Antioxidant Activity of Processed Table Beets (*Beta vulgaris var, conditiva*) and Green Beans (*Phaseolus vulgaris L.*)

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It has been shown that thermal processing of tomatoes and sweet corn results in increased antioxidant activities despite the loss of vitamin C. Until now, it is unclear whether this positive effect of thermal processing occurs with all crop produce. Therefore, analysis of a root vegetable (beets) and of a legume (green beans) was undertaken to address this question. Antioxidant activity of beets processed under typical commercial processing conditions remained constant despite an 8% loss of vitamin C, a 60% loss of color, and 30% loss of dietary folate. There was a slight but significant 5% increase in phenolic content of processed beets. In contrast, vitamin C and dietary folate content of green beans remained constant, whereas a 32% reduction in phenolic compounds occurred after typical commercial processing conditions. The antioxidant activity of green beans was reduced by 20%. These findings along with previous works suggest that the effects of thermal processing vary with the respective produce crop type. It also reinforces the concept that optimal health benefits may be achieved when a wide variety of plant foods (fruits, vegetables and whole grains) and preparation methods are incorporated into the diet.

KEYWORDS: phenolics; antioxidants; phytochemicals; ascorbic acid; folate; beets; green beans; thermal processing

INTRODUCTION

Epidemiological studies have consistently shown that consumption of diets high in fruits and vegetables may contribute to maintenance of health and possibly reduce risk for chronic diseases such as coronary heart disease, cataract, cancer, diabetes, and Alzheimer's disease (*1*, *2*). Complex mixtures of phytochemicals in whole foods are responsible for their health benefits and are better than single antioxidants due to a combination of additive and/or synergistic effects (*3*, *4*). The wide diversity and complexity of phytochemicals in fresh fruits and vegetables that contribute to these effects cannot be mimicked by individual dietary supplements (*4*). Therefore, the National Research Council (NRC) has recommended eating five or more servings of fruits and vegetables to sustain optimum health. It is thought that the key to preventing or reducing oxidative stress is to maintain the balance of antioxidants and endogenous oxidant levels. Tipping the balance toward oxidants causes oxidative stress, resulting in damages to DNA, lipids, proteins, and other biomolecules and ultimately leading to degenerative diseases (*5*, *4*). While the general belief has been that the greatest health benefits are obtained from fresh fruits and vegetables, and that processing destroys nutrients, current research has begun to question this concept (*6*, *7*).

Fruits and vegetables rich in pigments such as anthocyanins, betalains, or carotenoids are potent sources of antioxidants (*8*). These diverse biological pigments are found as complex mixtures in most fruits and vegetables. Anthocyanins and particularly betalain are heat sensitive, resulting in color loss during food processing. The ruby-like red color of table beet (*Beta vulgaris var conditiva*) has sparked much interest because of its intensity and availability. The color of beet is a mixture of red betacyanin (BC) and yellow betaxanthin (BX) pigments (*9*) that belong to a group of compounds collectively known as betalain. Apart from rich pigmentation, table beets are also very good sources of dietary folate (*10*). In recent studies, it has been shown that folate may prevent neural tube defects (*11*), exhibit antioxidant activity (*12*), and play an important role in prevention of cardiovascular diseases and cancer (*13*−*15*).

Green bean (*Phaseolus vulgaris L.*), a commonly consumed vegetable, is also a source of dietary folate and other important phytochemicals (*16*); therefore, it is important to understand how thermal processing affects the nutritional quality of green beans. Previous works on green beans mainly addresses storage and vitamin retention (*17*−*20*). Although there has been a recent shift to the study of the phytochemical profile and antioxidant activity of legumes in general (*21*−*23*), the question of how processing impacts antioxidant activity of canned green beans is still unanswered.

In previous studies, our research group has demonstrated that processed tomatoes and sweet corn exhibit higher antioxidant

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activities due to the increased release of bound phenolic compounds (*6, 7*), despite the loss of vitamin C content. Our previous research also found that vitamin C in apples accounts for only approximately <0.4% of the total antioxidant activity (*3*). To compliment our previous work on fruit and grain foods and to determine trends or general effects of thermal processing on nutritional quality of fruits and vegetables, we investigated the effects of thermal processing on a legume (green beans) and a root crop (beets). The objectives of this study were to: (1) evaluate the effects of thermal processing on the nutritional quality of beets and green beans by assessing the vitamin C content, total pigments (beets), dietary folate content, total phenolic content, total flavonoid content, and total antioxidant activity in raw and heat-treated samples; and (2) establish whether an increase in antioxidant activity is prevalent among canned fruits and vegetables.

MATERIALS AND METHODS

Chemicals. Sodium nitrate, (+)-catechin, Folin−Ciocalteu reagent, hydrochloric acid, α-keto-γ-methiobitiric acid (KMBA), L-ascorbic acid (AA), dehydroascorbic acid (DHAA) and trifluoroacetic acid were obtained from Sigma-Aldrich (St. Louis, MO). Aluminum chloride, sodium hydroxide, acetone and methanol were purchased from Fisher Scientific (Pittsburgh, PA). Gallic acid was purchased from ICN Biomedical Inc. (Costa Mesa, CA). Hexane and ethyl acetate were purchased from Mallinckrodt (Paris, Kentucky). 2,2′-Azobis-amidino-propane (ABAP) was obtained from the Wako Chemicals (Richmond, VA). *Lactobacillus casei* subspecies *rhamnosis* (ATCC 7469) was purchased from American Type Culture Collection laboratory (Parkville, MD). Bacto-MRS broth, Bacto-micro inoculum broth, folic acid casei medium, and pteroylmonoglutamic acid (PGA) were purchased from Difco (Sparks, MD). All reagents used were of analytical grade.

Sample Preparation. Raw table beets (10 kg) purchased from a local supermarket were peeled, trimmed and sliced. The sliced beets were mixed to obtain a homogeneous sample before being packaged in 8.8-cm (H) by 6.2-cm (D) cans, using a manual continental can sealer. A total of forty-eight cans were placed into the retort apparatus (Welding and Steel Fabrication Steam Kettle) in the pilot plant of the Department of Food Science, Cornell University. Groups of eight cans were subjected to six different heat treatments. Three groups were subjected to 15, 30, and 45 min of heat at 115 °C, where the commercial condition for canning beets is set at 115 °C for 30 min. Another two groups were subjected to 105 and 125 °C for 30 min. The control group was canned beets without a thermal processing treatment.

Raw green beans (15 kg) purchased from a local supermarket were washed and sliced. The green beans were mixed to obtain a homogeneous sample and packed in 8.8-cm (H) by 6.2-cm (D) cans with 150 mL of distilled water, enough to maintain a one-inch headspace. The 48 cans of green beans underwent similar sealing and retorting techniques as the canned beets. Groups of eight cans were subjected to six different heat treatments. Three groups were subjected to 10, 20, and 40 min of heat at 115 °C, where the commercial condition for canning green beans is set at 115 °C for 20 min. Another two groups were subjected to 100 and 121 °C for 20 min. Again, the control group was canned green beans without being thermally processed. All canned samples in each treatment were stored at −40 °C until analysis.

