned apples, some breeding programs have selected for high anthocyanin content. More recently, some fruit-breeding programs have included selection criteria related to antioxidant compounds, and in some cases new germ plasm has been introduced in an attempt to increase antioxidant content. The antioxidant capacity of domestic and wild Rubus species was evaluated and correlated to total phenolic content and anthocyanin content. Among the genotypes surveyed, a wild species, R. caucasis, was found to be highest in antioxidant capacity and phenolics, but it had only an intermediate level of anthocyanins (Deighton and others 2000). Thus, although highly colored fruits with a high anthocyanin content are generally high in antioxidant capacity, colorless phenolics also contribute substantially to the antioxidant capacity of foods. The sensory properties of certain phenolics, such as tannins, limit their increased content in fruit because this group of compounds contributes to astringency.

Two *Vaccinium* species, cultivated highbush and wild lowbush blueberries, were surveyed for their total phenolic content, their anthocyanin content, and their antioxidant capacity (Kalt and others 2001). Among the 135 genotypes of wild blueberries and 80 genotypes of cultivated blueberries, antioxidant capacity (measured as oxygen radical absorbing capacity) spanned a 1.75-fold range, whereas anthocyanins and total phenolic content spanned a 1.60-fold and 1.25-fold range, respectively. A wider range of values might have been expected from the wild blueberries because these phenotypes were not artificially selected.

The major phenolic components of apples have been determined and, like other crops, their content varies markedly among cultivars. Among 10 apple cultivars, the range in total phenolic content was 1.6-fold. Chlorogenic acid was the main phenolic component and spanned a greater than 10-fold range among the cultivars

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(Podsedek and others 2000). In a survey of 46 cider apple varieties, chlorogenic acid, epicatechin, and procyanidin B2 accounted for about 80% of the phenolics included in the analysis. Among the cultivars, chlorogenic acid ranged from 21 to 351 mg/L, epicatechin from 0 to 206 mg/L, and procyanidin B2 from 0 to 247 mg/L. Total phenolic content spanned a 10-fold range (Mangas and others 1999). Among a number of samples obtained from 4 dessert cultivars, procyanidin content varied by more than 2-fold, with Red Delicious having the highest and Golden Delicious the lowest level (Hammerstone and others 2000).

The phenolic antioxidant resveratrol and its isomers are of great interest because of their potent antioxidant properties, cardioprotective effects, and their myriad effects in various cancer research models (for review, see Pervais 2003). Best studied in grapes and wine, resveratrol is found in the fruit peel of both red and white grapes and red wines and has been identified in other crops such as peanuts and some berry crops. Not surprisingly, resveratrol content varies widely among and within species (Bhat and others 2001).

Onions have a high content of flavonoid antioxidants, in particular the flavonol quercitin and its glycosides. Among 12 onion cultivars, including yellow, red, and white types, total flavonoid content ranged from 1.2 to 980 mg/kg. Yellow onions had the highest flavonoid content, whereas white onions had the lowest levels (Marotti and Piccaglia 2002).

Localization of Antioxidants

Fruit and vegetable components including antioxidant compounds are differentially localized in the various structures, cells and organelles of these food plants (Table2). This differential localization becomes important when food plants are processed into products such as juice and wine. Vitamin C is localized in the apoplast, cytosol, mitochondria, vacuole, and the plastids (chloroplasts and chromoplasts) of plant cells. Certain antioxidants, such as anthocyanins and various carotenoids, are colored making it easier to see where they are localized in fresh crops. In tomatoes, 80% of lycopene content is in the peel and outer pericarp tissue (Shi and le Maguer 2000).

Anthocyanins are typically found in fruit peel, although species and cultivars can differ in this respect. A good illustration of potential variation in anthocyanin distribution is in strawberries. Ripe strawberries have a substantial level of anthocyanins, but these pigments may or may not be present in their internal tissues. Also, the specific types of anthocyanin can differ between internal and external tissues (Holcroft and Kader 1999). In apple and berry crops, anthocyanins, flavonols, and proanthocyanidins are found in the highest concentration in the peel, whereas hydroxycinnamic acids, such as chlorogenic acid, are most abundant in the flesh of these fruits (Golding and others 2001). Proanthocyanidins are more abundant in the peel and especially the seeds of berry crops. Another potent phenolic antioxidant, resveratrol, is localized in the peel of grapes (Bhat and others 2001).

