

# Antioxidant Capacity of Berry Crops, Culinary Herbs and Medicinal Herbs

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**Keywords:** antioxidants, anthocyanins, carotenoids, flavonoids, phenolics

## Abstract

Herbs and berry crops have been shown to contain high levels of antioxidant compounds. These antioxidants are capable of performing a number of functions including acting as free radical scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors, and synergists. The different antioxidant components found in herbs and berry crops provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease, in addition to a number of other health benefits. Herbs have been used for many purposes including medicine, nutrition, flavorings, beverages, and industry. Since prehistoric times, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the Nineteenth Century. Today herbs are still found in 40 percent of prescription drugs. Culinary herbs also have been grown and used for their ability to enhance and complement the flavors of a wide variety of foods. Even though a variety of herbs are known to be remarkable sources of phenolic compounds, data on the composition and antioxidant activities of herbs and berry crops are insufficient. We found herbs and berry crops to contain a number of healthful phytochemicals such as vitamin E, vitamin C, beta carotene, flavonoids, phenolic acids and are an effective and potential source of natural antioxidants. The results from this presentation will be useful to plant breeders, other researchers, and the general public who are interested in the antioxidant potentials of various herbs and berry crops as dietary supplements.

## INTRODUCTION

Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu et al., 1998). The antioxidant activity of phenolic compounds are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxide (Osawa, 1994). In general there are two basic categories of antioxidants, natural and synthetic. Recently, interest has been increasing considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity (Ito et al., 1983).

Berry fruits, and herbs contain high levels of antioxidant compounds, which provide protection against harmful free radicals and have been associated with a number of health benefits (Ames et al., 1993; Cao et al., 1996; Velioglu et al., 1998; Wang et al., 1996). Herbs have been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking, and industrial uses. Since prehistoric times, herbs were the basis for of nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Today herbs are still found in 40 percent of prescription drugs (Smith and Winder, 1996). Culinary herbs have been grown and used for hundreds of years, and they are becoming increasingly popular in the United States for their ability to enhance and complement the flavors of a wide variety of foods (Hacskeylo, 1996).

A variety of herbs are known to be sources of phenolic compounds and various herbs along with fruits contain numerous phytochemicals in addition to phenolic

compounds, such as nitrogen compounds, carotenoids, and ascorbic acid (Larson, 1988; Velioglu et al., 1998). Many of these phytochemicals possess significant antioxidant capacities (Larson, 1988). Diets rich in fruits, vegetables and herbs significantly reduce the incidence and the mortality rates of cancer, cardiovascular disorders, and other degenerative diseases caused by oxidative stress. Eating fruits, vegetables and herbs also reduces blood pressure, enhances the immune system, detoxifies contaminants and pollutants, and reduces inflammation (Ames et al., 1993; Larson, 1988; Velioglu et al., 1998). Therefore, modification of dietary and lifestyle habits can lead to significant improvements in human health. This paper summarizes the antioxidant capacities of various berry fruits and herbs and the factors which affect their antioxidant activities. This paper also suggests some strategies for establishing new research and production paradigms for enhancing antioxidant capacity and nutrient quality.

## **DIETARY ANTIOXIDANTS**

The phytochemicals in plant tissues responsible for antioxidant capacity can largely be attributed to the phenolics, anthocyanins, carotenoids, and other flavonoid compounds. Fruits, vegetables and herbs contain many of these components.

### **Anthocyanins and Other Flavonoids**

In the typical U.S. diet, daily intake of anthocyanins is approximately 180-215 mg/day, and represents the largest group of phenolic compounds in the human diet (Kühnau, 1976). The content of anthocyanins in fruit of blackberry, blueberry, cranberry, raspberry, strawberry and boysenberry range from 200 to 4950 mg.kg<sup>-1</sup> of fresh weight. In blueberries, the anthocyanin content is 10 to 200 times of the quercetin (Prior et al., 1998). The anthocyanins are glycosides and acylglycosides of anthocyanidins. Some common anthocyanidins have varying different hydroxyl or methyl substitutions in their basic structure, flavylium. Over 250 naturally occurring anthocyanins exist, and are differentiated further by their *O*- glycosylation with different sugar substitutes (Francis, 1989). Glucose, rhamnose, xylose, galactose, arabinose, and fructose are the most common sugars substituted on the aglycon (anthocyanidin). The common anthocyanins are either 3- or 3, 5-glycosylated. Free radical scavenging properties of the phenolic hydroxy groups attached to ring structures are responsible for the strong antioxidant properties of the anthocyanins (Rice-Evans and Miller, 1996; Wang et al., 1997). They help reduce damage caused by free-radical activity and low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilation of arteries (Heinonen et al., 1998; Rice-Evans and Miller, 1996).

