Processing Effects on Dietary Antioxidants from Plant Foods

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Epidemiological studies have revealed a lower risk of chronic diseases such as cancer and cardiovascular disease in populations that consume relatively large amounts of vegetables and fruits (Steinmetz and Potter, 1996). This effect has been attributed primarily to the antioxidant vitamins and their prevention of oxidative damage (Block et al., 1992; Byers and Guerrero, 1995; Elliott, 1999). Fruit, vegetable, and grain consumption provides a major portion of vitamins C and E and carotenoids in the diet, although fortified foods and supplements are becoming important sources. Most foods are eaten after they have been processed, so the physical and chemical changes that could affect their antioxidant content must be considered. Plant foods are usually consumed after cooking, a form of processing, as well as in their commercially canned, dehydrated, and frozen forms.

Fruit and vegetable consumption in the United States does not meet the recommendations of nutritionists and other health professionals (Nestle, 1997). Considerable data from national studies indicate that adults do not consume sufficient amounts of vegetables, fruits, and grains, and that children consume even less (Dennison et al., 1998; Muñoz et al., 1997; Patterson et al., 1990; Steinmetz and Potter, 1996; Subar et al., 1995). Government agencies, health organizations, and food processors have encouraged increased consumption of fruits and vegetables through advertisements, promotions, and Web sites (Kurtzwell, 1997; National Cancer Institute, 1997). Dietary recommendations suggest at least five servings each day of fruits and vegetables, as well as cereals and grains. However, the vegetables that are nutrient dense and good sources of antioxidant vitamins, such as the crucifers, represent a small fraction of the total intake for Americans (Kantor, 1998).

Considerable time and effort have been spent assessing changes in nutrients, particularly vitamins, during processing (see for example, Erdman and Klein, 1982; Williams and Erdman, 1998). In the last several years there has been increasing interest in the positive aspects of “phytochemicals” or “phytonutrients” (plant chemicals believed to be of benefit to the body that do not fit the definition of a vitamin) or “functional foods” (Hasler, 1998; Steinmetz and Potter, 1991). Health organizations recognize the potential value of these compounds as well (American Dietetic Association, 1995; Howard and Kritchevsky, 1997). Antioxidant vitamins in vegetables fall into the category of phytochemicals, but other compounds, such as lycopene, may be as important (Di Mascio et al., 1989; Giovannucci, 1999). More studies are focusing on changes in specific phytochemicals, such as the glucosinolates found in crucifers, that occur during processing and cooking (Howard et al., 1997).

From a review of the literature, the actions of some of the beneficial compounds in fruits and vegetables are obviously multifactorial—that is, they may serve as vitamins, but have other activities that are only peripherally related to their vitamin activity. This has led to controversy about recommended intakes. For instance, the amount of vitamin C required to prevent the deficiency disease scurvy is ≈10 $mg \cdot d^{-1}$ (Weber et al., 1996). Some researchers urge intakes of 200 $mg \cdot d^{-1}$ (Levine et al., 1996), but gram amounts were suggested by others (Hornig and Moser, 1981). The increased amounts are often connected with reported antioxidant activity.

In this brief overview of antioxidants in processed plant foods, we will focus on the antioxidant vitamins because these are the most studied. We will describe the major antioxidants, their stability in general, and the impact of processing. Other possible antioxidants present in plant foods will also be mentioned. In the next few years, some of these other compounds may become more prominent in health recommendations.