Maturity

Vitamin C

The content of vitamin C, carotenoids, and phenolics change in different ways during the development and maturation of fruits and vegetables. Immature citrus fruits are reported to have the highest, whereas ripe fruit have the lowest concentration of vitamin C. The absolute content of vitamin C increases of course as fruit expands (Nagy 1980). In strawberries, ascorbic acid content increases from essentially nil when fruit is green and immature to a maximum level when fruit is fully ripe (Maas and others 1995). When fruit become overripe, vitamin C content declines; this decline may be concurrent with the degradation of fruit tissue. Among different types of colored peppers, ascorbic acid increased, decreased, or remained the same during ripening (Simonne and others 1997).

Carotenoids

Colored antioxidants (for example, carotenoids and anthocyanins) often reach their highest level when fruit are at their optimal ripeness. This dramatic increase in the content of antioxidant pigments during the ripening of fruit (for example, tomatoes and berries) is often accompanied by fruit softening and a decline in tartness and astringency, which results in an improvement in overall palatability. Concurrent with these changes is the ripening of fruit seeds. In this way, antioxidant pigments provide a visual cue that fruit is palatable and seeds are mature, and therefore ready for ingestion and dispersal.

The total carotenoid content of 7 cultivars of colored peppers, representing 3 Capsicum species, generally increased as fruit became more mature. Among the genotypes, the provitamin A carotenoids increased from less than 5% of the RDA in immature fruit to between 10% and 34% of the RDA for vitamin A in mature fruit (Howard and others 2000). However, in a survey of 19 pepper cultivars of the green-yellow type, total provitamin A carotenoid content remained the same or decreased with advanced ripeness, possibly because of the interconversion of provitamin A to nonprovitamin A colored carotenoids (Simonne and others 1997). In the same study, the carotenoid profile generally became more complex as fruit matured. Changes in the profile of carotenoid antioxidants during ripening appear to be complex and require further study. Of particular interest are those types of carotenoid antioxidants that are precursors of vitamin A.

Lycopene, which is not a provitamin-A carotenoid, makes up about 75% of the total carotenoid content of ripe tomatoes. The lycopene content of 4 tomato cultivars increased from less than 10 μ g/100 g in green fruit to about 5000 μ g/100 g as fruit matured to the red-ripe stage. Lycopene can increase to more than 7000 μ g/100 g when tomatoes become overripe, soften, and begin to decay (Thompson and others 2000).

Phenolics

Fruit ripening is typically accompanied by substantial changes in the profile of phenolic antioxidants. For example, as fruit color changes during ripening, anthocyanin pigment content increases dramatically. The relationship between fruit maturity, phenolic content, and antioxidant capacity differs among fruit crops. Cultivated blueberries that were unripe and still white or pink in color had a similar antioxidant capacity as blue, ripe fruit. Although the anthocyanin content was substantially higher in the ripe fruit, the content of all the phenolic antioxidants, of which anthocyanins were a portion, was fairly constant (Kalt and others 2003). This shows that both colored and colorless phenolics contribute to the antioxidant capacity of blueberry fruit at different stages of ripeness. Black raspberries and strawberries had their highest antioxidant capacity when immature, whereas red raspberries were highest in antioxidant capacity when fruit were ripe (Wang and Lin 2000). Although immature fruit are not very palatable, underripe, poorer quality, or culled fruit may have value as raw material for antioxidant extraction because of their relatively high phenolic antioxidant content. Citrus have their highest content of the flavonoid hesperidin when fruit are immature (Ortuño and others 1997). Hesperidin is extracted from citrus byproducts and used in pharmaceutical products that are designed to reduce blood cell membrane permeability and fragility (Girard and Mazza 1998).