The other flavonoids, flavones (e.g., apigenin, luteolin, diosmetin), flavonols (e.g., quercetin, myricetin, kaempferol), and their glycosides are the most common compounds. They occur in nearly all our common fruits, vegetables and herbs. These flavonoids have been showed antioxidant and antitumor properties and also have applications as antibiotics, antidiarrheal, antiulcer and anti-inflammatory agents as well as in the treatment of diseases such as hypertension, vascular fragility, allergies, hypercholesterolemia (Kühnau, 1976; Larson, 1988; Rice-Evans and Miller, 1996; Heinonen et al., 1998).

### **Carotenoids**

Carotenoids are found in many fruits and vegetables such as carrots, dark leafy greens, orange and yellow vegetables and orange fruits. They are comprised of a set of several hundred pigmented, fat soluble antioxidants. The most abundant carotenoids in fruits and vegetables are  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, lutein, zeaxanthin, and  $\beta$ -cryptoxanthin. These carotenoids account for more than 90% of the carotenoids present in the human diet (Gerster, 1997). The nature of the carotenoids affects its bioavailability and lutein is five times more readily available in the human body than  $\beta$ -carotene (van het Hof et al., 1999). Combination of fatty foods with carotenoid-rich fruits and vegetables enhanced carotenoids uptake. Recent studies have shown that the bioavailability of lycopene from tomato has increased dramatically by heat treatment in the presence of oil

(Gartner et al., 1997). Lycopene was found to be more bioavailable from tomato paste than from fresh tomato due to heat treatment and the presence of higher oil content in the paste (Stahl and Sies, 1992). Epidemiological studies have shown that the increased consumption of foods rich in carotenoids is correlated with a diminished risk for several diseases.  $\beta$ -carotene is the essential precursor to retinol or vitamin A, and this conversion occurs in approximately 10% of the carotenoids. Recent studies have suggested that  $\beta$ -carotene itself may reduce the risk of cancer and heart diseases even before being converted into vitamin A and may account for much of the health benefits attributed to fruits and vegetables (Olson, 1999; Giovannucci, 1999). As an excellent antioxidant and radical trapping agent,  $\beta$ -carotene has the capability to protect membranes, DNA, and other cellular constituents from oxidative damage (Byers and Perry, 1992). Among all carotenoids, lycopene appears to have the greatest antioxidant activity, followed by  $\alpha$ -carotene,  $\beta$ -carotene, lutein, and  $\beta$ -cryptoxanthin and the singlet oxygen quenching ability of lycopene is twice that of  $\beta$ -carotene and 10 times that of  $\alpha$ -tocopherol (DiMascio et al., 1989; Liebler, 1993).

### **Vitamins E and C**

Vitamin E is a fat-soluble compound found in a variety of nuts, grains, and vegetable oils. No clinical deficiency syndrome has ever been identified, and thus the U. S. recommended dietary allowance for vitamin E ranges from only 5 U to 10 U (National Research Council, 1989). However, supplementation of vitamin E is thought to have multiple protective effects in a variety of conditions such as preventing heart attacks and destroying nitrite, an essential component in the food chain associated with the production of glandular stomach cancer (Weisburger, 1991).

Vitamin C occurs naturally in citrus fruits, green peppers, cabbage, strawberries, tomato, tomato-based products, potato, green leafy vegetables and herbs. It is highly water-soluble, in contrast to vitamin E which is lipid-soluble. Vitamin C has powerful antioxidant properties and indirectly contributes to several key oxidative and reductive enzyme systems, and has ability to regenerate other biologically important antioxidants, such as glutathione and vitamin E, into their reduced state (Halpner et al., 1998; Jacob, 1995). The biological function of vitamin C is based on its ability to donate electrons, which provide intra- and extra-cellular reducing power for a variety of biochemical reaction. It has been reported that the reducing power of vitamin C is capable of neutralization most of the physiologically relevant reactive oxygen and nitrogen species in the human body (Buettner, 1993). The substantially high cellular levels of vitamin C provide antioxidant protection in the eye against photosynthetically generated free-radicals (Delamere, 1996) and against plasma and low density lipoprotein oxidation (Frei et al., 1989; Jialal et al., 1990). Vitamin C also functions as a reducing agent for mixed-function oxidases involved in drug metabolism by inactivating a wide variety of xenobiotic substances and hormones (Tsao, 1997). Therefore, supplementation of vitamin C could enhance many life-supporting biochemical systems. Furthermore, like vitamin E, it plays an important role in reducing oxygen toxicity and is also an excellent nitrite trapping agent for preventing gastric cancer (Weisburger, 1991).