MAJOR ANTIOXIDANTS IN PLANTS

There are numerous compounds in plants that function as antioxidants. An antioxidant can be defined, chemically, as any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate. The Food and Nutrition Board of the National Academy of Science (1998) has proposed that “a dietary antioxidant is a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans.” In animals and humans, these reactive species are associated with lipid peroxidation and DNA damage, and with malignant transformation in vitro. Epidemiologically, the antioxidants are associated with lowered risk of cancer and heart disease (Byers and Guerrero, 1995; Steinmetz and Potter, 1996). Antioxidants have specific functionality, and phytochemicals that serve to delay oxidative damage must be in contact with substrate or reactive species, and able to react with free radicals of different types. Noguchi and Niki (1999) provide an excellent review of the chemistry of oxidation and antioxidant action. In both plant and animal tissues, antioxidants are associated either with lipid- or water-soluble fractions, so they are not interchangeable in oxidation reactions in vivo. Vitamin C, for example, is found in the aqueous cell phase, but vitamin E is lipophilic and located in membranes and lipoprotein fractions.

Epidemiological studies stress that intake of fruits and vegetables is associated with lower disease risk (Block et al., 1992). Correlating decreased risk with specific antioxidant nutrients or components is more difficult. Thus, recommendations to increase antioxidant intake emphasize consumption of plant foods, rather than individual compounds, because of the possible synergism among vitamins, minerals, and other phytochemicals that they contain.

Primary among the antioxidants are the micronutrient vitamins, such as ascorbate, vitamin E (tocopherols and tocotrienols), and carotenoids (some of which are vitamin A precursors). Vitamin functions require only small quantities (milligram or microgram). If higher quantities of a compound are needed to serve as antioxidants, sufficient amounts of vitamins may not be provided by a plant food. If we are interested in pharmacological doses, then fortification or nutrient supplementation would be needed, and many of these come from plant sources. In discussing the conservation or preservation of antioxidant vitamins in plant foods, one must keep in mind that they may act with other compounds to provide the nonvitamin function of protection from oxidative damage.

Ascorbic acid

Ascorbic acid (vitamin C) is a water-soluble antioxidant formed from a six-carbon compound derived from glucose (Jaffe, 1984). It is easily oxidized to form a free radical, semidehydroascorbic acid, that is relatively stable (Tolbert and Ward, 1982). Further oxidation generates diketogulonic acid, which has no biological function. The antioxidant activity of ascorbic acid is due to the ease of its loss of electrons, making it very effective in biological systems. Because it is an electron donor, it serves as a reducing agent for many reactive

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oxidant species. It protects compounds in the water-soluble portion of cells and tissues, and reduces tocopherol radicals back to their active form at the cellular membrane. It is also a cofactor for reduction of Fe^{3+} to Fe^{2+}. The interactions of ascorbic acid with minerals and with tocopherol are reminders of synergism in biological systems.

Carotenoids

Carotenoids are present in a great variety in plants, giving them color and serving a variety of functions (Simpson et al., 1985). There are >600 identified carotenoids, and only ≈50 of these have vitamin A activity (Boileau et al., 1999). From a nutritional standpoint, five carotenoids are of most importance for humans. These are α- and β-carotenes, lutein, cryptoxanthin, and lycopene (Mangels et al., 1993). Our primary food sources for naturally occurring forms are plant materials. Carotenoids interact with fat and possibly fiber, which is important from a health perspective. The food matrix, fat content, and dietary fiber (Boileau et al., 1999) affect intestinal absorption.

Carotenoids act as antioxidants in biological systems by quenching singlet oxygen and scavenging free radicals. They react with singlet oxygen to form a triplet excited carotenoid and a ground-state oxygen. The triplet excited carotenoid releases the energy in the form of heat and returns to ground state (Stahl and Sies, 1993). Carotenoids are potent singlet oxygen quenchers in biological systems (Papas, 1999), and lycopene appears to have the greatest antioxidant activity followed by α-carotene, β-carotene, lutein, and cryptoxanthin (Di Mascio et al., 1989; Liebler, 1993).

Vitamin E

Vitamin E is the major lipid-soluble antioxidant responsible for protecting the polyunsaturated fatty acids in membranes against lipid peroxidation, free radicals, and singlet oxygen species (Machlin and Bendich, 1987). The natural isomeric form (d-α-tocopherol) is most biologically active. The naturally occurring tocopherols (α, β, γ, δ) and tocotrienols are synthesized by plants (Papas, 1999). All chlorophyll a-containing tissues contain tocopherols, primarily in the chloroplasts. Tocotrienols have been identified in a number of plant tissues, ranging from kale (*Brassica oleracea* L. Acephala Group) and broccoli (*Brassica oleracea* L. Botrytis Group) to cereal grains and nuts (Piironen et al., 1986).