Apple fruit maturity interacts with both temperature and light

conditions to affect the anthocyanin content of the peel (Lancaster 1992). Peel components contribute substantially to the complexity and content of phenolic antioxidants in apples. Apple peel anthocyanins have been relatively well studied because of their importance to the color quality of fruit. When fruit are sufficiently mature, anthocyanin production is stimulated by cool temperatures and high light conditions. In an extensive examination of apple phenolics during the development of 2 apple cultivars, the concentration (mg phenolic per g apple tissue) of the major phenolics in the apple skin was greatest when fruit were newly set, 3 wk after bloom. However, the total flavonoid content of the peel on the basis of the whole apple increased as the fruit enlarged and ripened (de Jager and others 2001).

Environmental Factors

Light

Light exposure appears to have little or no effect on either the ascorbic acid or carotenoid content of the edible portion of fruits and vegetables, whereas in some cases, phenolic content can be increased by exposure to elevated light or UV irradiation. Pinot Noir grapes on sun-exposed clusters had a 10-fold higher quercitin glycoside content compared with grapes from the shaded part of the cluster (Price and others 1995). In the same study, anthocyanin content was not affected by light exposure. Because flavonoids are known to absorb UV irradiation, it is believed that flavonol production is stimulated to protect plant tissues from UV damage. In apple fruit, light is required to stimulate localized anthocyanin production during fruit ripening (Lancaster 1992). The content of both anthocyanins and quercitin was much greater in sun-exposed portions of both Elstar and Jonagold apple fruit. Not all phenolic synthesis is responsive to light. Although anthocyanins and quercitin compounds were much higher in sun-exposed apples, the contents of other major apple phenolics, including catechins, phloridzin, and chlorogenic acid, were not different between apples from the sun-exposed and shaded portions of the tree (Awad and others 2000).

Cultural conditions

Some research has been conducted on the influence of soil fertility on the content of fruit and vegetable antioxidants. When nitrogen fertilizer was increased, ascorbic acid content was found to be lower in several fruits and vegetables, including cauliflower, broccoli, various citrus crops, and some cultivars of potatoes (for review, see Lee and Kader 2000). However, higher ascorbic acid content was observed in tomatoes grown under saline conditions (Pascale and others 2001). Although lower productivity may be expected where saline conditions are unavoidable, higher vitamin C content would contribute to the nutritional content of the tomatoes. A 50% difference in the ascorbic acid content of broccoli was found between 2 y of study, but it is not clear whether this difference may have been caused by soil differences (Howard and others 1999).

Although large differences were seen in the ascorbic acid content of broccoli, β -carotene content was similar between 2 y of study (Howard and others 1999). Carotenoid content of carrots increased quadratically with the application of nitrogen fertilizer; the highest carotenoid levels were achieved with 160 kg/hectare (Hochmuth and others 1999). This level of nitrogen application also resulted in the greatest yield and highest carrot sugar content. No differences in carotenoid content were found in carrots grown in 6 locations in Norway spanning 11° N latitude (Skrede and others 1997). There were no differences in lycopene content between con-

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ventionally and hydroponically grown tomatoes, and lycopene content in tomatoes under both production systems increased during 2 wk of storage (Ajlouni and others 2001).

Production conditions have also been examined for their effect on the phenolic content of fruits and vegetables. In a 2-y study on the effects of water availability on the phenolic content of grapes, total phenolic content and the content of anthocyanins and tannins were higher in grapes on the basis of weight and per fruit from nonirrigated plots for almost all harvest dates (Esteban and others 2001). High soil levels of aluminum chloride were shown to stimulate the production of resveratrol in grape leaves, but the study did not examine the effects on the grapes themselves (Adrian and others 2000). No difference was found in the phenolic flavonol content of strawberries grown using either conventional or organic cultivation practices (Hakkinen and Torronen 2000). Phenolic content and antioxidant capacity was examined in blueberry cultivars over 2 growing seasons (Howard and others 2003). Although there were seasonal differences in the content of anthocyanins, total phenolic content, and antioxidant capacity, the differences among cultivars for these parameters were far greater. However, anthocyanin content differed by up to 45% between 2 y, whereas phenolic and antioxidant capacity differed by up to 56% and 60%, respectively. There was no consistent change among all cultivars in a given year; some cultivars had higher contents whereas others had lower.