### **ANTIOXIDANT CAPACITY OF BERRY FRUITS**

Berry fruits contain a wide range of flavonoids and phenolic acids. Main flavonoid subgroups in berries are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic acids present in berries and herbs are glycosides of hydroxylated derivatives of benzoic acids and cinnamic acid (Macheix et al., 1990). Antioxidants have long been used for food preservation, but there has been concern that synthetic antioxidants used for preservation such as butylated hydroxyanisole (BHA) and butylated hydroxytoluence (BHT) may cause liver damage and carcinogenicity (Gartner et al., 1997). Thus, the interest in natural antioxidants has increased considerably (Stahl and Sies, 1992). In addition, high levels of natural antioxidants have been shown to have multiple benefits to human health (Larson, 1988). Berry fruits had high antioxidant capacity against peroxy radical ( $ROO^{\cdot}$ ). Among the berry fruits, chokeberries, lingonberries, blackberries, blueberries, black raspberries

had higher antioxidant activities (oxygen radical absorbance capacity, ORAC) than red raspberries and, cranberries, and strawberries generally had the lowest values of antioxidant activity against  $\text{ROO}^\cdot$  (Wang and Lin, 2000). The influence of species on antioxidant activity was significant (Wang and Lin, 2000). The total anthocyanin, phenolic content and ORAC  $\text{ROO}^\cdot$  values in chokeberries were 4.28 mg of cyanidin 3-glucoside equivalents /g fresh weight, 25.56 mg of gallic acid (GRE) equivalents/g fresh weight and 160.2  $\mu\text{mol}$  of Trolox (TE) equivalents/g fresh weight, respectively. Previous research showed that a linear relationship existed between total phenolic or anthocyanin content and ORAC in various berry crops (Wang and Lin, 2000; Prior et al., 1998; Kalt et al., 1999). This indicated that the antioxidant activity of fruit is mainly derived from the contribution of phenolic and anthocyanin compounds in fruits.

Berries also possess antioxidant activities against radicals other than  $\text{ROO}^\cdot$  such as superoxide radicals ( $\text{O}_2^\cdot$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydroxyl radicals ( $\text{OH}^\cdot$ ), and singlet oxygen ( $^1\text{O}_2$ ) (Wang and Jiao, 2000). Different species and cultivars showed varying degrees of scavenging capacity on different active oxygen species ( $\text{O}_2^\cdot$ ,  $\text{H}_2\text{O}_2$ ,  $\text{OH}^\cdot$ , and  $^1\text{O}_2$ ) (Wang and Jiao, 2000). The scavenging capacity among the berries ranged from 40.8 to 72.0% for  $\text{O}_2^\cdot$ , from 50.7 to 73.9% for  $\text{H}_2\text{O}_2$ , from 52.4 to 77.3% for  $\text{OH}^\cdot$ , and from 6.3 to 17.4% for  $^1\text{O}_2$ . Among the small fruits of blackberry, strawberry, cranberry, raspberry, and blueberry, blackberries had the highest antioxidant capacity against  $\text{O}_2^\cdot$ ,  $\text{H}_2\text{O}_2$ , and  $\text{OH}^\cdot$ . Meanwhile strawberry was second best in the antioxidant capacity assay for these same free radicals ( $\text{O}_2^\cdot$ ,  $\text{H}_2\text{O}_2$ , and  $\text{OH}^\cdot$ ). With regards to  $^1\text{O}_2$  scavenging activity, strawberry had the highest activity, while blackberry was second. Among the least effective scavengers was raspberry for  $\text{O}_2^\cdot$ , cranberry for  $\text{H}_2\text{O}_2$  and for  $\text{OH}^\cdot$  and  $^1\text{O}_2$  (Wang and Jiao, 2000).

Berries contain a wide range of phenolic acids, flavones and flavonoids. The main flavonoid subgroups in berries are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic acids present in berries are glycosides of hydroxylated derivatives of benzoic acid and cinnamic acid (Macheix et al., 1990; Wang and Zheng, 2001). Acidic compounds incorporating phenolic groups have been repeatedly implicated as active antioxidants. Phenolic acids such as caffeic acid, chlorogenic acid, *p*-coumaric acid and vanillic acid are widely distributed in berries as natural antioxidants (Macheix et al., 1990, Shahidi and Wanasundara, 1992). Their antioxidant activities are associated with the number of hydroxyl groups in their molecule structure to some extent. For example, in comparing two hydroxycinnamic acids, caffeic acid and *p*-coumaric acid, the antioxidant activity of the former was higher than that of the latter. It was likely that dihydroxylation in the 3, 4 position could enhance antioxidant potency due to being more available as hydrogen donors (Shahidi and Wanasundara, 1992). Caffeic acid and its derivative were found to be two major phenolic acids in chokeberry and both had a high proportion of antioxidant activity with 20.6% and 17.6%, respectively.