Extraction of Free Water-Soluble Phytochemicals. Phytochemical extraction is shown in the flowchart of **Figure 1**. A 50-g sample of beets or 1/5 of the canned green bean's total contents (35 g of green beans along with 30 mL of liquid fill) were measured and combined with distilled water in a 1:2 (w/v) ratio. The sample was blended at highest speed for 5 min in a Waring Commercial Laboratory Blender (Waring Commercial, Torrington, Connecticut) and homogenized for 3 min using the Virtis Homogenizer (Virtis, Gardiner, New York) to extract water-soluble phytochemicals. The slurry was centrifuged in a Beckman GS-6R Centrifuge for 15 min at 2200*g*. The supernatants of all samples were stored at −40 °C until used for quantification of vitamin C and folic acid.
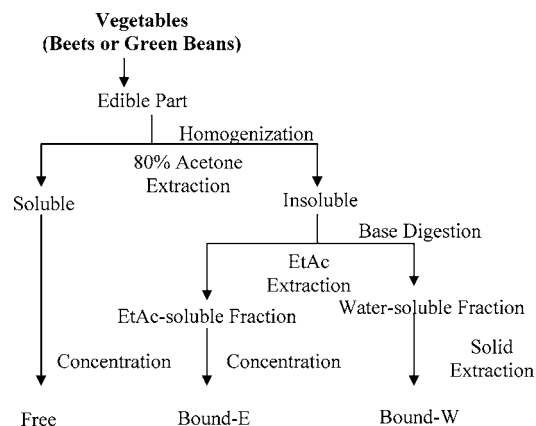

Figure 1. Flowchart of phytochemical extraction for beets and green beans.

Extraction of Free 80% Acetone-Soluble Phytochemicals. Free 80% acetone-soluble phytochemicals of canned beets and green beans were extracted by a modified method reported previously (*3, 6*). Briefly, 50 g of beets or 1/5 of the canned green bean's total content was measured and homogenized for 5 min with 80% acetone (1:2 w/v). After centrifugation, the 80% acetone supernatant was separated from the residue and evaporated under vacuum at 45 °C until ∼90% of the filtrate had evaporated. The residues were saved for the ethyl acetate extraction. The free 80% acetone-soluble phytochemical green bean extracts, designated as Free, were frozen at −40 °C until used for total phenolic, total flavonoid, and total antioxidant activity analysis (**Figure 1**).

Extraction of Bound Ethyl Acetate-Soluble Phytochemicals. Bound ethyl acetate-soluble phytochemicals of canned beets and green beans were extracted by the method previously reported (*7, 24*). Briefly, 2-g equivalents of beets or green beans were weighed from the acetone extraction residues and hydrolyzed directly with 4 N sodium hydroxide at room temperature for 1 h under shaking conditions. The bound phytochemicals were extracted with ethyl acetate. The remaining water-soluble residues were kept for further extractions. The ethyl acetate fraction was evaporated under vacuum at 45 °C to dryness. The phenolic compounds extracted by ethyl acetate were designated as bound-E (**Figure 1**). The extracts were stored at −40 °C until total phenolic, total flavonoids, and total antioxidant activity analysis.

Extraction of Bound Water-Soluble Phytochemicals. Bound water-soluble phytochemicals of canned beets and green beans were extracted by the method previously reported (*24*). Briefly, the water-soluble residues remaining from the ethyl acetate extractions were neutralized to pH 7 and then combined with an equal amount (w/w) of muffled Celite. The phytochemicals were eluted through the column by 20% methanol/ethyl acetate. The eluate was then evaporated under vacuum at 45 °C to dryness to obtain the bound water phytochemicals, which were designated as bound-W (**Figure 1**). The samples were stored at −40 °C until analysis.

HPLC Analysis of L-Ascorbic Acid and Dehydroascorbic Acid. L-Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents of beet or green bean extracts were determined by a modification of a method previously reported (*25, 26*). Free water-soluble extracts were filtered through a 0.45-μm filter and were injected into the HPLC. Separation, analysis, and quantification of AA and DHAA were accomplished using a 25-cm × 4.6-mm Supelcosil LC-18 (5-μm) column (Supleco) operated at ambient temperature. An isocratic mobile phase was used for the separation and was composed of acidified deionized water at a flow-rate of 0.5 mL/min. The pH of the mobile phase was adjusted to 2.7 with 0.01% trifluoroacetic acid. AA and DHAA were detected using a Waters 484 Tunable Absorbance Detector (Waters Corp., Milford, MA) set at 245 and 210 nm, respectively. Detector signals were acquired and integrated by Waters Millenium32 software (Waters Corp.). The AA and DHAA calibration curves were generated from peak heights of standards and sample concentrations were calculated by extrapolation on the calibration curves. Peak identification of AA and DHAA in sample extracts was based on retention time and co-chromatography of authentic AA or DHAA

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standards. The results were expressed as mean (mg AA or DHAA per 100 g of beets or green beans) ± SD. Beets were analyzed in triplicates and green beans in five replicates.

Determination of Dietary Folate. Dietary folate content of beet or green bean extracts was determined by the method previously reported (*27*). Briefly, after incubation with extracts, the turbidity of *Lactobacillus casei* subspecies *rhamnosis* (ATCC 7469) was measured at 650 nm with the aid of a spectrophotometer. *L. casei* ATCC 7469 was subcultured from Bacto-MRS stock into Bacto-micro inoculum broth. After 18 h of incubation at 37 °C, the cells were washed with 0.85% sterile sodium chloride and re-suspended to 80% transmittance at 650 nm in 0.85% sterile sodium chloride. A 20-mg sample of crystalline pterolymonoglutamic acid (PGA) was dissolved and diluted to make a PGA standard working solution containing 0.2 ng PGA/mL. Each assay set of PGA stock working solution included six levels of folic acid concentrations (0.05, 0.10, 0.20, 0.30, 0.40, and 0.50 ng), plus a blank control. Each assay tube in the set consisted of 2.5 mL of assay medium, various amounts of PGA stock working solution and correspondent distilled water to a final volume of 5.0 mL, respectively. The extracts were filter sterilized through a 0.45-μm filter and diluted 1:10 with sterile deionized water. Each assay tube in the set consisted of 2.5 mL of assay medium, 1.0 mL of sample solution, and 1.5 mL of distilled water to make a final volume of 5.0 mL per tube. A 10-μL aliquot of standardized (80% T) inoculum of *L. casei* was aseptically added into each assay tube, except the blank control. At the end of a 20-h incubation period at 37 °C, the assay tubes were thoroughly vortexed, and the transmittance of each assay tube at 650 nm relative to un-inoculated control was recorded spectrophotometrically. The dietary folate content of each entry was determined by the regression equation of the standard curve. Results were reported as mean (μg folic acid per 100 g of beets or green beans) ± SD. Beets were analyzed in six replicates and green beans in five replicates.

Determination of Total Pigment of Beets. The total betalain pigment content of beets was measured using Beckman DU 640B spectrophotometer (Beckman, Fullerton, California). The wavelengths of 535 and 476 nm were used for betacyanin and betaxanthin analysis, respectively (*8*). Extract from the raw sample was diluted in a series of halves with distilled water until a dilution factor of 300 was reached or absorbance falls in the range of 0.18−1.8 for both wavelengths. Distilled water was used as the control.

The pigment concentration in beet extract was calculated using the following formula:

$$c = A/\alpha b$$

where A is the absorbance; α is the molecular absorptivity constant; b = 1 cm, and c is the percent concentration. Betacyanin has an α of 1120 cm^{-1}1%$^{-1}$ at 535 nm (*8*), and betaxanthin has an α of 650 cm^{-1}1%$^{-1}$ at 476 nm (*8*). Total pigment of beets was the sum of betacyanin and betaxanthin values expressed as mean (mg of pigments per 100 g of beets) ± SD for triplicates. Ratios of red to yellow pigments were calculated by dividing betacyanin by betaxanthin values (BC/BX) (*9*).