Disease

Vitamin C and carotenoid content appear to be affected by plant disease only inasmuch as disease contributes to the decay of fruit and vegetables and to the oxidation of their components. However, with respect to phenolics, there is a well-documented example of fungal disease affecting the content of the phenolic phytoalexin, resveratrol. In grapes, the synthesis of resveratrol and other related stilbenes is stimulated by fruit infection with fungal pathogens including Botrytis cinera, Plasmopara viticola (see references in Romero-Perez and others 2001), and Uncinula nectora. In powdery mildew infection by Uncinula nectora, total resveratrol, including trans-isomers and cis-isomers of resveratrol and their corresponding glycosides, increased by about 8-fold. Interestingly, some pathogens, including Botrytis cinerea, possess enzymes that are capable of degrading stilbenes so that resveratrol levels in highly infected grapes may be even lower than those found in healthy grapes (Adrian and others 2000). However, grapes on the same cluster or near clusters of infected grapes can have high levels of resveratrol (Adrian and others 2000). In a study with controlled infection, the highest levels of resveratrol were observed on clusters that were about 10% infected with Botrytis gray mold (Jeandet and others 1995).

Handling and Storage

Vitamin C

Whether raw or processed, all fruits and vegetables are subjected to handling and storage by producers and consumers, which may affect the integrity of their antioxidants. Compared with other major fruit and vegetable antioxidants, ascorbic acid is more susceptible to significant loss during postharvest handling and storage. In contrast, the content of certain carotenoids and phenolics is more stable and can actually increase under appropriate storage conditions.

Ascorbic acid requires an acidic environment for stability. Its stability in fresh produce can be quite variable and may depend on several factors, including the level of ascorbate oxidase (Lee and Kader 2000). Strawberries have a high vitamin C (ascorbic acid +

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dehydroascorbic acid) content compared with many fruit crops. The effects of storage conditions on ascorbic acid retention in strawberries have been relatively well studied. More ascorbic acid was retained in strawberries that were stored at either 1 or 10 °C compared with those stored at 20 °C. However, wrapping fruit with plastic film to reduce water loss helped retain ascorbic acid even more than optimal storage temperature. Fruit that were stored at 1 °C or 10 °C with plastic over wrap lost little ascorbic acid after 8 d of storage, whereas those stored at 20 °C without wrapping lost between 55% and 70% of their ascorbic acid after only 4 d (Nunes and others 1998). Irradiation, which is used as a disinfection treatment for fresh produce, had some effect on the vitamin C content of strawberries. Although dehydroascorbate increased immediately after strawberry irradiation, indicating some ascorbic acid oxidation, the overall loss in vitamin C because of irradiation was only about 5% (Graham and Stevenson 1997).

Ascorbic acid content decreased between 25% and 30% in 3 apple cultivars during 6 mo of conventional storage (Lachman and others 2000). In a separate study, the vitamin C content of apples stored in controlled atmosphere declined to a greater extent than those stored in air, although those in controlled atmosphere were stored for a longer period. Storage of fruit crops in a high carbon dioxide atmosphere with or without reduced oxygen gave different results in strawberries, raspberries, currants, and blackberries. Under controlled atmosphere storage, vitamin C declined by 42% in strawberries, whereas losses in blackberries and currants were less. In red raspberries, vitamin C content declined only by about 15% (Agar and others 1997). In a study in which broccoli ascorbic acid content at harvest differed by almost 50% between 2 seasons, after 3 wk of storage, ascorbic acid had declined by 52% in the florets that had a high content at harvest, whereas ascorbic acid declined by about 13% in the florets with lower ascorbic acid at harvest (Howard and others 1999). When different methods of broccoli packaging were investigated, there was essentially no loss of vitamin C in broccoli florets stored in a modified atmosphere package, whereas unpackaged florets, or florets packed in perforated film, lost between 75% and 85% of their vitamin C after 6 d of storage. Keeping florets moist with misting helped to retain vitamin C (Barth and Zhuang 1996). Green beans did not retain their vitamin C very well. After 16 d of refrigerated storage, only 8% of the original ascorbic acid content was present. Lee and Kader (2000) categorize different vegetables as being good, moderate, or poor in their retention of ascorbic acid; green beans were designated as poor. During modified atmosphere storage of spinach, vitamin C actually increased slightly between 3 and 7 d. After 7 d of storage, vitamin C content was back to prestorage levels. However, since most vitamin C was present as dehydroascorbic acid, this translated to a net decline in antioxidant capacity during this period (Gil and others 1999).