Chlorogenic acid was found to be the most abundant phenolic acid in the blueberry extract and also the most active antioxidant. A  $1.2 \times 10^{-5}$  M solution of chlorogenic acid inhibited over 80% of peroxide formation in a linoleic acid test system (Larson, 1988). We found that chlorogenic acid was effective in inhibiting  $\text{OH}^\cdot$ ,  $\text{O}_2^\cdot$ , and  $\text{H}_2\text{O}_2$  free radicals. Vanillic acid is a benzoic acid derivative and was found in cranberries (49.3  $\mu\text{g/g}$  fresh weight) and contributed 4.4% of total antioxidant activity in cranberries. The activity of cinnamic acid derivatives with two hydroxyl groups is superior to other phenolic acids with only one free OH group (Larson, 1988; Pratt, 1992). *p*-Coumaric acid and *p*-coumaroyl glucose occurs naturally in lingonberries and strawberries and also had antioxidant activity. Ellagic acid, a putative anticarcinogenic compound, was detected in many berry crops (Maas et al., 1991; Wang et al., 1994) and contributed 9.8% of total antioxidant activity in blackberries. *p*-Coumaroyl-glucose and ellagic acid together constituted 12.7% of the total antioxidant activity in strawberries.

The most important single group of phenolics in berries are flavonoids which consist mainly of proanthocyanidins, anthocyanidins, flavones, flavonols and their glycosides (Macheix et al., 1990). Proanthocyanidins are polyflavonoid in nature,

consisting of chains of flavan-3-ol units. Proanthocyanidins have relatively high molecular weights and have the ability to complex strongly with carbohydrates and proteins. Flavones (quercetin, myricetin and kaempferol) showed high antioxidant activity and have a structure which allows them to have more effective antioxidant activity than that of anthocyanins. The 2, 3 double bond in conjunction with a 4-oxo function in the C ring of quercetin allowed electron delocalization from the B ring and showed extensive resonance, thereby resulting in significant effectiveness for radical scavenging (Rice-Evans et al., 1995). An additional OH group at C5' of quercetin, as in myricetin, increases the ORAC value. Wang et al. (1997) also reported that the antioxidant activity of myricetin was higher than quercetin when comparing ORAC values (4.32 vs 3.2). Kaempferol, which is a related structure of quercetin, has only a single 4'-OH group in the B ring, and has much lower antioxidant activity compared to quercetin. The antioxidant capacities (ORAC value) for quercetin and kaempferol are 3.29 and 2.67, respectively (Cao et al., 1997).

The occurrence of *p*-coumaroyl glucose, dihydroflavonol, quercetin 3-glucoside, 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide have been detected in berry fruits and are effective antioxidants (Macheix et al., 1990; Pratt, 1992; Bakker et al., 1994; Gil et al., 1997; Maybry et al., 1970; Van Buren, 1970). Kaempferol 3-glucoside and Kaempferol 3-glucuronide constituted 4.6 and 4.3% of the total antioxidant activity in strawberries. Quercetin contributed 15.3%, 21.9%, 8.8% and 27.5% of total antioxidant activity in blueberries, cranberries, chokeberries and lingonberries, respectively. Kaempferol and quercetin are potent quenchers of ROO<sup>·</sup>, O<sub>2</sub> and <sup>1</sup>O<sub>2</sub> (Larson, 1988). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing bioavailability of carcinogens (Starvick et al., 1992).

Anthocyanins are probably the largest group of phenolic compounds in the human diet. Anthocyanins have been used for several therapeutic purposes including the treatment of diabetic retinopathy, fibrocystic disease, and visual disorders (Leonardi, 1993; Scharrer and Obe, 1981). Berry fruits contained a large number of anthocyanins (cyanidin, delphinidin, pelargonidin, peonidin, and malvidin). The total ORAC value of 11 identified anthocyanins in blueberry accounted for 56.3% of the total ORAC value. Pelargonidin 3-glucoside was the predominant anthocyanin in strawberries and contributed 27.3% of total antioxidants, while pelargonidin 3-rutinoside constituted 8.3%. Peonidin 3-galactoside comprised 20.8% of the total ORAC value in cranberry extract. Cyanidin 3-galactoside was the predominant anthocyanin in chokeberry and lingonberry and 43.5% of antioxidant capacity in lingonberry was derived from cyanidin 3-galactoside. Andersen (1985) identified cyanidin 3-galactoside (88%) as the main anthocyanin in Norwegian lingonberry. In addition to cyanidin 3-galactoside, cyanidin 3-arabinoside was also a major constituent in chokeberry and had a remarkably high ORAC value (17.49 μmol of TE/g).