Determination of Total Phenolic Content. The total phenolic content was analyzed spectrophotometrically using a Folin−Ciocalteu colorimetric method reported previously (*28*) and modified in our laboratory (*6*). All values were expressed as mean (μg of gallic acid equivalents per 100 g of beets or green beans) ± SD. Beets were analyzed in six replicates and green beans in five replicates.

Determination of Total Flavonoid Content. Total flavonoid content was determined by a colorimetric method described previously (*29*) and modified in our laboratory (*6*). The results were expressed as mean (μg of catechin equivs per 100 g of beets or green beans) ± SD. Beets were analyzed in six replicates and green beans in five replicates.

Quantification of the Total Antioxidant Activity. The total antioxidant activity of the free phytochemical extracts from beets and green beans was measured by a modified total oxyradical scavenging capacity (TOSC) assay (*30*, *6*). Antioxidant activity was assessed at four different time points of 15-minute intervals for 1 h and six different concentrations to determine the TOSC value. The TOSC values for each concentration of beets or green beans were calculated using the

Table 1. Effects of Thermal Processing at a Constant Temperature of 115 °C for 0, 15, 30, and 45 Minutes or at Various Holding Temperatures (105, 115, 125 °C) at a Constant Time of 30 min on Vitamin C (Mean ± SD, $n = 3$) and Dietary Folate Contents in Beets (Mean ± SD, $n = 6$)

treatment groups		ascorbic acid (mg/100 g beet)			free folic acid (μg/100 g beet)
		AA	DHAA	total	
constant temp (115 deg C)	0 min	14.83 ± 0.41[a]	158 ± 1[a]	173 ± 1[a]	27.4 ± 1.3[a]
	15 min	14.97 ± 1.1[a]	148 ± 4[b]	163 ± 4[b]	21.2 ± 2.4[b]
	30 min	13.20 ± 0.13[b]	146 ± 4[b]	159 ± 4[b]	19.0 ± 1.6[b,c]
	45 min	12.46 ± 0.08[b]	146 ± 4[b]	159 ± 4[b]	16.8 ± 1.6[c]
constant time (30 min)	raw	14.83 ± 0.41[a]	158 ± 1[a]	173 ± 1[a]	27.4 ± 1.3[a]
	105 deg C	13.66 ± 0.05[b]	157 ± 3[a]	171 ± 3[a]	20.7 ± 0.7[b]
	115 deg C	13.20 ± 0.13[b]	146 ± 4[b]	159 ± 4[b]	19.0 ± 1.6[b,c]
	125 deg C	12.08 ± 0.33[c]	150 ± 2[b]	162 ± 2[b]	16.6 ± 1.4[c]

[a] In the same column, values with no letters in common are significantly different ($p < 0.05$).

integration of the area under the kinetic curve. The TOSC value for each concentration was quantified according to the following equation:

$$TOSC = 100 - (\int SA/\int CA \times 100)$$

where $\int SA$ is the integrated area from the sample reaction and $\int CA$ is the integrated area from the control reaction.

For the extracts of each treatment, the median effective dose (EC50) was determined from the dose−response curve of concentration of beets or green beans versus TOSC. Using the median effective dose for each treatment, the TOSC value was determined and expressed as mean (μmol vitamin C equivalents per gram beets or green beans) ± SD. Beets were analyzed in six replicates and green beans in five replicates.

Statistical Analysis. Statistical analysis was conducted using MiniTab (12th Edition) software (MiniTab, State College, Pennsylvania). Differences among each treatment were determined using Tukey's pairwise comparisons of the analysis of variance (ANOVA). Means were considered significantly different if *p*-values were less than or equal to 0.05.

RESULTS

Vitamin C Content. Thermal processing of beets showed a slight linear degradation of free vitamin C content (**Table 1**). Treatment under constant temperature of 115 °C at various time intervals of 15, 30, and 45 min showed a small but significant reduction of vitamin C as compared to the control (**Table 1**), which is consistent with previous results (*31*). Treatment under a constant time of 30 min at various temperatures of 105, 115, and 125 °C showed similar results as the former treatment group. There was a significant reduction ($p < 0.05$) in vitamin C content (**Table 1**), which was consistent with previous results (*31*). Dehydroascorbic acid (DHAA) content of processed beets showed a small and significant decrease compared to the control ($p < 0.05$) (**Table 1**). Total vitamin C content of processed beets did decrease with thermal processing, as was seen with free vitamin C content. A small, but significant difference ($p < 0.05$) was seen comparing the results from the commercial condition treatment to the control for both groups.

Thermal processing of green beans under constant temperature of 115 °C at various time intervals of 10, 20, and 40 min showed a small but significant initial reduction of vitamin C content ($p < 0.05$) followed by a increase to pre-thermal processing levels (**Table 2**), which is consistent with previous studies (*32*, *33*). The commercial processing conditions of canned green beans were 115 °C for 20 min (5.00 ±. 017 mg AA/100 g green bean), which was not significantly different from the control ($p > 0.05$). Treatment under constant time of 20 min at various temperatures of 100, 115, and 121 °C showed no significant difference from
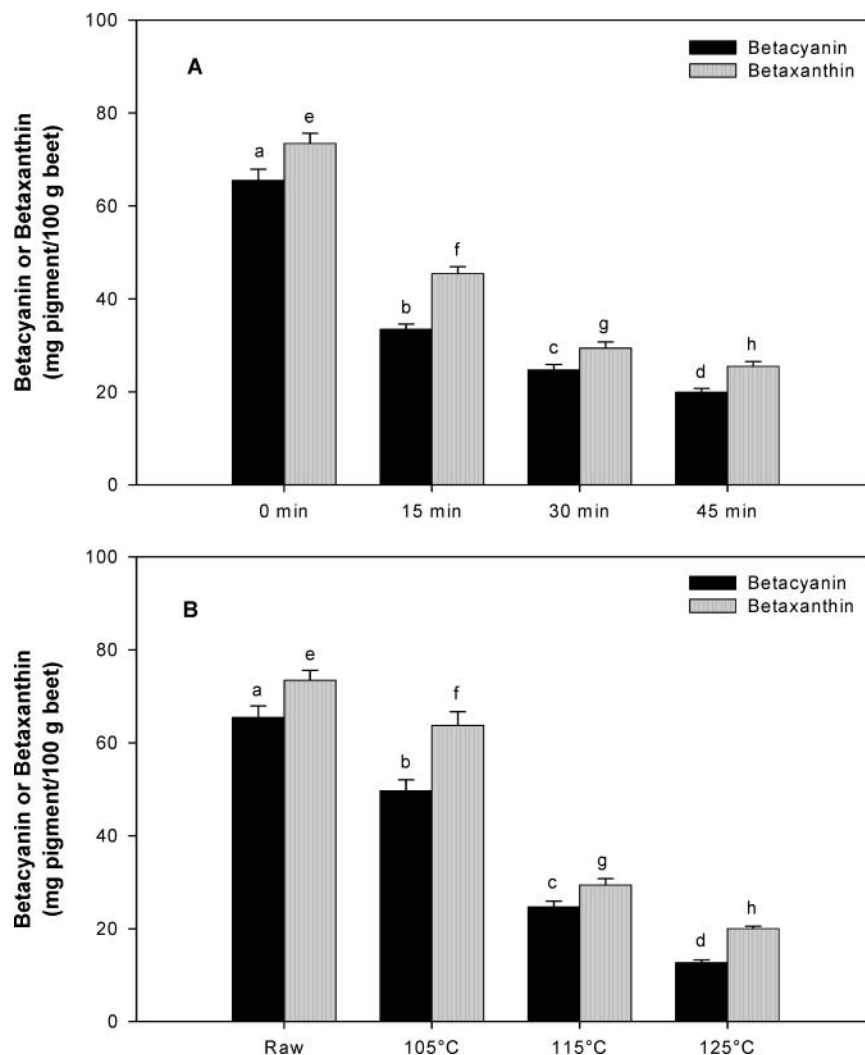
Figure 2. Effects of thermal processing at a constant temperature of 115 °C for 0, 15, 30, and 45 min (**A**) or at various holding temperatures (105, 115, 125 °C) at a constant time of 30 min (**B**) on betacyanin and betaxanthin pigments in beets (Mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

