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The Effect of Commercial Canning on the Flavonoid Content of Blueberries

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Introduction

Many chronic diseases, including cardiovascular disease, cancer, diabetes, asthma, rheumatoid arthritis, cataracts, Alzheimer's disease, Parkinsons's disease and multiple sclerosis, are related to oxidative stress. Organisms are constantly exposed to free radicals either from exogenous sources such as electromagnetic radiation or endogenous sources as, for example, by products of normal cellular metabolism. Oxidative stress occurs when the exposure to reactive oxygen species exceeds the ability of the cell to defend against them. This is most likely to occur in areas of the body with high metabolic rates such as the brain. Antioxidants disrupt the oxidation process thereby protecting against the deleterious effects of radical oxygen species.

Epidemiological studies suggest that diets rich in botanical antioxidants such as antioxidant vitamins, carotenoids and polyphenolics are beneficial in protecting against chronic diseases (Yang et al, 2001; Agarwal and Rao, 2000). Accordingly, there is tremendous interest in dietary intake of antioxidants and their potential protective effects. Flavonoids, polyphenolic compounds present in fruits, vegetables, nuts and seeds, represent the largest source of antioxidants in the diet. There are more than 5,000 different flavonoid compounds that fall into five major categories: flavonols, flavones, flavan-3-ols, flavanones and anthocyanins. The most common flavonoid in the diet is quercitin, a flavonol estimated to account for 70% of dietary flavonoid intake (Sesso et al, 2003). In a meta-analysis of prospective cohort studies, Huxley and Neil (2003) found an inverse correlation between the intake of flavonones and mortality from coronary heart disease. Likewise, using data from the Finnish Mobile Clinic Health Examination Survey, Knekt et al (2002) observed significant inverse associations between flavonoid intake and lung caner, asthma, diabetes, death from ischemic heart disease, cancer at all sites, thrombotic stroke and all causes of mortality.

Food processing may influence the bioavailability and stability of plant-derived antioxidants. For instance, uptake of lycopene is greater from heat-processed than from unprocessed tomato juice, and mild heat treatment of carrots and spinach enhances the bioavailability of □-carotene (Stahl et al, 1992, Rock et al, 1998). On the other hand, cooking or heat processing of blueberries contributes to the degradation of resveratrol (Lyons et al, 2003). While there is intense

interest in the flavonoid content of foods in the diet, there is very little information available in the literature regarding the effects of food processing on flavonoid values. The data that are available in many databases can be misleading because values reported for fresh and processed foods often do not come from the same study, may not be from the same country and are unlikely to be of the same strain of vegetable or fruit. The purpose of the current study was to investigate the effects of commercial canning on the flavonoid content and antioxidant status of blueberries as compared to fresh blueberries.

Methods

Blueberry samples were obtained from the Oregon Fruit Products cannery in Salem, OR. One pallet of high bush (Blue Crop) blueberries (80 flats) was selected for sampling from a field truck within 15 minutes of arrival at the facility. Random samples (50 cc each) were taken from every other flat (40 samples) within 60 minutes of arrival from the fields. The samples were placed in a large bowl and thoroughly stirred to mix the samples. Fifty ml samples were randomly selected from the mixed berries and assigned to various conditions. One set of samples (fresh) was immediately frozen in liquid nitrogen and stored at –80˚C. A second set (refrigerated) was stored in a refrigerator at 4˚C for 14 days before being frozen at –80˚C. A third set (heated) was heated in a saucepan over medium heat for three minutes and allowed to cool before being frozen in liquid nitrogen and stored at –80˚C. A fourth set (frozen) was frozen for two weeks at –20˚C before being frozen at –80˚C.

A fifth set of samples represented pre-canning (pre-can). Berries for this sample came from the same pallet but were randomly sampled from the sorting line just before they would have been placed in cans. The run was timed and sampling continued throughout the time frame that berries from the selected pallet were being canned. Samples were mixed as described above and random samples were selected and flash frozen in liquid nitrogen and stored at –80˚C.

Blueberries were canned in a light syrup. Each 444 ml can contained 65.8% blueberries, 8.4% sugar and 25.8% water by weight. The syrup was added at 71˚C. The cans were heated for eight minutes in a rotary cooker to a final temperature of 93˚C and were cooled to 38˚C in a rotary water bath within 10 minutes. Cans of blueberries from the same pallet were randomly sampled as they came off the canning line.