Carotenoids

Carotenoid content of fresh fruits and vegetables may be affected by storage conditions. In a study of the lycopene content of 8 varieties of tomatoes, maturity at harvest, ethylene treatment, and storage time all played a role in the lycopene accumulation in tomato fruit during storage. In general, fruit that were at the breaker stage (10% red) at harvest had a greater accumulation of lycopene during room temperature storage than tomatoes that were green at harvest and received ethylene to stimulate ripening (Thompson and others 2000). There were no differences in lycopene content between conventionally and hydroponically grown tomatoes at harvest, and tomatoes from both production systems increased their lycopene content substantially during 2 wk of storage at 22 °C; lycopene increased 2.5-fold and 3.2-fold in hydroponically and conventionally produced tomatoes, respectively (Ajlouni and others 2001). Interestingly, during storage at 4 °C, there was no change in the lycopene content of the conventionally grown tomatoes, whereas hydroponically grown fruit increased their lycopene content by about 70% (Ajlouni and others 2001).

The retention of total broccoli carotenoids during storage was affected by packaging. Modified atmosphere packaging prevented any loss in carotenoids of broccoli florets during 6 d of 5 °C storage, whereas unwrapped florets or florets wrapped in perforated film lost about half of their carotenoid content under the same storage conditions (Barth and Zhuang 1996). The content of β -carotene was not substantially affected for green beans and broccoli stored for 3 wk and carrots for 6 mo at 4 °C (Howard and others 1999).

Phenolics

Phenolic content may either increase or decrease in fruits and vegetables, depending on storage conditions. Apples stored at either 1.5 °C or 4 °C, with or without controlled atmosphere, retained their flavonoid phenolics throughout their storage life. Controlled atmosphere approximately doubled the storage life of the fruit from about 25 to 50 wk (van der Sluis and others 2001). However, in another study in which apples were stored at 5 °C in air, 27% of the total phenolic content was lost after about 6 mo of storage. The discrepancy between these 2 apple studies may be due, in part, to differences in the methods used to measure the phenolics.

A substantial accumulation of anthocyanins was observed in strawberries and raspberries, and to a lesser extent in cultivated blueberries, when ripe fruit were stored for various periods at 20 °C. Anthocyanin accumulation was less at 30 °C and 15 °C. Although anthocyanins in strawberries increased during storage, total phenolic content and antioxidant capacity did not change. In raspberries, however, there was an increase in total phenolic content, including anthocyanins, as well as an increase in antioxidant capacity during 20 °C storage (Kalt and others 1999). Holcroft and Kader (1999) reported increases in anthocyanins and other phenolics in strawberries when stored for up to 10 d in air. In the same study, strawberries stored in a controlled atmosphere of high CO₂ accumulated less anthocyanin and other phenolics than strawberries stored in air. The activity of enzymes involved in phenolic synthesis was lower, and tissue pH was higher, in fruit stored in the high CO₂ atmosphere. Anthocyanins are most stable and their color intensity is greatest at low pH.

The content of table grape phenolics was examined during storage at 0 °C for 10 d followed by 5 d at 15 °C to simulate typical storage and marketing conditions. Although many phenolics, including anthocyanins, flavonol glycosides, and cinnamate acid esters, did not change during storage, resveratrol derivatives increased by almost 2-fold during storage. Furthermore, grapes that received a treatment of UV-B had resveratrol glucoside (piceid) 2 to 3 times greater than grapes that did not receive UV-B (Cantos and others 2000). After harvest, exposure of apples to UV-B irradiation was found to increase both anthocyanins and quercitin glycosides, although the response depended on both the storage temperature and the cultivar (Lancaster and others 2000).