Table 2. Effects of Thermal Processing at a Constant Temperature of 115 °C for 0, 10, 20, and 40 Minutes or at Various Holding Temperatures (100, 115, and 121 °C) at a Constant Time of 20 min on Vitamin C (Mean ± SD, $n = 5$) and Dietary Folate Contents in Green Beans (Mean ± SD, $n = 5$)

		ascorbic acid (mg/100 g green bean)			free folic acid
treatment groups		AA	DHAA	total	(μg/100 g beet)
constant	0 min	5.33 ± 0.07^a	ND	5.33 ± 0.07^a	4.82 ± 0.22^a
temp	10 min	4.68 ± 0.08^b	ND	4.68 ± 0.08^b	4.52 ± 0.08^a
(115°C)	20 min	5.00 ± 0.17^a	ND	5.00 ± 0.17^a	4.56 ± 0.09^a
	40 min	5.11 ± 0.11^a	ND	5.11 ± 0.11^a	4.71 ± 0.073^a
constant	raw	5.33 ± 0.07^a	ND	5.33 ± 0.07^a	4.82 ± 0.22^a
time	100 deg C	5.32 ± 0.26^a	ND	5.32 ± 0.26^a	4.68 ± 0.16^a
(20 min)	115 deg C	5.00 ± 0.17^a	ND	5.00 ± 0.17^a	4.56 ± 0.09^a
	121 deg C	5.15 ± 0.13^a	ND	5.15 ± 0.13^a	4.89 ± 0.23^a

[a] In the same column, values with no letters in common are significantly different ($p < 0.05$).

the control ($p > 0.05$) (**Table 2**). Unlike canned beets, there were trace amounts of DHAA in canned beans (data not reported), which is consistent with previous studies performed on water-packed canned green beans (*32*).

Betalain Content. The total betacyanin content of the raw canned beets was 65.5 ± 2.5 mg BC/100 g beet (**Figure 2**). With thermal processing at 115 °C for 15, 30, and 45 min, the

total betacyanin content in the heat-treated beets decreased by 48.8 ($p < 0.05$), 62.2 ($p < 0.05$), and 65.3% ($p < 0.05$), respectively (**Figure 2A**). After treatment at 105, 115, and 125 °C for 30 min, the betacyanin content was significantly decreased by 24.0 ($p < 0.05$), 62.2 ($p < 0.05$), and 80.6% ($p < 0.05$), respectively, when compared to the unprocessed raw beet (**Figure 2B**). A similar trend was seen with betaxanthin pigments. With thermal processing at 115 °C for 15, 30, and 45-minute intervals, the total betaxanthin content in the heat-treated beets was significantly decreased by 38.1 ($p < 0.05$), 59.9 ($p < 0.05$), and 65.3% ($p < 0.05$), respectively (**Figure 2A**). After treatment at 105, 115, and 125 °C for 30 min, the betaxanthin content was decreased by 13.1 ($p < 0.05$), 59.9 ($p < 0.05$), and 72.7% ($p < 0.05$), respectively, when compared to unprocessed raw beet (**Figure 2B**). The decrease of beta-xanthin content across both heating time and heating temperature parameters was statistically significant between the control and each heat-treated beet sample ($p < 0.05$).

Dietary Folate Content. Folic acid content of thermally processed beets did show a significant reduction ($p < 0.05$) in both treatment groups as compared to the unprocessed set. Approximately 25% of folic acid content was initially lost with application of heat in both treatment groups, and a total of 40% reduction was measured in the most severe processing condition at 125 °C for 30 min (**Table 1**).
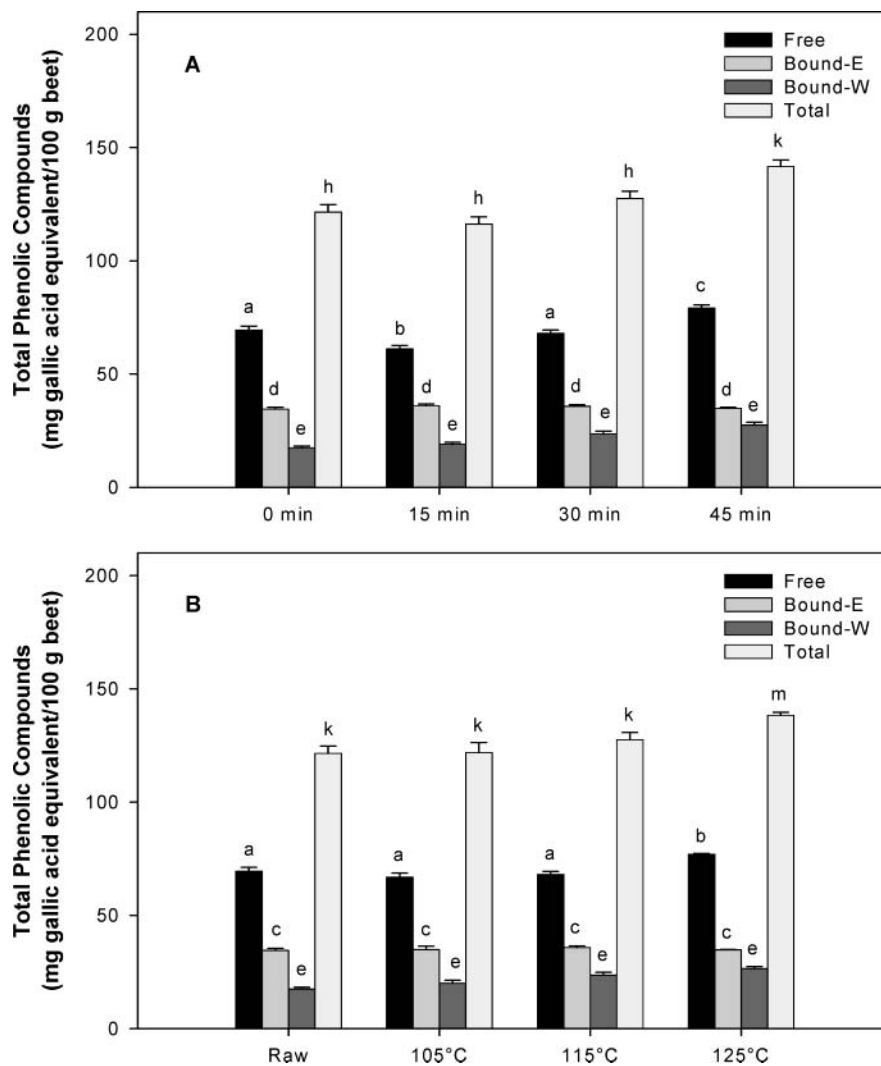
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Figure 3. Effects of thermal processing at a constant temperature of 115 °C for 0, 15, 30, and 45 min (**A**) or at various holding temperatures (105, 115, 125 °C) at a constant time of 30 min (**B**) on total phenolic content in beets (Mean ± SD, $n = 6$). Bars with no letters in common are significantly different ($p < 0.05$).