It has been shown that anthocyanidins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups attached to ring structures (Rice-Evans et al., 1995; Rice-Evans and Miller, 1996; Wang et al., 1997). The hydroxyl radical scavenging activities of flavonoids increase with the number of hydroxyl groups substituted on the B-ring, especially at C-3' (Ratty and Das, 1988). A single hydroxy substituent generates little or no antioxidant. All flavonoids such as cyanidin, with 3', 4'-dihydroxy substitution in the B ring and conjugation between the A- and B-rings, possess antioxidant activity (Dziedzic and Hudson, 1983) and have antioxidant potentials four times that of Trolox (Rice-Evans et al., 1995). Cyanidin showed higher antioxidant activity and the order of antioxidant potency defined by ORAC values was as follows: cyanidin > delphinidin > malvidin ≈ peonidin ≈ petunidin > pelargonidin (Satué-Gracia et al., 1997; Wang et al., 1997). The results from our experiments showed that chokeberries, and lingonberries had higher antioxidant activities than cranberries and that strawberries generally had lower antioxidants. Chokeberries and lingonberries contained high amounts of cyanidin, strong antioxidant, whereas strawberries are rich in pelargonidin and ascorbic acid, which are weak antioxidants (Macheix et al., 1990). The antioxidant capacities (ORAC value) for cyanidin 3-glucoside and pelargonidin 3-glucoside were found to be 2.24 and 1.54, respectively (Cao et al., 1997; Wang et al., 1997). The anthocyanin content

in berries was found to correlate with oxygen radical absorbance capacity against  $\text{ROO}^\cdot$ ,  $\text{O}_2^\cdot$ ,  $\text{H}_2\text{O}_2$ ,  $\text{OH}^\cdot$ , and  $^1\text{O}_2$  with correlation coefficients from 0.855 to 0.980 (Wang and Zheng, 2001).

Berry crops showed remarkably high scavenging activity of chemically generated active oxygen species. For example, with 100 g of 'Earliglow' strawberries, the antioxidant capacity against  $\text{ROO}^\cdot$ ,  $\text{O}_2^\cdot$ ,  $\text{H}_2\text{O}_2$ ,  $\text{OH}^\cdot$  and  $^1\text{O}_2$  was equal to 375.6 mg of Trolox, 188.9 mg of  $\alpha$ -tocopherol, 44.2 mg of ascorbic acid, 155.7 mg of chlorogenic acid and 33.8 mg of  $\beta$ -carotene, respectively (Wang and Zheng, 2001). Clearly, consumption of berry crops indeed is beneficial to our health.

## ANTIOXIDANT CAPACITY OF HERBS

Herbs contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids. The antioxidant effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. Herbs have been investigated for their quenching activity of reactive oxygen species (Masaki et al., 1995). Kim et al. (1994) had determined the antioxidant activities from the methanol extracted of 180 selected Oriental herbs. Among the herb extracted, 44 species had antioxidant activities. *Psoralea corylifolia* L. (Malaytea Scurfpea), *Epimedium koreanum* Nakai (Koreanum Epimedium), *Syringa dilata* Nakai (Clove), *Prunus mume* Sieb. et Zucc. (Mumeplant), and *Aconitum rocyanum* Raymund (Arbor Monkshood) showed particularly high antioxidant activity. The antioxidant activities of selected medicinal plants are also reported by Pietta et al. (1998). Green tea, oligomeric procyanidins (from grape seed and pine bark), bilberry, and ginkgo exhibited a valuable antioxidant capacity. Zheng and Wang (2001) studied the antioxidant capacity of 39 culinary and medicinal herbs and found that the medicinal herbs with the highest antioxidant values were *Catharanthus roseus*, *Thymus vulgaris*, *Hypericum perforatum*, and *Mentha x piperita*. *Catharanthus roseus* is also known by its common name, Madagascar periwinkle. The alkaloids, vinblastine and vincristine, extracted from the dried whole plant are widely used today in the treatment of leukemia, lymphomas and cancer. *Hypericum perforatum* is also called Saint-John's-Wort. The fresh or dried leaves and flowers of this plant have traditionally been used externally to treat wounds and burns, and internally as a tonic, antidepressant and tranquilizer. *Thymus vulgaris* is also known as Garden Thyme and is employed topically in lotions, creams, and ointments because of its antibacterial and antifungal action. *Mentha x piperita* is more commonly called peppermint. Peppermint is a natural hybrid that first made its appearance in the late Seventeenth Century. Tea leaves is used primarily for its gas-relieving properties in treating colic, indigestion, and flatulence. *Aloe vera* had the lowest antioxidant value. The gel obtained from freshly cut leaves of *Aloe vera* can be applied to the skin for minor burns and irritations (Smith and Winder, 1996).

