Antioxidants in Fruits and Their Possible Anticancer Property

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Abstract
Fruits are good sources of natural antioxidants such as carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites. 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. Active oxygen species are generated as by-products of normal metabolism. Increased levels of these active oxygen species or free radicals create oxidative stress, which leads to a variety of biochemical and physiological injuries often resulting in impairment of metabolism, and eventually, cell death. The different antioxidant components found in fruits 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. This paper summarizes the antioxidant capacities of various fruits, and the factors which affect their antioxidant activities such as crop genotype variation and maturity, pre-harvest conditions, post-harvest handling and processing. Many attractive opportunities exist for enhancing the quantity and quality of essential nutrients present in fruits. Strategies for establishing a new research and production paradigm will be reviewed, such as improving selection criteria among different horticultural cultivars and improving pre-harvest conditions and post-harvest handling to enhance nutrient quality. Evidence will also be presented on the prevention and inhibition of tumor growth by fruit extracts and bioactive compounds isolated from fruits.

INTRODUCTION
In the past, the agricultural industry has focused on maximizing the quantity of fruits and vegetables produced for commercial markets. However, modern consumers are now interested in optimizing the nutritional composition of foods in order to promote health. Therefore, much attention has now been placed on the agricultural practices that will enhance the nutritional content of fruits and vegetables being produced today. This trend is likely to continue in the future. Fresh fruits and vegetables have been shown to provide not only the usual nutrients such as vitamins and minerals, but also health-promoting antioxidants, free radical scavengers, and other cancer-fighting agents. Eating more fruits and vegetables has been associated with lowering the incidence and mortality rates of cancer and heart disease in addition to a number of other health benefits (Ames et al., 1993; Gey, 1990). Research is in full force to find the best genotypes, optimum cultural practices, and most desirable postharvest handling techniques to enhance and preserve the nutritional components in fruits and vegetables that are beneficial to our health. This paper summarizes the antioxidant capacities of various fruits and describes the factors affecting these antioxidant activities. In addition, the effectiveness of fruit extracts and bioactive compounds isolated from fruits on the prevention and inhibition of tumor growth are also discussed.

ANTIOXIDANT CAPACITY OF FRUITS
Fruits contain a wide range of flavonoids and phenolic acids. The main flavonoid subgroups in fruits are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic
acids present in fruits are glycosides of hydroxylated derivatives of benzoic acid and cinnamic acid (Macheix et al., 1990). Good sources of natural antioxidants are found in many fruits, most notably prunes, raisins, blackberries, black currants, blueberries, raspberries, strawberries, grapes, and pomegranates (Prior et al., 1998; Wang and Lin, 2000; 1996; Gil et al., 2000).

Among the small fruits, black raspberries and blackberries have higher antioxidant activities than red raspberries, while strawberries generally have lower values of total antioxidants (Wang and Lin, 2000). These results correlate with the findings that black raspberries and blackberries contain high amounts of cyanidin glycosides, a strong antioxidant (Macheix et al., 1990), while strawberries contain pelargonidin 3-glucoside (Gil et al., 1997) and ascorbic acid, which are weak antioxidants. Among the anthocyanins, the relative antioxidant strength in preventing oxidation of human low-density lipoprotein is as follows: delphinidin > cyanidin > malvidin > pelargonidin (Satué-Gracia, 1997).

EFFECT OF PRE-HARVEST FACTORS ON ANTIOXIDANT ACTIVITY OF FRUITS

Genotypes and Maturity
For many crops, a large variety of cultivars exist, and thus there is potential for genetic variability leading to varying amounts of antioxidant activity. For example, in blackberry the ‘Hull Thornless’ cultivar yields higher antioxidant values compared to the ‘Chester Thornless’, and ‘Triple Crown’ cultivars. For raspberry fruits, ‘Jewel’ (a black raspberry) has higher values compared to red raspberry cultivars (‘Autumn Bliss’, ‘Canby’, ‘Sentry’, and ‘Summit’) (Wang and Lin, 2000). Various species and cultivars of blueberry and bilberry contain different antioxidant capacities (Prior et al., 1998).

Many phytonutrients are synthesized in parallel with the overall development and maturation of fruits and vegetables. Therefore, antioxidant capacity varies considerably with different levels of maturity. Blackberry, raspberry and strawberry fruits harvested during their ripe stage consistently yield higher antioxidant values than when harvested during the pink stage. Antioxidant activity decreases steadily in strawberry fruit from the green stage to the 50 percent red stage, which has the lowest antioxidant value. Beyond the 50 percent red stage, the antioxidant value steadily increases again with fruit maturity. Immature blueberries harvested at an early stage (immediately after turning blue) have lower oxygen radical absorbance capacity (ORAC) values and total anthocyanin content when compared to more mature blueberries harvested 49 days later (Prior et al., 1998). Both ascorbic acid and total carotenoids increased with maturation and ripening in ‘clingstone’ peaches (Kader et al., 1988).