Tocopherols scavenge free radicals by reacting with lipid peroxyl radicals to produce a tocopheroxyl radical. The form most studied, α-tocopherol, is present in cell and organelle membranes (Papas, 1999). In vitro, the tocopheroxyl radical reacts with ascorbate to regenerate the tocopherol and produce an ascorbate radical. Glutathione is then required for the regeneration of ascorbate (Sies and Stahl, 1995). Although α-tocopherol also prevents lipid oxidation in lipoproteins, tocotrienols are thought to be more effective (Papas, 1999). The antioxidant properties of the tocopherols and tocotrienols appear to have no direct relationship to vitamin E activity.

OTHER ANTIOXIDANTS IN PLANT FOODS

A number of other compounds from plants are believed to have antioxidant activity, but their behavior during processing is not well established. Nonnutrients, such as flavonoids and glutathione, are possible contributors to antioxidant capacity. Some minerals, such as selenium in its role as a cofactor for glutathione peroxidase, can be considered as antioxidants. We know the most about vitamin concentration changes during processing, and they are well established as "natural antioxidants." However, the importance of fruits and vegetables in the diet goes beyond that of providing vitamins. We know much less about other phytochemicals, but recent studies suggest that they play a role in the battery of antioxidants in foods.

At least 2000 chemically distinct flavonoids that may have antioxidant activity occur in nature. These include flavonols (e.g., quercetin), flavones (e.g., apigenin), flavanones (e.g., naringenin), isoflavones (e.g., genistein), and flavanols (e.g., epicatechin), as well as anthocyanins (Formica and Regelson, 1995; Williamson et al., 1998). These compounds fall in the category of low molecular weight polyphenolics (Rice-Evans et al., 1995). They are associated with color in fruits and vegetables, and may contribute to some flavor sensations such as astringency and bitterness. Flavanols and flavones occur in numerous plant foods (Cao et al., 1996), notably tea [*Camellia sinensis* (L.) Kuntze] and red wine, and in crucifers (Plumb et al., 1997). Cereals, as well as vegetables and fruits, were identified as a possible major dietary source of a variety of flavonoids (Andlauer and Fürst, 1998). Anthocyanins provide the bright red and purple colors of fruits and flowers (Cook and Sammon, 1996).

Of the flavonoids, high molecular weight plant polyphenols (tannins) show the most promise in human health (Hagerman et al., 1998). Polyphenolic compounds have no vitamin function, but can be classified as phytochemicals that are biologically active. Many, such as the anthocyanins, flavanols, and isoflavones, have been associated with anticarcinogenic activity in animal or cell systems (Huang and Ferraro, 1991; Papas, 1999). Their mechanisms are not clearly defined, and there are very few studies on the effects of processing on these components. Hagerman and coworkers (1998) proposed that tannins (naturally occurring polyphenols that precipitate protein) could have unique roles as both antioxidants and protectors of other nutrients from oxidative damage. However, research to date has focused on structurally well-defined purified polyphenolic compounds.

Nicoli and coworkers (1997) suggested that the loss of antioxidant vitamins in food [e.g., tomato (*Lycopersicon esculentum* Mill.) puree] during processing could be compensated for by the appearance of other antioxidants. Specifically, they suggested that Maillard reaction products that result from the condensation of sugars with free amino acids might have both antioxidant and antimutagenic characteristics. This concept is yet to be proven, but emphasizes the importance of recognizing that losses of a known antioxidant could be counterbalanced by the development of others during processing (Nicoli et al., 1997).