Processing

Processing can alter and often damage fruit and vegetable antioxidants. Maceration, heating, and various separation steps can result in oxidation, thermal degradation, leaching, and other events that lead to lower levels of antioxidants in processed food compared with fresh. This is particularly true in the case of vitamin C and phenolic antioxidants. However, in the case of carotenoids, processing can lead to a dissociation of antioxidants from plant matrix components, an increase in carotenoid antioxidants, and improved digestive absorption (for review, see Shi and le Maguer 2000).

As discussed in an earlier section, the localization of components within plant materials becomes important when tissues such as peels and seeds are separated from other components during processing, as in juice and wine production (Waterhouse and Walzem 1998). This can reduce the level of certain antioxidant components from processed food products and yield processing byproducts that still contain substantial levels of antioxidant compounds (Waterhouse and Walzem 1998; Skrede and others 2000).

While most studies examine the impact of specific factors on the content of antioxidant phytochemicals, few studies have examined their impact on the actual antioxidant activity of foods. In a survey of commercially available spinach, peas, green beans, and carrots, the highest antioxidant activity was generally found in the fresh, and then frozen produce. The antioxidant activity of canned and jarred products was about equal and was less than the same vegetables that were fresh or frozen. Ascorbate accounted for 47% and 23% of the total antioxidant activity in fresh peas and spinach, respectively (Hunter and Fletcher 2002).

Steam blanching followed by -20 °C frozen storage for up to a year resulted in vitamin C declines in carrots, broccoli, and green beans. Most of the loss occurred during blanching and early in the storage period, after which the rate of loss slowed. Ascorbic acid was much more stable after blanching and storage in broccoli and carrots than in green beans, which lost about 50% of their ascorbic acid content (Howard and others 1999). Additional relatively minor losses of ascorbic acid occurred in these foods during microwave cooking (Howard and others 1999). Peas lost about 20% of their ascorbate during blanching, and there was no further loss during 21 d of frozen storage (Hunter and Fletcher 2002). When fresh spinach was boiled in water, approximately 60% of the vitamin C was found in the cooking water and 40% in the cooked tissue (Gil and others 1999). In a study of various cooking methods of 4 different vegetables (bean sprouts, green beans, nappa cabbage, and spinach), microwave cooking and stir-frying in oil retained more vitamin C than either boiling or stir-frying in water (Masrizal and others 1997). Because of its water solubility, larger losses of vitamin C occur in processes that use water. Steam blanching is therefore more effective in retaining vitamin C before freezing than hot water blanching. Blanching inactivates enzymes, such as ascorbate oxidase, that are capable of breaking down vitamin C during frozen storage. A recent comprehensive study of the effects of processing on the antioxidants in peas, carrots, spinach, potatoes, and several brassicas found that losses in vitamin C during blanching and frozen storage ranged from about 10% to 40% (Puupponen-Pimiä and others 2003).

Carotenoids appear to be less adversely affected by processing compared with the other major fruit and vegetable antioxidants. In the same study (Puupponen-Pimiä and others 2003) in which vitamin C content was examined in carrots, spinach, potatoes, and several brassicas, there was essentially no loss in α -carotene and β -carotene after blanching. In fact, some increases were observed that were attributed to the dissociation of carotenoids from plant matrix materials. After blanching and freezing, losses in β -carotene content occurred between 6 and 12 mo of frozen storage. Pumpkin and carrot β -carotene retention was compared after pressure cooking and normal boiling (Gayarthri 2004). Retention was substantially greater in carrots compared with pumpkin. Although pressure cooking helped to retain more β -carotene was lost during pressure cooking compared with boiling.