Folic acid content of thermally processing green beans did not show any significant reduction ($p > 0.05$) in both treatment groups as compared to the unprocessed set (**Table 2**). The folic acid content of the bound ethyl acetate extracts was not detectable, whereas trace amounts were detected in the bound water residues (data not reported).

Phenolic Content. Free phenolic content of beets was not greatly affected by the thermal processing required for commercial canning at 115 °C. Initial application of heat reduced phenolic content of the beets by 12%, but further processing raised its content back to the equivalent of unprocessed beets, and eventually a 14% increase was noted after processing at 115 °C for 45 min (**Figure 3***A*). Similarly, the phenolic content of bound ethyl acetate extracts was also not affected by thermal processing and contributed 25−30% of total phenolic content of processed beets (**Figure 3A**). Analysis of bound water extracts did not show a significant change of phenolic compound as compared to the control (**Figure 3A**). Compared to the control, processing at 15 and 30 min did not significantly change the total phenolic content in processed beets, but a 17% ($p < 0.05$) increase in total phenolic content was seen in the 45-minute heat treatment.

A 30-minute treatment time at temperatures of 105, 115, and 125 °C showed a free phenolic compound profile similar to that

of the previous treatment group (**Figure 3B**). Similarly, there was no significant change in free phenolics at 105 and 115 °C for 30 min when compared to the control, and ultimately, ended with an 11% increase at 125 °C for 30 min (**Figure 3B**). Similar to the previous bound ethyl acetate group, thermal processing at a constant holding time of 30 min for 105, 115, and 125 °C did not show any effect on beet phenolic content. Again, bound ethyl acetate extracts represented 25−30% of the total phenolic content of the samples. Analysis of bound water extracts showed no significant changes at 105 and 115 °C when compared to the control, but there was a 14% increase at 125 °C ($p < 0.05$).

Initial thermal processing of green beans at 115 °C for 10 min degraded approximately 40% of free total phenolic compounds ($p < 0.05$) (**Figure 4A**). Further application of heat for 20 min, however, did not result in further reduction of phenolic content of the green beans, but there was an increase in phenolic content after 40 min of processing when compared to the samples at 10 and 20 min. (**Figure 4A**). Evaluation of the bound ethyl acetate extracts showed a significant increase ($p < 0.05$) of phenolic content as compared to the control treatment where phenolic contents were at undetectable levels. Although significant, the phenolic content from these extracts represented roughly 10 percent of the free counterpart (**Figure 4A**). Analysis of bound water extracts showed no detectable phenolic com-
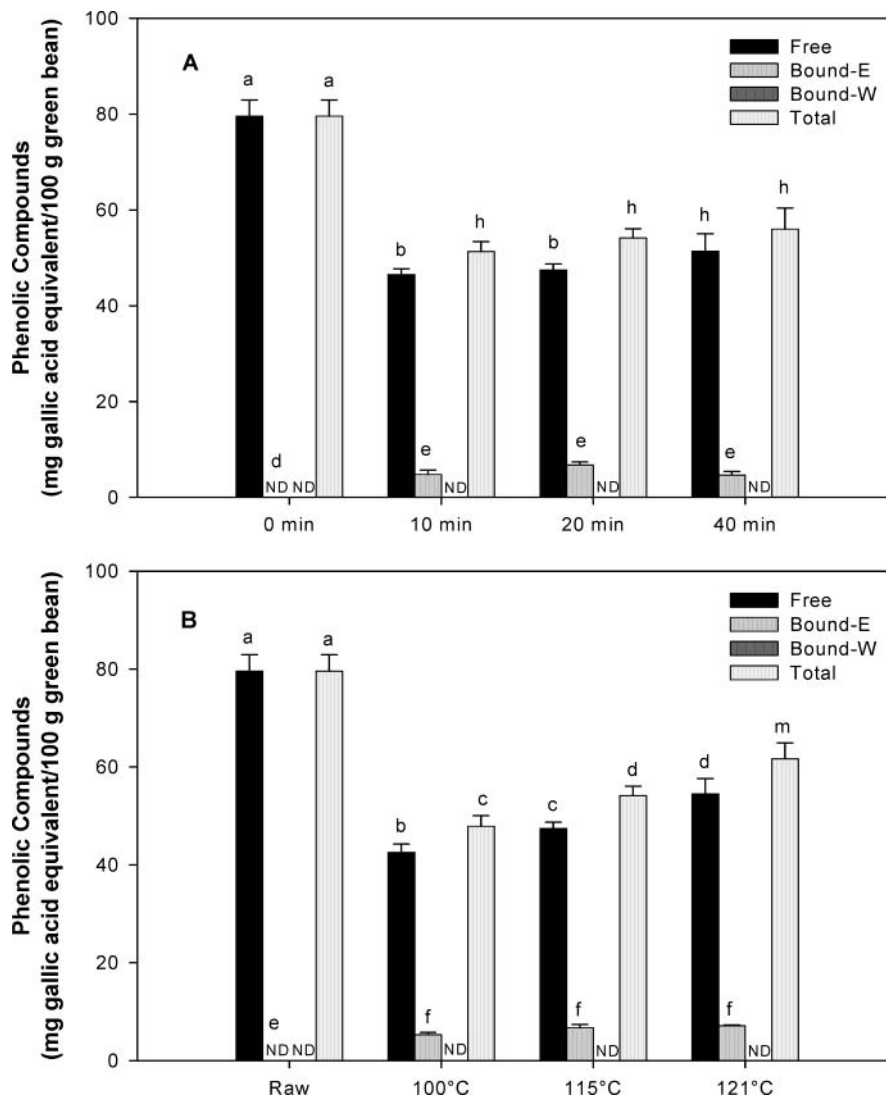
Figure 4. Effects of thermal processing at a constant temperature of 115 °C for 0, 10, 20, and 40 min (**A**) or at various holding temperatures (100, 115, and 121 °C) at a constant time of 20 min (**B**) on total phenolic content in green beans (Mean ± SD, $n = 5$). Bars with no letters in common are significantly different ($p < 0.05$).

pounds, therefore the total phenolic content of processed green beans were mainly comprised of "Free" phytochemicals (**Figure 4A**). Total phenolics in green beans processed at 115 °C for 10, 20, and 30 min were decreased by 36 ($p < 0.05$), 32 ($p < 0.05$) and 30% ($p < 0.05$), respectively, when compared to the control.

Treatments of 100, 115, and 121 °C for 20 min resulted in a slightly different profile of phenolic compounds than measured in the previous treatment group (**Figure 4B**). Similarly, there was an initial 40% reduction of free phenolics. The phenolic compounds measured for processing at 121 °C for 20 min showed levels lower than the control group; however, the levels were significantly higher when compared to the 100 and 115 °C treatment ($p < 0.05$) (**Figure 4B**). Similar to the previous group, thermal processing at constant holding time of 20 min for 100, 115, and 121 °C showed a significant increase ($p < 0.05$) in bound-E phenolic compounds as compared to its control, at undetectable levels. As described above, the phenolic content from this extraction set represents roughly less than 10% of total phenolic compounds found for each thermal treatment (**Figure 4B**). Analysis of bound water extracts showed no detectable phenolic compounds in any group (**Figure 4B**). The total phenolic content of the 100, 115, and 121 °C groups was

40, 32, and 23% lower, respectively, than for the unprocessed group ($p < 0.05$).