Flavonoid Assay

Frozen samples were shipped overnight on dry ice to the United States Department of Agriculture (USDA) facility in Beltsville, MD where they were assayed for flavonoids in the Food Composition Laboratory.

Catechins and flavanones were extracted from homogenized samples in a 90% methanol solution. Samples were centrifuged and the supernatant was removed. This process was repeated three times before the sample was assayed using high-pressure liquid chromatography. Flavones, flavonols, and anthocyanidins were extracted in a 40 ml solution containing 62.5% MeOH in H_2O with 0.4 g/L TBHQ and 7.5 ml of HCl, and 2.5 ml of H_2O.

Two samples were assayed from each condition. Each sample was run in duplicate and the average value was reported.

Determination of ORAC

Frozen samples were shipped overnight on dry ice to Brunswick Laboratory (Wareham, MA). Samples were thawed on ice and aliquots were homogenized for extraction in a 50/50 mixture of acetone/H_2O. Samples were centrifuged at 5,000 RPM at 4°C in an International Centrifuge (IEC). The supernatant was assayed on a COBAS FARA II spectrofluormetric centrifugal analyzer. The procedure was based on a previous report of Ou et al (Ou, 2001). With the exception of samples and Trolox standards, which were made in 7% RMCD solvent, all other reagents were prepared at 75 mM phosphate buffer (pH 7.4). In the final assay mixture (0.4 ml total volume), FL (6.3×10^{-8} M) was used as a peroxyl radical generator. Seven percent RMCD was used as the blank, and Trolox (12.5, 25, 50, and 100 □M) was used as the control standard. The analyzer was programmed to record the fluorescence of FL every minute after the addition of AAPH. All measurements were expressed relative to the initial reading. Final results were calculated using the differences of areas under the FL decay curves between the blank and a sample. These results were expressed as micromoles Trolox equivalent (TE).

Data Analysis

Separate one-way analyses of variance were performed for each flavonoid and for ORAC using SPSS for Windows software version 10.1. Least significant difference tests were used for follow-up. Data for canned blueberries were adjusted by a factor of 1.342 to account for the added fluid and sugar in the can. Data are reported as means and standard deviations.

Results

Values for each of the flavonoids are presented in Table 1.

TABLE 1

	Delphinidin	Cyanin	Peonidin	Quercitin	Malvidin	Petunidin
Fresh	49.0 ± 13	26.0 ± 2	45.3 ± 7	52.9 ± 21	249 ± 96	162 ± 69
Refrigerated	47.3 ± 5	22.9 ± 1	39.9 ± .1	39.0 ± 2	118 ± 8	161 ± 34
Pre-can	48.9 ± 7	31.1 ± 4	39.7 ± 4	58.0 ± 7	130 ± 33	204 ± 58
Frozen	50.3 ± 10	26.6 ± 1	48.8 ±8	40.7 ± .1	130 ± 43	162 ± 37
Canned	110.3 ± 16	35.8 ± 5	78.1 ±9	74.7 ± 22	166 ± 18	365 ± 48
Heated	208.7 ± 50	76.6 ± 9	91.8 ±	133.7 ±43	402 ± 39	697 ± 77

Data represent means and standard deviations. All values are in mg/kg.