The culinary herbs *Poliomintha longiflora*, *Origanum x majoricum*, and *Origanum vulgare ssp. hirtum* had high antioxidant values. These three herbs are also known as Mexican oregano, Italian oregano, and Greek mountain oregano, respectively. The Italian oregano is a versatile herb used to season meats, egg dishes, soup, and vegetables. The Greek mountain oregano is known for its pepper-flavored leaves that provide a piquant flavor associated with Italian pizza and classic Greek cuisine (Hacskeylo, 1996).

Typical phenolics that possess antioxidant activity in herbs were found to be mainly phenolic acids and flavonoids (Kähkönen, 1999). Phenolic acids are acidic compounds incorporating phenolic groups and have been repeatedly implicated as natural antioxidants in fruits, vegetables, herbs and other plants. For example, caffeic acid, ferulic acid and vanillic acid are widely distributed in the plant kingdom. Caffeic acid has been found to have high activity comparable to that of quercetin. Ferulic acid was shown to inhibit the photo-peroxidation of linoleic acid at the somewhat high concentration of  $10^{-3}\text{M}$  (Larson, 1988). The most widespread and diverse phenolics are the flavonoids which have low molecular weights, have the same  $\text{C}_{15}$  ( $\text{C}_6$ - $\text{C}_3$ - $\text{C}_6$ ) basic skeleton, and possess antioxidant capacity toward a variety of easily oxidizable compounds (Robards et al., 1999). In many herbs, the main flavonoid constituents are flavonol aglycones such as quercetin, myricetin,

kaempferol, and their glycosides (Kähkönen, 1999). In general, flavonoids containing multiple hydroxyl groups have higher antioxidant activities against peroxy radicals than do phenolic acids. However, the flavonoid glycosides (including rutin, naringin and hesperidin) usually have low antioxidant activity values (Robards et al., 1999).

Sage (*Salvia officinalis*) is not only widely used as a natural source of food flavoring but also has medicinal properties for the treatment of various diseases (Areias et al., 2000). Rosmarinic acid and luteolin were the most abundant phenolic constituents in the sage extracts. Other compounds such as vanillic acid, caffeic acid, ferulic acid, luteolin 7-O-glucoside, rosmarinic acid, 4',5,7,8-tetrahydroxyflavone, scutellarein, apigenin, hispidulin, cirsimarin, carnosol, carnosic acid, and methyl carnosate were also detected. In addition, many volatile constituents of sage have been studied such as 1,8-cineole, thujone, isothujone and camphor (Boelens, 1991).

In early pharmacological works, the extracts of sage showed multiple biological activities including antimutagen, antiviral, and antioxidant activity. Cuvelier et al. (1996) measured the correlation between antioxidant efficiency and the composition of sage and recognized that carnosol, rosmarinic acid, and carnosic acid had the greatest antioxidant activities followed by caffeic acid and cirsimarin. Vanillic acid had only half of the antioxidant activity of caffeic acid and the relative antioxidant activities among caffeic acid, luteolin, and apigenin was 1.3, 2.1, and 1.5 (Robards, 1999).

*Ginkgo biloba* leaf extract, which is a complex product containing different active compounds (mainly flavonoids and terpenes), which are used as phytomedicines to increase peripheral and cerebral blood flow. Caffeic acid, quercetin-3-O-rhamnosyl-(1→2)-rhamnosyl-(1→6)-glucoside and kaempferol-3-O-rhamnosyl-(1→2)-rhamnosyl-(1→6)-glucoside were major components in the *Ginkgo biloba* leaf extract. A great variety of flavonoid glycosides was also found in *Ginkgo biloba* leaf extract. Its characteristic constituents were biflavones (bilobetin, ginkgetin, isoginkgetin), vanillic acid, and quercetin-3-rutinoside which possess medicinal value and antioxidant activities (Hasler et al., 1992).