Climate, Temperature, and Light
Pre-harvest conditions of fruits and vegetables such as growing temperature and light intensity can affect their content of vitamins, especially carotenoids, thiamine, ascorbic acid, and riboflavin. For example, strawberry grown under high temperature conditions significantly contain an enhanced content of flavonoids and antioxidant capacity. Strawberry grown in cool day and night temperatures generally have the lowest antioxidant capacity in fruit. One explanation for this difference could be related to different flavonoid concentrations (Wang and Zheng, 2001). Month-to-month variability in vitamin C content dependent on growing conditions has been documented in processed Florida citrus products such as orange and grapefruit juices (Lee and Coates, 1997). The composition of flavonols in red raspberry juice has also been reported to be influenced by cultivar, processing, and environmental factors (Rommel and Wrolstad, 1993).

Agricultural Practices
1. Compost. Composts have been utilized in agriculture as a significant source of organic matter. Compost, as a soil supplement, significantly enhanced content of ascorbic acid
(AsA), glutathione (GSH) and the ratios of AsA/dehydroascorbic acid (DHAsA) and the GSH/oxidized glutathione (GSSG) in fruit of strawberry (Wang and Lin, 2003). The oxygen absorbance capacity for peroxyl radical, as well as the superoxide radical, hydrogen peroxide, the hydroxyl radical and singlet in strawberries increased significantly with increasing compost use (Wang and Lin, 2003). Plants grown with compost yielded fruits with high levels of phenolics, flavonol, and anthocyanin content (Wang and Lin, 2003). It is possible that compost causes changes in soil chemical and physical characteristics, increases beneficial microorganisms, and increases nutrient availability and uptake, thus favoring plant and fruit growth. Woese et al. (1997) reported that the optimal conditions for plant growth generally result in the highest levels of antioxidants.

2. Mulch. Using different mulches for growing strawberries affected strawberry fruit quality. Different mulches probably led to differences in canopy temperature, soil temperature and moisture content, and the quantity and quality of light transmitted, reflected or absorbed. These differences in turn may have affected plant growth, development, fruit quality and carbohydrate metabolism in strawberry plants (Wang and Lin, 2002). Fruit from a hill plasticulture (HC) production system had higher flavonoid content and antioxidant capacities compared to fruit from plants grown in a matted row (MR) system (Wang et al., 2002). In general, phenolic acid and flavonol content, as well as cyanidin- and pelargonidin-based anthocyanins and total flavonoids were greatest in the HC system. Fruits from plants grown in the MR system generally had the lowest content of phenolic acids, flavonols, and anthocyanins. Fruit grown under HC conditions had the highest peroxyl radical absorbance capacity (Wang et al., 2002).

3. Elevated Carbon Dioxide. Increased CO₂ (300 and 600 μmol mol⁻¹ above ambient) concentrations in the field resulted in increases in anthocyanins, phenolics, AsA, GSH, and ratios of AsA to DHAsA and GSH to GSSG in strawberry fruit (Wang et al., 2003). These increases in strawberries may allow for the removal of free radicals, which is associated with increased antioxidant capacity. Growing strawberry plants under CO₂ enrichment conditions significantly enhanced fruit flavonoid content (Wang et al., 2003). High flavonoid content was associated with high antioxidant activity. Fruit of strawberry plants grown in CO₂ enrichment conditions also had high oxygen radical absorbance activity against all radicals: peroxyl radical, superoxide radical, hydrogen peroxide, the hydroxyl radical and singlet (Wang et al., 2003). This indicated that strawberry fruit grown with CO₂ enrichment had high scavenging activity for chemically generated active oxygen species.

4. Methyl Jasmonate. Jasmonic acid (JA) and its methyl ester (methyl jasmonate, MJ) are a class of oxylipins derived from the lipoxxygenase-dependent oxidation of fatty acid. Both compounds have been found to occur naturally in a wide range of higher plants. JA/MJ play key roles in plant growth and affect a great diversity of physiological and biochemical processes (Sembdner and Parthier, 1993). Pre-harvest spray MJ significantly enhanced anthocyanin, total phenolic, flavonoid content and antioxidant capacity in raspberries. The oxygen radical absorbance capacity (ORAC) value was positively correlated with anthocyanins and total phenolics. Previous research also showed that a linear relationship exists between anthocyanin or total phenolic content and ORAC in various berries (Wang and Lin, 2000).