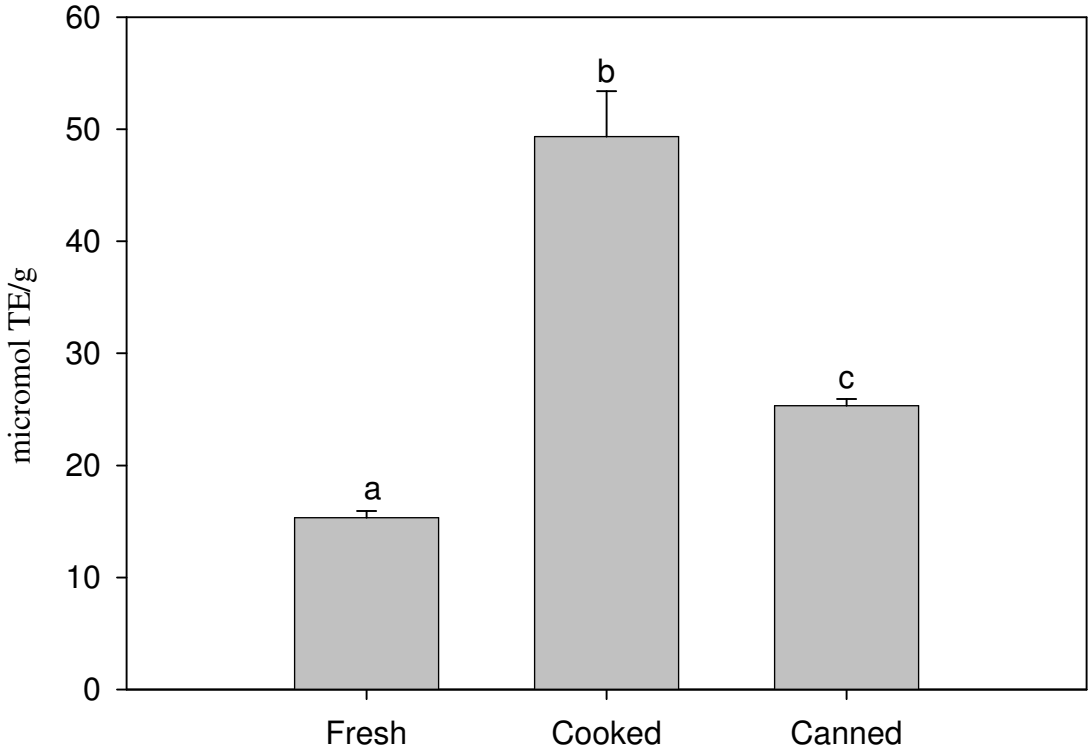
One-way analyses of variance (ANOVA) for each flavonoid indicated that there were significant differences between treatment groups for delphinidin ($p<.005$), cyanidin ($p<.001$), petunidin ($p<.001$), peonidin ($p<.005$), quercitin ($p<.05$) and malvidin ($p<.01$). Least significant difference tests indicated that the heated samples had higher values than all other samples ($p<.05$) for each flavenoid except peonidin where the heated sample was not included in the analysis because only one value was available. Canned samples had significantly higher values ($p<.05$) than all other samples for delphinidin, petunidin, and peonidin. The canned samples also had significantly higher values ($p<.05$) for cyanidin than the refrigerated sample. In addition, catechins were detectable in the heated and canned samples that were below detection limits in all other samples (see Table 2).

Table 2

	Epigallocatechin	Catechin	Epigallocatechingallate	Epicatechin
Fresh	ND	ND	ND	ND
Refrigerated	ND	ND	ND	ND
Pre-can	ND	ND	ND	ND
Frozen	ND	ND	ND	ND
Canned	22.6 ± 0.6	20.8 ± 1.2	16.3 ± 13.4	15.8 ± 13
Heated	21.8	21	ND	18

Data represent means and standard deviations for the canned group. There was only one sample reported for the heated group so there is no standard deviation. Values are in mg/kg.

ANOVA for total antioxidant activity, measured as ORAC, indicated that there were significant differences between treatment groups. Total antioxidant activity ranged from15.3 □mol of Trolox equivalents TE/g for fresh berries to 49.3 □mol TE/g for cooked berries (Fig 1). Canned berries had an intermediate antioxidant capacity of 25.3 TE/g. The ORAC analysis provides a measure of the scavenging capacity of antioxidants against the peroxyl radical, which is one of the most common reactive oxygen species (ROS) found in the body.



Discussion

The results show an increase in flavonoid activity in samples that were heated or canned relative to fresh, refrigerated or frozen samples. Increases in anthocyanins, total phenolics and antioxidants have previously been observed in two strains of blueberries (Connor et al, 2002) stored at 5˚C. In one of those strains, the increase in activity was attributed to continued ripening but that did not appear to be the case for the other, which was fully mature at the outset. Likewise, increases in flavenoid activity have been observed in strawberries and raspberries (Kalt et al, 1999) during storage at different temperatures. Anthocyanin content of strawberries increased by 1.7 fold over eight days at 0˚C but increased 6.8 fold in the same time frame at 30˚C. In the same study, anthocyanins in high bush blueberries increased 1.2 fold at 20˚C. The authors suggested that the post-harvest increase in anthocyanins may be related to interconversion of

organic acids with carbohydrates thus providing carbon skeletons for the synthesis of phenolics.