A variety of other phytochemicals may have antioxidant potential. These include the sulfur-containing glucosinolates in cruciferous vegetables (Williamson et al., 1998), which are thought to be anticarcinogenic. Evidence to date, however, indicates that the importance of these compounds is not related to antioxidant activity. Antioxidants from spices and herbs, such as rosemary (*Rosmarinus officinalis* L.) and basil (*Ocimum basilicum* L.), are more likely candidates for food applications (Nakatani, 1992).

PROCESSING EFFECTS ON ANTIOXIDANT VITAMINS

The stability of nutrients in foods varies considerably, based on their chemical form, solubility, and susceptibility to environmental conditions (Table 1). Carotene and tocopherols are fat-soluble, so they are relatively unaffected by an aqueous environment. If the presence

Table 1. Stability of selected nutrients in food.[z]

		Effect of pH			Effect of environment		
Nutrient	Solubility	Acid	Base	Neutral	Air or O_2	Light	Heat
Vitamin A	Fat-soluble	U	S	S	U	U	U
Carotenes	Fat-soluble	U	S	S	U	U	U
Ascorbate	Water-soluble	S	U	U	U	U	U
Tocopherols	Fat-soluble	S	S	S	U	U	U
Minerals	Water-soluble in some forms	S	S	S	S	S	S

[z]S: Stable (not easily destroyed); U: unstable (easily destroyed). These are generalizations and do not take into account the stability of all forms of the micronutrient. Data adapted from Gregory (1996).

of oxygen affects a micronutrient, it is likely to be a good antioxidant. If light and heat affect it as well, processing conditions of high heat in the presence of light could be deleterious. On the other hand, absence of oxygen, as in canning, will help stabilize these nutrients, and the environment (water or fat) will also influence retention. Some types of processing will reduce the antioxidant potential of fruits and vegetables. For example, dehydration, whether by sun or artificial heat sources, will affect not only ascorbate, but also tocopherols and carotenoids, as a result of exposure to ultraviolet light, air, and heat. Dried fruits have essentially no vitamin C content, for example. Minerals, on the other hand, are well retained because they occur as relatively insoluble complexes, and may be bound to membranes or other components of the food matrix, and thus are stable to oxygen and to heating.

Note that considerable variation exists in the initial plant material, even within a species, depending on the genotype and environmental conditions. Recent studies completed at the Univ. of Illinois show wide variations in glucosinolate content among cruciferous vegetable genotypes (Kushad et al., 1999), as well as antioxidant vitamins (Kurilich et al., 1999). These differences suggest that the health values of these vegetables vary, and that there is opportunity for enhancement of antioxidant and vitamin levels through genetic manipulation.

Ascorbic acid

Ascorbic acid, specifically the reduced form, is used as an index or indicator nutrient for the effects of processing (see review by Erdman and Klein, 1982). Because it is water soluble and easily oxidized, especially at neutral pH in aqueous solutions, ascorbate is the least stable micronutrient in fruits and vegetables. Research on vitamin retention during processing usually focuses on vitamin C, assuming that if this labile nutrient is well retained, others that are similar in stability will also be preserved. Many studies over the last 50-plus years have shown that ascorbic acid losses are related to the amount of water used, heat applied, and duration of heating (Erdman and Klein, 1982; Gregory, 1996).

Foods that are high in vitamin C content before processing tend to be high afterwards, but losses can range from as little as 10% to 20% to almost 100% during cooking or canning (Erdman and Klein, 1982). For example, the vitamin C content of citrus fruits and juices is well known and its stability during storage and processing is established. The low pH of citrus products and the relatively high concentrations of ascorbic acid stabilize the water-soluble antioxidant. The exclusion of trace transition minerals, such as iron and copper, will further enhance stability.