EFFECT OF POST-HARVEST HANDLING ON ANTIOXIDANT ACTIVITY OF FRUITS

Storage Duration and Temperature
The level of antioxidants in ‘Red Delicious’ apples increased 2 to 10 times during the first two months of storage at -1°C (Curry, 1997). In ‘Granny Smith’ apples, the antioxidant content also increased almost 10-fold two months after the initiation of cold storage. The ORAC and anthocyanin content of strawberries and raspberries increased during storage at 0°C. There was also an increase in the total phenolic content during storage of raspberry fruit (Kalt et al., 1999). Phenolics and anthocyanins were found to be
strongly correlated to antioxidant capacity. In strawberry fruit, the magnitude of the increase in anthocyanin content is related to storage temperature. Increases in anthocyanin content during storage have also been reported for lowbush blueberries (Kalt et al., 1993).

**Modified Atmosphere Packaging**

Modified atmosphere packaging (MAP) was very effective in the retention of \( \alpha \)-carotene in jalapeno pepper rings; \( \alpha \)-carotene retention was 92% in MAP compared to 52% in air after 12 days of storage at 4.4°C plus an additional 3 days at 13°C (Howard and Hernandez-Brenes, 1998). The retention of \( \beta \)-carotene was 87% in MAP compared to 68% in air after the same 15-day period. The retention of ascorbic acid was 83% in MAP compared to 56% in air after the same period. Therefore, MAP treatment resulted in greater preservation of antioxidants and quality attributes including texture, color, and moisture content in comparison to air storage.

**Carbon Dioxide Treatment and Low Oxygen Treatment**

High carbon dioxide atmosphere has been used to retard microbial growth and suppress respiration rate, ethylene production, softening, and other metabolic activities associated with the senescence of fresh fruits and vegetables, in an effort to maintain quality and extend storage life of these fresh produce. Carbon dioxide-enriched atmospheres (10–20%) are especially effective in retarding decay and softening of strawberries. However, exposure to high concentrations of carbon dioxide could adversely affect the color change in strawberry fruit (Gil et al., 1997). High carbon dioxide caused a reduction in red color intensity and a decrease in anthocyanin content of internal tissue of strawberry fruit. As carbon dioxide levels increased, the concentration of pelargonidin glycosides in the internal tissue decreased (Gil et al., 1997). It is possible that high carbon dioxide caused an increase in pH which in turn affected the stability of anthocyanins.

**Heat Treatment and Irradiation**

One of the postharvest techniques which have been demonstrated to effectively maintain quality of fresh fruits and vegetables is prestorage heat treatment and irradiation treatment. In addition to controlling diseases and insects, adequate heat treatment and irradiation treatment can also retard senescence or degradative processes in fresh produce. Irradiation treatments have resulted in increased anthocyanin and phenolics in sweet cherry and grapes (Katoka et al., 1996; Cantos et al., 2000). However, heat treatment (48°C air, 3 h) reduced anthocyanin accumulation in strawberries (Civello et al., 1997).

**Treatment with Naturally Occurring Substances**

The application of natural products has been shown to maintain quality of fruits and vegetables. A naturally occurring compound, methyl jasmonate, was found to reduce chilling injury, retard decay, inhibit sprouting, and improve storage quality of several fruits and vegetables. Methyl jasmonate treatment also maintained higher levels of oxygen radical absorbance capacity in blueberries compared to untreated control fruit, especially during the later part of storage (Wang, 2001). High antioxidant activities were associated with better overall quality of methyl jasmonate-treated blueberries which included high levels of sugars, organic acids, and low incidence of decay.

**HUMAN HEALTH – ANTICANCER PROPERTY**

Cancer is one of the major causes of death in the United States. Epidemiological studies have shown that a large number of cancer-related deaths could be prevented by increased consumption of fruits and vegetables (World Cancer Research Fund, 1997). Fruits and vegetables contain various antioxidant components that could provide protection against cancer and heart disease, in addition to a number of other health benefits (Ames et al., 1993; Gey, 1990). Fruits and vegetables have shown protective effects on several types of cancer such as those involving the lung, oral larynx,
esophagus, stomach, pancreas, cervix, bladder, colon, rectum, breast, ovary/endometrium and prostate (Block, 1992).