Oregano belongs to the *Lamiaceae* family of herbs, and has been extensively studied as an effective antioxidant in the lipid system (Lagouri and Boskou, 1996). We found that certain species of Oregano (*Poliomintha longiflora*, *Origanum vulgare* ssp. *hirtum* and *Origanum xmajoricum*) had extremely high antioxidant activity values (Zheng and Wang, 2001). Their antioxidant activities were higher than  $\alpha$ -tocopherol and were comparable to that of BHA against linoleic acid oxidation (Nakatani, 1992). The *Oregano* species extracts had high contents of rosmarinic acid and other hydroxycinnamic acid compounds. Rosmarinic acid and hydroxycinnamic acid compounds had been demonstrated to possess strong antioxidant activity (Larson, 1988; Chen and Ho, 1997). The antioxidant activity of rosmarinic acid was much higher than that of  $\alpha$ -tocopherol and BHT (Chen and Ho, 1997).

The herbs thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) are known to have high antioxidant capacities (Economou et al., 1991) and some methylated flavones have been isolated from the thyme herb as antioxidants (Miura and Nakatani, 1989). In the essential oil of thyme, thymol and carvacrol were recognized as major components that showed high antioxidant and antimicrobial activity (Kähkönen, 1999; Schwarz et al., 1996). In addition, a biphenyl compound (3,4,3',4'-tetrahydroxy-5,5'-diisopropyl-2,2'-dimethylbiphenyl) and a flavonoid (eriodictyol) had also been isolated from thyme and reported to be potent antioxidants inhibiting superoxide anion production in the xanthine/xanthine oxidase system and mitochondrial and microsomal lipid peroxidation (Haraguchi et al., 1996). The biphenyls, dimers of thymol, and flavonoids isolated from thyme showed antioxidant activity as strong as BHT (Nakatani, 1992). High contents of rosmarinic acid and luteolin were found in the extract of thyme. Several phenolic compounds of rosemary; i.e., rosmanol, rosmarinic acid, naringin and carnosic acid are very potent antioxidants and are utilized in many food products. Rosmanol is an active antioxidant and has more activity than  $\alpha$ -tocopherol or BHT (Nakatani, 1992). Compared with the commercial antioxidants BHA and BHT, the

phenolic antioxidants from rosemary provide desirable flavors in frying operations (Fisher, 1992). Rosmarinic acid has been shown to possess more antioxidant activity than rosmannol (Cuvelier et al., 1996). Each herb generally contained different phenolic compounds and each of these compounds possessed differing amounts of antioxidant activity. The antioxidant activities of phenolic compounds increased with the number of hydroxyl groups substituted on to the B-ring, especially at C-3', and a single hydroxy substituent generates little or no additional antioxidant capacity (Rajalakshmi and Narasimhan, 1996).

The antioxidant defense system is composed of different antioxidant components. Some antioxidant activities in fruits and herbs may be attributable to other unidentified substances or to synergistic interactions. The antioxidant activity in many herbs was higher compared to berries, fruits and vegetables (Wang and Lin, 2000; Wang et al., 1996; Zheng and Wang, 2001). There was a positive linear correlation between the phenolic content and antioxidant capacity of fruits, vegetables and herbs. Therefore, the total antioxidant potential of berry fruits, vegetables and herbs is more important than individual levels of specific antioxidants (Miura and Nakatani, 1989). Thus, supplementing fruits, vegetables and herbs with a balanced diet is more effective than consuming high doses of an individual antioxidant such as ascorbic acid, vitamin A or E, in protecting the body against various oxidative stresses.

## **FACTORS AFFECTING ANTIOXIDANT ACTIVITY**

### **Genotypes and Maturity**

Genetic factors have the potential to influence antioxidant capacity in crops. For many crops, a large variety of cultivars exist and thus there is great potential for genetic variability in antioxidant activity. For example, in blackberry the 'Hull Thornless' yielded the highest antioxidant value compared to the 'Chester Thornless', and 'Triple Crown'. For raspberry fruits, 'Jewel' (a black raspberry) had the highest value compared to red raspberry cultivars ('Autumn Bliss', 'Canby', 'Sentry', and 'Summit'). On the basis of the wet weight of fruit, ripe fruit of 'Jewel' black raspberry had higher antioxidant values than the three cultivars of ripe thornless blackberry fruit ('Chester Thornless', 'Hull Thornless', and 'Triple Crown'). Compared to blackberries and raspberries, strawberries had generally lower mean values of total antioxidant capacity (Wang and Lin, 2000). Prior et al. (1998) and Ehlenfeldt and Prior (2001) had found that various species and cultivars of blueberry and bilberry contain different antioxidant capacities.