Flavonoid Content. Analysis of the free flavonoid content of processed beets at 115 °C for 15 min showed a significant 47% ($p < 0.05$) increase compared to the control. Further thermal treatment for 30 and 45 min at 115 °C showed a significant increase of flavonoids by 63% ($p < 0.05$) and 75% ($p < 0.05$), respectively, as compared to the control (**Figure 5A**). Analysis of flavonoid content in the bound ethyl acetate and bound water extracts showed no significant change as compared to the controls (**Figure 5A**). Combined, both groups of bound flavonoids contributed approximately 22−30% of the total flavonoid content. A significant and gradual increase of total flavonoid content was seen in the 15, 30, and 45 min treatment groups as compared to the control.

Processing beets for 30 min at 105 °C significantly increased the free flavonoid content (**Figure 5B**). Processing at 115 °C did not show any significant difference from the 105 °C treatment group; however, treatment at 125 °C resulted in significant increase compared to the control and the two previous treatment groups (**Figure 5B**). The analysis of bound ethyl acetate extracts in the three thermally processed groups showed no significant change in the flavonoid content from the control
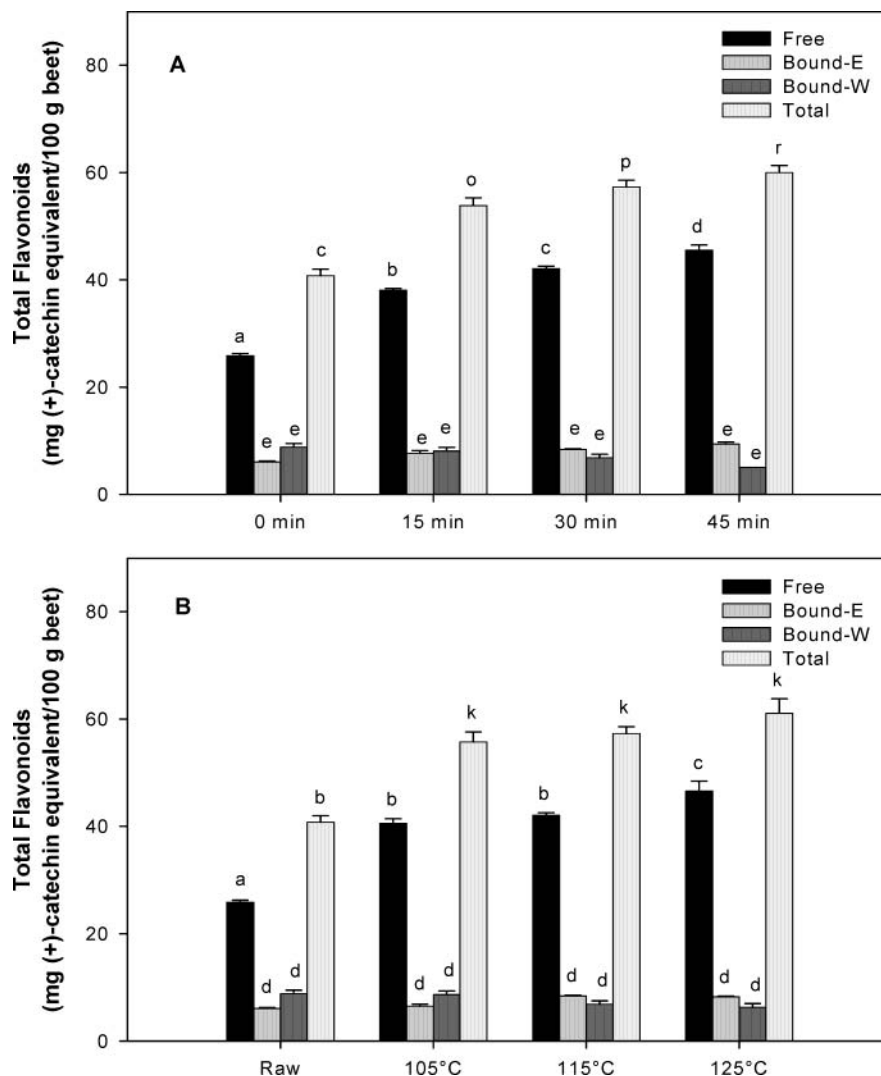
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Figure 5. Effects of thermal processing at a constant temperature of 115 °C for 0, 15, 30, and 45 min (**A**) or at various holding temperatures (105, 115, 125 °C) at a constant time of 30 min (**B**) on total flavonoid contents in beets (Mean ± SD, $n = 6$). Bars with no letters in common are significantly different ($p < 0.05$).

and represented 11−15% of total flavonoid content (**Figure 5B**). The bound water extracts yielded flavonoid values that were similar to the bound ethyl acetate extracts. Beets processed at 105 °C for 30 min showed a significant increase in total flavonoid content as compared to the control. Processing at 115 and 125 °C for 30 min also showed a significant increase in flavonoids as compared to the control; however, no significant difference was detected among the three thermal treatments (**Figure 5B**).

Unlike processed beets, processed green beans showed a significant decrease in free flavonoid content compared to the control. There was an initial 60% reduction of free flavonoid compounds in green beans processed for 10 min at 115 °C as compared to the control (**Figure 6A**). Treatments at 20 and 40 min also showed a significant decrease in flavonoids compared to the control (**Figure 6A**). There were no significant differences among the three thermally processed groups. Analysis of bound ethyl acetate extracts of processed green beans showed no significant different from the control. Flavonoids were not detected in any of the bound water extracts; therefore, the major contribution to the total flavonoid content of processed green beans came from the free extracts. Thermal processing decreases

total flavonoid content by approximately 60% in all three treatment groups as compared to the nonprocessed control (**Figure 6A**).

Processing at 100, 115, and 121 °C for 20 min also showed a significant decrease in free flavonoid contents as compared to the control (**Figure 6B**). Again, analysis of bound ethyl acetate extracts showed an approximately 15% contribution of flavonoid detected among thermally treated green beans (**Figure 6B**). There was no contribution to the total flavonoid content from any of the bound water-soluble green bean extracts. Total flavonoid content of green beans processed at 100 °C for 20 min showed a 60% decrease as compared to the control. Subsequent treatment of green beans at 115 °C and 121 °C for 20 min showed no significant difference from the 100 °C group (**Figure 6B**).