In Vitro Anticancer Activity of Fruit Extracts

Fruit extracts have shown chemopreventive effects in different systems, based on striking inhibition of diverse cellular events associated with tumor initiation, promotion, and progression (Bode and Dong, 2002). Extracts of grape, strawberry, apple peel, blackberry and four Vaccinium species (lowbush blueberry, bilberry, cranberry, and lingonberry) showed anticancer activity (Jang, et al., 1997; Wolfe et al., 2003; Bomser et al., 1996). Resveratrol, which occurs naturally in grapes and other fruits, suppressed tumor-promoter-induced cell transformation and strongly induced apoptosis through a p53-dependent pathway (Huang et al., 1999). We found that pretreatment of JB6 cells with berry fruit extracts or cyanidin 3-glucoside (CG, the major anthocyanidin in blackberries) produced a dose-dependent inhibition of activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB). In addition, these berry fruit extracts also inhibited cell transformation induced by either 12-O-tetradecanoylphorbol-13-acetate (TPA) or ultraviolet-B (UVB). Berry fruit extracts also blocked UVB-induced phosphorylation of the mitogen-activated protein kinase (MAPK) family members ERK1, ERK2, and p38 but not JNK. In addition, berry fruits extract also prevented TPA-induced phosphorylation of ERK1 and ERK2. Results of soft agar assays indicated that berry fruit extracts suppressed TPA-induced neoplastic transformation of JB6 P⁺ cells in a dose-dependent manner. These results suggest that ERK1 and ERK2 may be the primary targets of berry fruit extracts, resulting in suppression of AP-1 and neoplastic transformation in JB6 cells.

Inhibition of Papillomagenesis and Malignant Transformation by Fruit Extracts

1. Animals and Two-Stage Skin Carcinogenesis. To study the chemopreventive activity of a bioactive compound (e.g., CG) isolated from blackberry, the anti-tumorigenic effects of CG were evaluated using experimental animals [(AP-1-luciferase reporter transgenic mice [C57BL/6 crossed with DBA2 were used]. In the mouse skin model, initiation of tumor can occur as a result of a single dose of DMBA that mutationally activates H-ras. Fourteen days following initiation, all mice (except those in the negative control group) were exposed to TPA twice a week for 22 weeks. For the CG-treated group, the dorsal skin was pre-treated topically with CG for 30 min before each application of TPA. The incidence of papillomas was detected by palpation, and the number of papillomas appearing on each mouse was recorded once a week. We found that mice treated with CG had significantly decreased size and number of skin tumors in the two-stage mouse skin model, compared to untreated mice.

2. Effect of CG on A549 Tumor Xenograft Growth in Athymic Male Nude Mice. Male nude mice (AthymicNCr-nu) aged 8 weeks were used. Human lung cancer cell line, A549 cells (2×10⁶/flank), were injected in both the right and left flanks of each mouse to initiate tumor growth. After 2 days, the mice were treated with either PBS or CG dissolved in PBS (9.5 mg/kg, 3 times/week). Once tumor xenografts started growing, their sizes were measured twice weekly in two dimensions, throughout the study. Metastatic dissemination into the other organs was evaluated macroscopically and by microscopic examination of tissue sections. In the nude mice study, we also found the control mice (treated mice carrying A549 cells) showed a large subcutaneous tumor mass at the site of injection. The tumor invaded through the abdominal wall and extended into the abdominal cavity, resulting in peritoneal carcinomatosis. Multiple small tumor nodules were seen on the peritoneal surface of the abdominal wall. The tumor also extensively involved the mesenteric fat with malignant ascites. Tumors were found not only on the surface, but within the parenchyma of several organs, such as the liver, kidney, pancreas, and lymph nodes. In contrast, the CG treated mice showed much less tumor involvement in the abdominal cavity, although a smaller subcutaneous tumor mass at the injection site was seen. CG treated mice had significantly fewer tumor nodules in the abdominal cavity and mesenteric fat. Microscopically, the injection site of the CG
treated mice showed collection of macrophages with pigments, consistent with the phagocytosed pigment derived from the CG compound. There were viable tumor cells in the deep skeletal muscle distant from the subcutaneous injection site. There was no tumor involvement of organ parenchyma in the CG treated mice.

CONCLUSIONS

Of the 10.3 million new cases of cancer diagnosed around the world each year, as many as two-thirds may be caused by dietary and lifestyle factors. Diets rich in fruits and vegetables may decrease the risk of many types of cancer (World Cancer Research Fund, 1997). What is it about fruits that make them such effective weapons in our fight against cancer and other degenerative diseases? The answer is phytonutrients, which are natural substances found in plants. Many of these phytonutrients have antioxidant capacity. The antioxidant capacity in fruits are affected by genetic variation, maturation, preharvest conditions (climate, temperature, light, cultural practices) and postharvest handling (storage, modified atmosphere packaging, carbon dioxide treatment, heat treatment, irradiation, treatment with naturally occurring compounds). The goal of our research is to maximize phytonutrient content in fruits and vegetables. By selecting genotypes with high antioxidant content for breeding, and searching for the best preharvest conditions and postharvest techniques for enhancing and maintaining high fruit quality and high antioxidant content, it is possible to create foods with high nutrient content. Maximizing antioxidant capacity in fruits will help lead to better chemoprevention interventions in the future.

Literature Cited

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