In a recently completed study of vitamin retention in fresh and processed vegetables, broccoli, carrots (*Daucus carota* L.), and green beans (*Phaseolus vulgaris* L.) from a single field were either stored under refrigeration, canned, or frozen (Howard et al., 1999). During refrigerated storage (4 °C), ascorbic acid declined slightly in both carrots and broccoli, but substantial losses were apparent in green beans within hours postharvest (Fig. 1). The stability of ascorbic acid in broccoli during the 2 years of harvest and storage is shown in Fig. 2 for both noncooked and cooked forms. Blanching raw broccoli reduces the content of vitamin C from ≈1.2 g·kg^{-1} to ≈0.8–0.9 g·kg^{-1} (0 time in Fig. 2). Ascorbic acid is well retained over the recommended storage period of 6 months for frozen broccoli; there was no significant difference between 0 time and 225 d of storage. Although ≈20% of the ascorbic acid was lost in the blanching, thereafter the decline was very gradual. Microwave cooking had no significant effect on loss of vitamin C. This confirms that the losses after blanching are due to solubility and leaching of ascorbic acid (Selman, 1993). It also shows that a cooking method that uses little water (microwave cooking) and relatively short heating time helps conserve the vitamin C. Under the same conditions, β-carotene in broccoli was conserved, because of its relative insolubility in water. Other studies have shown similar results (Martin et al., 1960; Wu et al., 1992).

Carotenoids

Processing methods, such as dehydration, blanching, and canning, are thought to result either in loss of carotenoids or their isomerization from *trans* to *cis* isomers. Carotenoids are much more stable than ascorbic acid, but present a unique challenge in accurate measurement (Williams and Erdman, 1998). Early studies of carotene content in fruits and vegetables used assays that measured total carotenes. With the advent of HPLC, methods were developed that can separate not only the α- and β-carotenes (Kurilich et al., 1999), but also the *cis* and *trans* forms (Emenhiser et al., 1996; Simpson et al., 1985). Thus, large discrepancies in carotenoid content in vegetables among studies can be attributed to measurement variability and variation in the original material (Granado et al., 1997).

Another unique characteristic of carotenes is that they can be bound in carotenoproteins or may be associated with the plant matrix (Boileau et al., 1999). Following heating, the carotenes are released, resulting in apparent increases in carotene content after cooking or thermal processing. This can be viewed as an artifact of the assay procedure, because the carotenes are more easily extracted when they are not bound. Almost uniformly, we find, for example, that cooked carrots or pumpkin (*Cucurbita pepo* L.) have more measurable carotene than the noncooked food. One of the challenges in nutrition is to establish whether the protein-bound carotenes are as bioavailable as those that have been released. Evidence suggests that they are not, and that release of carotenoids, such as lycopene in tomatoes, is needed for their effective utilization (Giovannucci, 1999; Giovannucci et al., 1995; Tonucci et al., 1995). Heating tomato juice or steaming spinach (*Spinacia oleracea* L.), for example, increases the amount of carotenoids extracted (Dietz et al., 1988). Studies of the effects of cooking or thermal processing show little effect on β-carotene retention, although other carotenoids may be lost (Boileau et al., 1999).

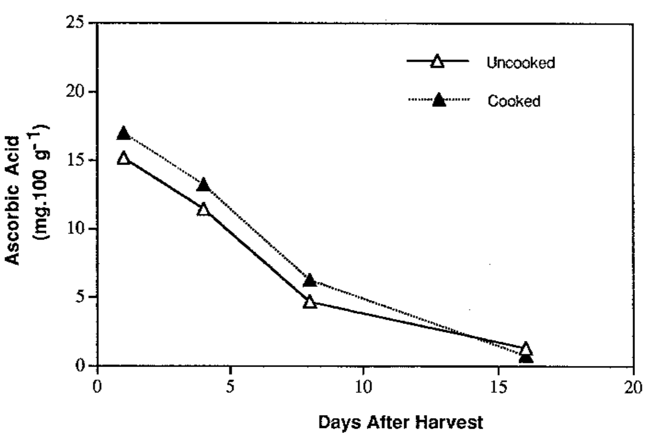

Fig. 1. Ascorbic acid content (mg·100 g^{-1}) of green beans harvested in 1995 and stored at 4 °C for 16 d, uncooked and microwave-cooked.