The effect of processing on tomato lycopene has been well stud-

ied, partly because of the abundance of processed tomatoes in the Western diet. Compared with other antioxidants, including other carotenoids, lycopene is relatively stable. Lycopene can be lost as a result of thermal degradation and oxidation. During thermal processing, the naturally occurring trans-isomer, which is a linear molecule, can be converted to the cis-isomer, which is kinked. The degree of isomerization is directly correlated to the degree of thermal treatment (Shi and le Maguer 2000). Formation of cis-lycopene may be of benefit because cis-lycopene, although less stable, is believed to be more bioavailable in the body than *trans*-lycopene (Boileau and others 2002). Lycopene, like other carotenoids, is often higher in processed products because of its dissociation from plant matrix materials. Lycopene is readily degraded by exposure to air, especially in the presence of cationic pro-oxidant metals such as copper. This may be apparent by a loss in redness in products. Lycopene retention can be improved by storage at low temperature, low light, and low water content (Shi and le Maguer 2000).

Phenolic antioxidants are subject to degradation during processing. In the same study in which vitamin C, β -carotene, and α -carotene were examined in carrots, spinach, potatoes, and several brassicas, losses in total phenolic content after blanching and long-term frozen storage ranged from 20% to 30% (Puupponen-Pimiä and others 2003). In this study, total phenolic content was positively correlated with the total antioxidant capacity of these foods, suggesting that among vitamin C, carotene, and phenolics, phenolics make an important contribution to the antioxidant capacity of these foods. Like vitamin C, phenolic antioxidants are water-soluble and can be leached from fruit and vegetable tissues by processing in water. After fresh spinach was boiled in water, approximately half of the flavonoid content was found in the cooking water and the other half in the cooked tissue (Gil and others 1999). In processing blueberries into juice, substantial losses of phenolics occurred; the recovery of anthocyanins, procyanidins, and chlorogenic acid were 32%, 43%, and 53%, respectively. Heat-labile enzymes in blueberry fruit (for example, polyphenol oxidase) made a large contribution to the loss in anthocyanins. Approximately 20% of the anthocyanins in blueberries were retained in the press cake after juicing (Skrede and others 2000).

Conclusions

There is unprecedented awareness and interest in both the positive and negative impacts of dietary selection in human health. In focusing on approaches to maintain and improve health through dietary means, consumers, public health organizations, and the food industry wish to know more about the content of health-promoting phytochemicals, including antioxidants, in fruits and vegetables. They also wish to know how health-promoting components can be best preserved during storage and processing. Research that has addressed the content and retention of phytochemical antioxidants can help the horticulture and food industries to develop and promote foods based on their antioxidant content.

Some of the major conclusions of this review are summarized in Table 3. It is clear that, after food choice, the most effective means of improving the antioxidant content of the diet is by fruit and vegetable varietal selection. The substantial variation that exists in phytochemical antioxidant content means that breeders can select genotypes and develop varieties with high levels of antioxidants. Once high antioxidant varieties are available, new technologies in cultivar identification and traceability can make it possible to offer the consumer a wide selection of health functional varieties and to obtain premium prices for high-antioxidant produce.

In addition to genetic factors, environmental factors, before and

Factor	Ranking
Influence of genotype Influence of preharvest environmental factors	Vitamin C = carotenoids = phenolics Phenolics > Vitamin C > carotenoids
Losses during fresh storage	Vitamin C >> carotenoids >> phenolics
Increases during fresh storage	Carotenoids, phenolics
Losses during processing	Vitamin C > phenolics > > carotenoids

after harvest, can influence antioxidant content. Among the antioxidant groups discussed, phenolic antioxidants appear to be more responsive to environmental factors such as water availability, light quality, and temperature. These factors undoubtedly contribute to the seasonal variability in fruit and vegetable phenolic content. Phenolics also seem to be more affected by storage factors such as temperature, atmosphere, and light, than either carotenoids or vitamin C. Cultivar selection and manipulation of storage conditions could lead to specific conditions for optimal phenolic antioxidant enhancement. Because vitamin C is the most labile antioxidant and prone to loss during storage and processing, it may serve to indicate the quality of all antioxidant groups in a product. In developing processing technologies that optimize antioxidant retention, it is important to understand more fully the chemical properties of antioxidants, the enzymes that affect their content in plant tissue, and the partitioning of antioxidants among plant tissues.

Increasing the consumption of fruit and vegetable antioxidants requires the development of foods with optimal phytochemical antioxidant content without sacrifice to taste or convenience. Continued research into the many avenues presented here will underpin the development of food products with enhanced antioxidant content.

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