Total Antioxidant Activity. Total antioxidant activity of processed beets at 15, 30, and 45 min under constant temperature showed no significant change as compared to its control ($p > 0.05$) (**Figure 7A**). Processing at a constant time of 30 min at various temperatures also showed no significant change as compared to the control ($p > 0.05$) (**Figure 7B**). Overall, thermal processing of beets had no impact on the total antioxidant activity, which was expected.
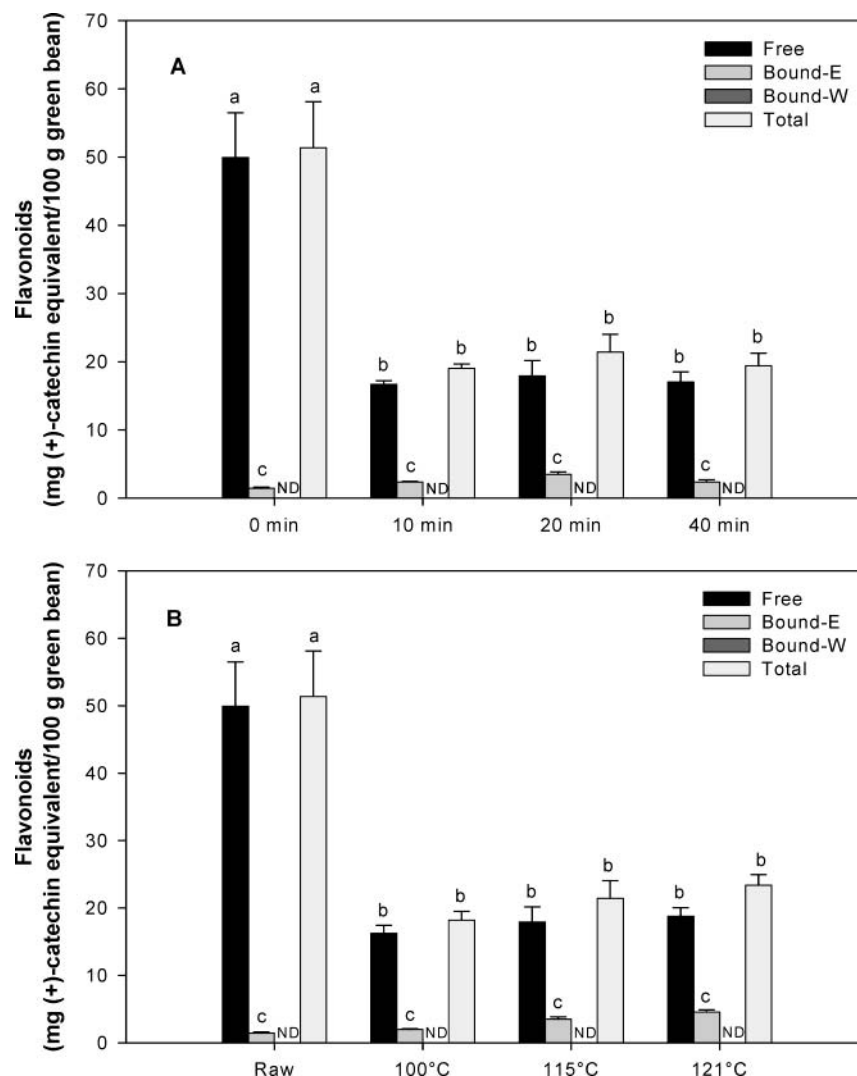
Figure 6. Effects of thermal processing at a constant temperature of 115 °C for 0, 10, 20, and 40 min (**A**) or at various holding temperatures (100, 115, and 121 °C) at a constant time of 20 min (**B**) on total flavonoid contents in green beans (Mean ± SD, $n = 5$). Bars with no letters in common are significantly different ($p < 0.05$).

The total antioxidant activity of processed green beans for 10 min at constant temperature showed a 9% decrease in total antioxidant activity as compared to the control ($p < 0.05$) (**Figure 8A**). Subsequent treatment at 20 and 40 min showed a constant 9% decrease as compared to the control. There was no significant change in total antioxidant activity among the processed groups ($p > 0.05$). Total antioxidant activity of green bean processed at 100, 115, and 121 °C for a constant time of 20 min were statistically lower as compared to the control ($p < 0.05$) (**Figure 8B**), but there were no differences in total antioxidant activity among the processed groups.

DISCUSSION

Processed fruits and vegetables, especially canned products, have long been perceived to have lower nutritional values (*34, 35*) due to degradation of vitamin C content, but recent findings from our lab suggests that this loss did not significantly impact antioxidant activity (*6–7*). AA content was observed to be approximately 10% of total vitamin C content, where the remaining 90% was due to DHAA, which was thought to accumulate during storage of beets after harvest as a result of continued metabolic processes of this root crop. We observed the loss of total vitamin C in canned beets to be minimal. In

contrast, analysis of canned green beans showed only trace amounts of DHAA present, and AA content was responsible for the total vitamin C reported. Discrepancy of these two sets of results can be explained by the different packing method used prior to thermal treatment. Neither vegetable was blanched before canning, and more importantly, beets were canned with no liquid fill, whereas the green beans were packed with water. The liquid fill was thought to produce a sufficient reducing environment in the can to recycle DHAA back into AA (*32*). In previous works (*36*), it was shown that dehydration-heating methods cause more severe vitamin degradation. Leaching of vitamin C during blanching prior to packaging for processing is also a key step in vitamin C loss. The estimated $D_{115°C}$ value for canned beets (the time taken for 90% reduction of the initial vitamin C content at 115 °C) was approximately double of the previously reported values of 218−276 min (*35, 6–7*) and even longer values for canned green beans because there was no degradation of vitamin C. The consistent values reported for vitamin C content in green beans among the thermal heating conditions was reinforced by previous findings that time and temperature parameters contribute directly very little to vitamin C degradation (*33*).

Apart from the reducing environment created within the can, enzyme antioxidant activities in combination with phytochemi-
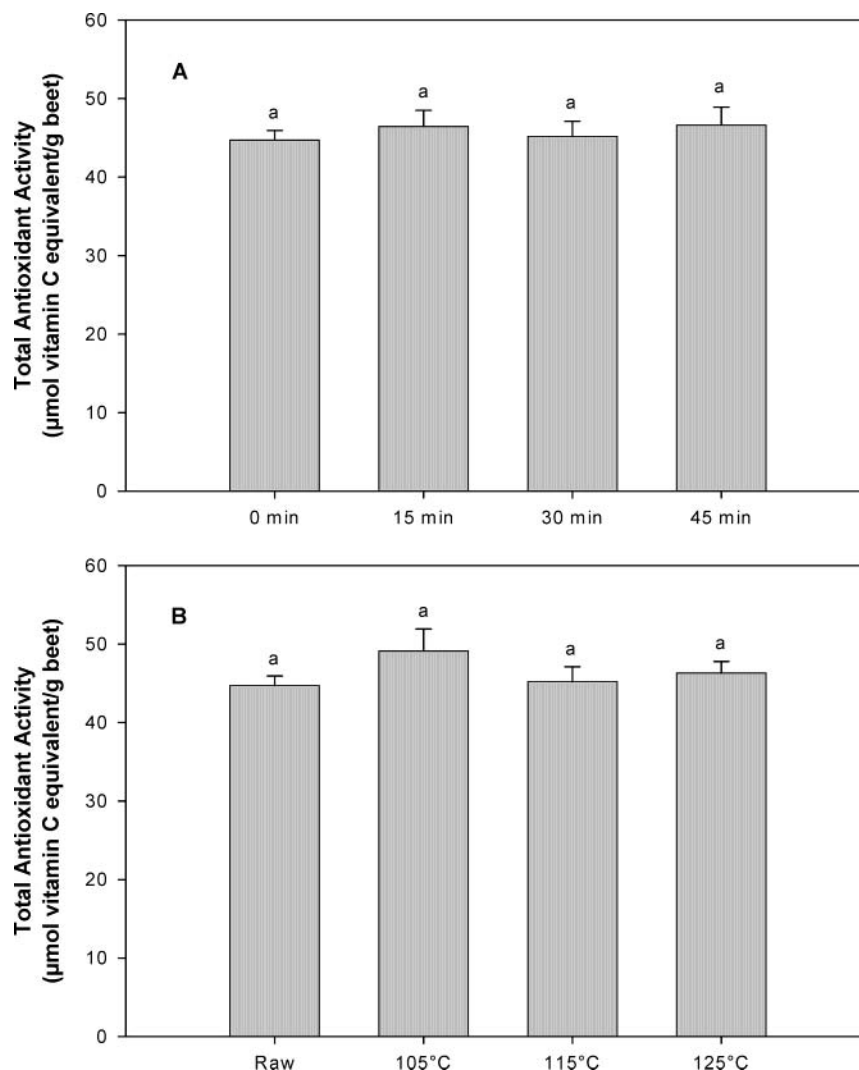
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Figure 7. Effects of thermal processing at a constant temperature of 115 °C for 0, 15, 30, and 45 min (**A**), or at various holding temperatures (105, 115, 125 °C) at a constant time of 30 min (**B**) on total antioxidant activities in beets (Mean ± SD, $n = 6$). Bars with no letters in common are significantly different ($p < 0.05$).