The effects heating or canning on flavonoids have not been systematically studied. Most available data on canned products appear to be based on convenience samples obtained from supermarkets. For example, both Stewart et al (2000) and Lavelli et al (2000) used convenience samples to compare canned and fresh tomato products. Gu et al (2004) report proanthocyanidin data for a number of common foods including both fresh and canned peaches. The canned peaches contained a fraction of the total proanthocyanidins of fresh and, based on the table, it would be assumed that canning has a detrimental effect on proanthocyanidins. However, that may not be the case, especially since the same fruit was not being compared.

In the current study, berries from the same lot were compared before and after heating or canning. The results indicate that the commercial canning process did not diminish the levels of any flavonoid measured and actually increased the abundance of some anthocyanins. Similarly, some forms of catechin that were undetectable in fresh samples were detectable in the heated and canned samples.

One potential explanation for the increase in flavonoids with canning is heating. Although there has not been a great deal of research on heating and flavonoids, there are indications that heating may increase total phenolic content along with antioxidant capacity in some products. Gahler et al (2003) reported that total phenolic concentration of tomatoes increased significantly with heating even as lycopene levels declined. The effect of heating was significant within 15 minutes at 180C˚, the lowest temperature tested, and increased further with continued heating. Whether or not the lower heating temperatures used in canning (93˚C) would cause an increase in the total phenolic content of canned blueberries remains to determined. With tomatoes, heating at 88˚C did not cause an increase or decrease in total phenols although it did increase the total antioxidant capacity (Dewanto et al, 2002). In corn, however, total phenolic activity was increased by heating to 115˚C for 25 minutes (Dewanto et al, 2002).

It is noteworthy that in the current study the samples were homogenized before being assayed. Consequently, the solids and liquids were combined in a slurry before the extraction process. Since flavonoids are water soluble, this procedure retained any flavonoids that otherwise may have been lost in the liquid. This could have important implications for different canned products as some products such as blueberries are typically consumed with the liquid while others are not.

It is likely that the increase in anthocyanins in heated or canned blueberries would have a beneficial impact on human health. Certainly, the antioxidant values for the heated and canned samples increased as would be expected from the increase in anthocyanin and flavan-3-ol levels. McGhie et al (2003) reported that anthocyanins from blueberries were absorbed from the gut and excreted unmetabolized in both humans and rats. Wu et al (2002) reported much the same although they did find evidence of in vivo methylation of cyanidin to peonidin and glucuronide conjugate formation.

Absorption of anthocyanins has been shown to increase the total antioxidant capacity of human serum ex vivo (Mazza et al, 2002). However, it should be noted that a detectable increase in total antioxidant capacity is not necessary for the neuroprotective effects of blueberries. Sweeney et al (2002) reported that supplementing rats for six weeks with 14.3% blueberries in the diet reduced by 50% the neuronal damage caused by ischemia in the hippocampus. There was no detectable effect of the blueberry diet on plasma or urine antioxidant capacity. Likewise, it does not appear that detectable levels of polyphenolics are necessary for a protective antioxidant effect. Youdim et al (2000) reported that supplementing rats with polyphenols from blueberries resulted in protection of red blood cells from oxidative damage six and 24 hours later yet polyphenols could not be detected in plasma at 24 hours post supplement.

Dajas et al (2003) suggest that quercetin and structurally related flavonoids may be responsible for the neuroprotective effect of flavonoids. In their study, catechins were not found to be neuroprotective. This finding is somewhat surprising given that catechins facilitate nitric oxide activity and increased nitric oxide activity mitigates against damage by stroke (Ignarro, 2002). For example, Karim et al (2000) reported that catechins from cocoa extracts cause endothelium-dependent vasodilation through activation of nitric oxide synthase in rabbit aortic rings. Fisher et al (2003) fed human subjects cocoa extract and assessed peripheral vasodilation. Using plethysmography, they found that both acute and chronic consumption of cocoa extract caused increased vasodilation that was sensitive to blockade by n-nitro-L-arginine methyl ester. The results were consistent with those of Karim et al (2000) and were attributed to catechins. Besides a beneficial effect on stroke, blueberries have been found to limit the progression of Alzheimer's disease in an animal model (Joseph et al, 2003) as well as reverse age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits (Joseph et al, 1999).

In summary, commercial canning of blueberries increased the anthocyanidin and flavan-3-ol content relative to fresh or frozen blueberries. The only sample with more content was heated. These data strongly suggest that canning blueberries may increase their health benefit in the diet while preserving the fruit.

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