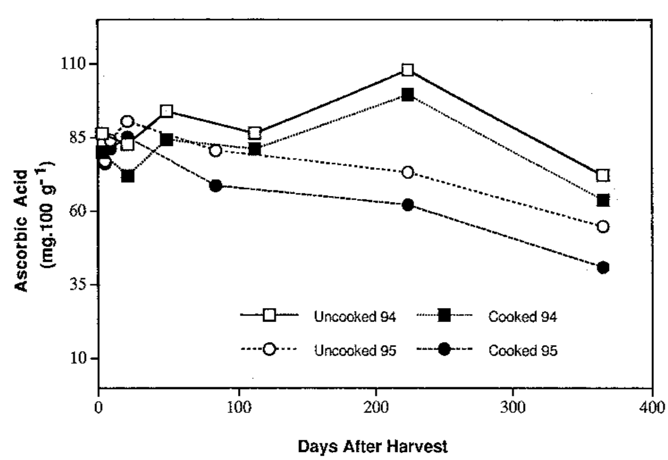

Fig. 2. Ascorbic acid content of broccoli (mg·100 g^{-1}) harvested in 1994 and 1995, blanched, frozen, and stored at –20 °C for up to 1 year, before and after microwave cooking.

The *trans* form of β-carotene is dominant in plant foods, but some studies show that thermal processing converts the all-*trans* forms to *cis* forms (Chandler and Schwartz, 1987, 1988). Many of the *cis*-isomers are found in human tissue, so transformation of *trans* to *cis* takes place in the body, as well as during processing. As part of the study by Howard et al. (1999), retention of *trans*-β-carotene in canned carrots was examined in consecutive harvests in 1994 and 1995 (Fig. 3). Significant amounts of *trans*-β-carotene were lost during the canning process, but its concentration remained stable thereafter. Conversion to *cis*-β-carotene may have occurred during canning, as noted by Chandler and Schwartz (1988) in sweet potatoes [*Ipomoea batatas* (L.) Lam.]. Isomerization of *trans*-β-carotene can also be induced by exposure to light, acid, and processing chemicals, such as hypochlorite (Gregory, 1996), resulting in loss of activity.

Vitamin E

Oils from seeds are the major food source of vitamin E, with safflower (*Carthamus tinctorius* L.), sunflower (*Helianthus annuus* L.), olive (*Olea europaea* L.), and canola (*Brassica napus* L.) oils being highest in α-tocopherol (Bauernfeind, 1980). Losses during oil processing can occur during bleaching, deodorizing, and refining, as well as during hydrogenation. Retentions as low as 30% during oil hydrogenation were estimated. Some tocopherols can be found in the sludge from vegetable oil processing; heating during processing or cooking also results in significant losses of tocopherols, estimated at 30% to 35% of initial levels (Bauernfeind, 1980).

When grains, grasses, and corn (*Zea mays* L.) are exposed to sunlight during drying or ensilage, tocopherol content is reduced. Sun-drying means exposure to ultraviolet light and heat. Artificial drying methods under more controlled light and heat are less destructive. Certain treatments of grains with a variety of acids or water can result in a lowered amount of vitamin E–active compounds. This is believed to result from an acceleration of oxidation of vitamin E at low pH, so organic acids that are added as antifungal agents to malted barley (*Hordium vulgare* L.) or during water-soaking of wheat (*Triticum aestivum* L.) increase this effect (Bauernfeind, 1980). Milling removes portions of the grain, such as wheat germ, that are high in vitamin E. Because the germ is also high in oil, it is usually removed to prevent flavor changes that occur with lipid oxidation. Other steps in milling of flour or bread dough formation encourage the oxidation of carotenoids or gluten, and in the process reduce the tocopherol content.