Many phytonutrients are synthesized in parallel with the overall development and maturation of berry fruits, and herbs. Therefore, antioxidant capacity varied considerably with different levels of maturity. Blackberry, raspberry and strawberry fruits harvested during their ripe stage consistently yielded higher antioxidant values than harvest in the pink stage. The small green stage of strawberry fruit had the highest antioxidant value and steadily decreased at the 50 percent red stage, which had the lowest antioxidant value. Beyond the 50 percent red stage, the antioxidant value steadily increased with fruit maturity (Wang and Lin, 2000). Younger blueberries harvested at an early stage (immediately after turning blue) had lower ORAC and total anthocyanins compared to more mature blueberries which were not harvested until 49 days later (Prior et al., 1998).

### **Preharvest Conditions**

Preharvest conditions of fruits and herbs such as growing temperature and light intensity can affect their content of vitamins, especially carotenoids, thiamine, ascorbic acid, and riboflavin. For example, strawberries grown with higher light intensity results in increased levels of ascorbic acid. Month-to-month variability in vitamin C content depending on growing conditions has been documented in processed Florida citrus products such as orange and grapefruit juices (Lee and Coates, 1997). Strawberry fruit contains flavonoids with potent antioxidant properties. Different hydroxylation and glycosylation may modulate their antioxidative properties. The phenolic acid (*p*-coumaroylglucose), flavones (dihydroflavonol, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide), and anthocyanins



(cyanidin 3-glucoside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, cyanidin 3-glucoside-succinate and pelargonidin 3-glucoside-succinate) have been detected in strawberries. Strawberry growth under high temperature conditions significantly enhanced content of flavonoids and the antioxidant capacities. Plants grown in cool day and night temperature generally had the lowest antioxidant capacity in fruit. One explanation for this difference could be related to different flavonoid concentrations (Wang and Zheng, 2001). The composition of flavonols in red raspberry juice also was reported to be influenced by cultivar, processing, and environmental factors (Rommel and Wrolstad, 1993).

### **Cultural Practices**

Different cultural systems had varying effects on antioxidant activity, anthocyanin, and total phenolic content in various strawberry cultivars. The hill black plasticulture system consistently produced the highest levels of antioxidant activity in strawberries, while the straw-vetch mulch treatment resulted in the lowest. Additional compost in the soil also enhanced antioxidant content in the strawberry fruit.

### **Postharvest Handling**

Postharvest handling can also affect phytonutrient levels in fruits and vegetables. In cranberries, postharvest storage temperatures between 0 and 15°C increased antioxidant capacity, anthocyanins and total phenolic content (Wang and Stretch, 2001). Strawberries and raspberries stored at temperatures greater than 0 °C also resulted in an increase in antioxidant capacity (Kalt et al., 1999). Different postharvest packaging methods have also affected nutrient content. In strawberry, controlled atmosphere (CA) storage did not affect anthocyanin content in external tissues, but decreased anthocyanin content in internal tissues (Gil et al., 1997).

### **FUTURE RESEARCH**

Fruits and herbs contain many nutritionally significant, health-promoting components of benefit to humans. Recent advances in structural and functional genomics, as well as technical advances in plant breeding, bioengineering, and biotechnology, make it possible now more than ever to create designer foods for consumer. Therefore, enhanced antioxidant capacity of horticulture crops can be achieved through plant breeding.

Selection and breeding of berry crops and herbs from a diverse genetic base with known concentrations of phytochemicals is a key step in developing an improved food supply. In addition, pre-harvest factors may influence the concentration and stability of phytochemicals with possible nutritional value. For example, climate, temperature and light all affect antioxidant activity in various horticultural crops. The stage of crop maturity also significantly influences the antioxidant capacity of blueberries, blackberries, raspberries and strawberries. One of the most nutritionally significant applications of modern technologies may well be to retard the softening process so that fruits can be harvested and marketed at a more mature stage, when more of the phyto-compounds have already been biosynthesized.

Post-harvest evaluation of the phytochemical composition of food crops is an important component in assessing the impact of handling procedures on the nutritional content of a fruit, vegetable, or herb. New research is being initiated to develop a better understanding of how transport and storage influence their chemical composition.

Opportunities in biotechnology are one of the most important factors driving producer interest in the nutritional and health values of foods and food components.

Despite increasing media attention in the past few years, the science of phytonutrients is still an embryonic discipline. A number of priority research areas on phytonutrients need to be emphasized and evaluated: (a) Improve selection criteria among different horticultural cultivars including variety selection, molecular genetic breeding and stage of maturity at time of harvest; (b) Investigate the influence of production practices on formation of selected certain phytonutrient compounds; (c) Evaluate post-harvest conditions of transport, storage and processing on the stability of phytonutrient compounds.

Knowledge gained from these research studies will be helpful in improving human health by optimizing the nutritional content and quality of the human diet.

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