The relatively few studies on the stability of vitamin E during baking, freezing, and canning were performed in the 1960s, when measurement of tocopherols was not well standardized, so there are many discrepancies in the findings. Two studies of bread baking showed that during the baking process, bread loses 35% to 40% of initial total tocopherol content, with α-tocopherol being the most easily destroyed form. Less destruction (≈10%) occurred during baking of rye (*Secale cereale* L.) bread (Bauernfeind, 1980).

Few comparisons of vitamin E stability in raw and processed plant foods appear in the literature (Hogarty et al., 1989; Piironen et al., 1986). There are reported losses of 40% to 65% for vitamin E in beans, corn, and peas (*Pisum sativum* L.) during the canning process. However, Hellendoorn and coworkers (1971) showed that α-tocopherol was stable both during canning and storage of canned vegetables. In the most recent study, Piironen et al. (1986) found that the tocopherol content appeared to be stable during freezing and storage of vegetables. These Finnish researchers found that green, leafy vegetables [broccoli, brussels sprouts (*Brassica oleracea* L. Gemmifera Group)] were high in α-tocopherol, but that levels in white cabbage (*Brassica oleracea* L. Capitata Group) and cauliflower (*Brassica oleracea* L. Botrytis Group) were extremely low. They reported that there was little difference in α-tocopherol in fresh vs. frozen vegetables. This suggested that α-tocopherol was stable during blanching and freezing, although their work did not confirm an earlier study.

Recently, Wyatt et al. (1998) examined α- and γ-tocopherol content of commonly consumed Mexican foods. Processing of raw corn into tortillas, which involves treatment with alkaline solution, destroyed most tocopherols. Cooking losses for other grains were 22% to 55%, and ranged from 9% to 59% for legumes. Vegetable oils supplied a major proportion of the vitamin E in diets of Mexican consumers.

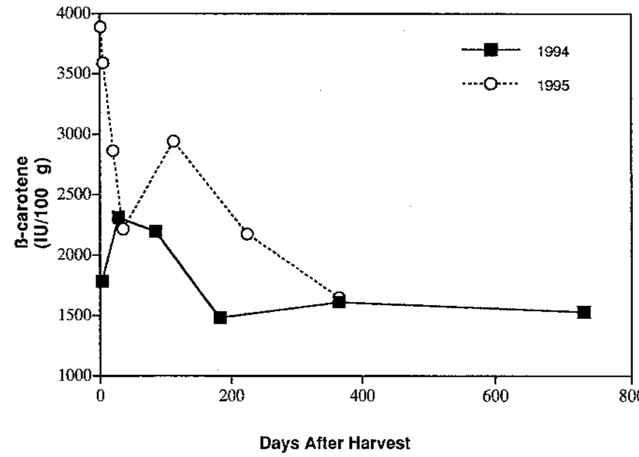

Fig. 3. β-Carotene content (expressed as IU Vitamin A/100 g) of carrots harvested and canned in 1994 and 1995, stored for up to 750 d.

IMPLICATIONS

Diets that are high in plant foods, whether they are cereals, fruits, or vegetables, confer significant benefits to human health. Epidemiological data, as well as in vitro studies, strongly suggest that foods containing phytochemicals with antioxidation potential have protective effects against major disease risks, including cancer and cardiovascular disease. A wide spectrum of compounds with the ability to counter oxidative damage has been identified, chief of which are the micronutrient vitamins C and E and carotenoids. Polyphenolics, Maillard reaction products, and glucosinolates are among the other antioxidants that may be obtained from plant foods, although their efficacy is less well established.

During processing, the antioxidant compounds may undergo physical and chemical changes that alter their potential benefits. Generally, thermal processing results in a decrease in ascorbate and tocopherols and an increase in available carotenes. Once processed, frozen or canned fruits, vegetables, and grains retain ascorbate, carotene, and tocopherol levels for prolonged periods. Since other micronutrient phytochemicals may act in concert with vitamins, future studies should be considered to investigate the effects of processing on other naturally occurring antioxidants.

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