cals within the green bean extracts could be responsible for ascorbic acid regeneration. Enzymes such as ascorbate oxidase and glutathione-dependent oxidoreductase along with plasma membrane bound cytochrome b_{561} and polyamines have been found to regenerate ascorbic acid from oxidized intermediates (*37, 38*). Nonacclimate heat shock studies have also shown increasing protective effects of polyamines, which has recently been shown to have antioxidant activities, on membrane and cell wall stability (*38, 39*). The protective role that polyamines play against thermal stress prolongs the regeneration of ascorbic acid from membrane bound enzymes and transport channels (*38*). Once temperatures exceed biological ranges, heat stable phytochemicals continue the ascorbic acid regeneration process. Ubiquinone (UQ) or coenzyme Q_{10} has been suggested as an important molecule in this system (*40*). Recent data reported by Mattila and Kumpulainen (*41*) show that beans are good vegetable sources of UQ. Thermal degradation and homogenization of green beans could increase the distribution of UQ and consequently increase the regeneration of oxidized intermediates back to ascorbic acid within the extracts. It was also suggested that other parameters such as acidity, salinity, and metal content played significant roles (*32*). Generally, loss of vitamin C occurs primarily by chemical degradation involving oxidation of AA to DHAA and 2,3-diketogulonic acids, which further degrades to nutritionally inactive products. Without taking pH, salt metal

ion, enzyme, and phytochemical concentration into account, fruits and vegetables should loose their vitamin C content during processing because heat accelerates this reaction (*42*). Our previous findings that vitamin C only makes up roughly less than 0.4% of antioxidant activity in apples (*3*) suggest that degradation of vitamin C might not affect the total antioxidant activity found in canned beets and green beans.

The influence of thermal processing on betacyanin and betaxanthin pigment was determined by a spectrophotometric assay. Thermal processing for 15 min at 115 °C reduced betacyanin content to approximately half; a linear decline was seen in subsequent treatments. A similar trend was observed for the betaxanthin compounds. The half-lives of betacyanin and betaxanthin were 22.31 ± 0.7 and 26.19 ± 0.9 min at 115 °C, respectively. The longer half-life for betaxanthin was expected because yellow pigment degrades slower than the red pigments of betacyanin. Savolainen et al. (*43*) reported half-life values for purified pigments to be roughly one-half of those reported here. This discrepancy strengthens the suspicions that antioxidants, such as AA, in the beet extract help maintain and preserve some of the pigments. The red to yellow (BC/BX) ratio is a comparison tool between different cultivars of table beets (*9*). The BC/BX ratio in this cultivar was slightly higher than 1; some cultivars are known to have ratios as high as 2.08 (*9*). Thus, it is important to consider the cultivars when concentrating
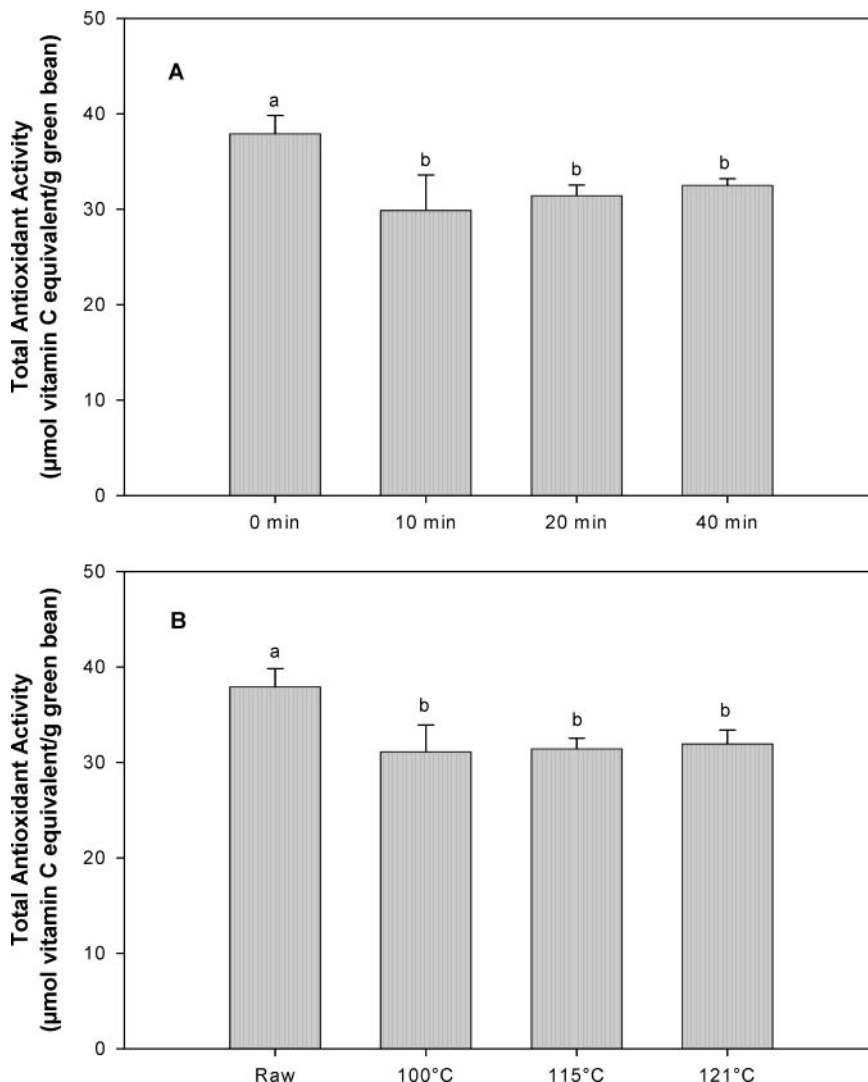
Figure 8. Effects of thermal processing at a constant temperature of 115 °C for 0, 10, 20, and 40 min (**A**), or at various holding temperatures (100, 115, and 121 °C) at a constant time of 20 min (**B**) on total antioxidant activities in green beans (Mean ± SD, $n = 5$). Bars with no letters in common are significantly different ($p < 0.05$).

and purifying of dye applications. The approximately linear loss of the two compounds did not translate into a consistent trend when their ratios were compared (data not shown). The fluctuations in the ratio suggested that degradation of betacyanin and betaxanthin were not constant, which was expected. The commercial time and temperature combination gave the highest response among the thermally processed beet samples in both sets. An approximate 60% loss of pigment was observed under commercial thermal processing conditions; however, the BC/BX ratios remained almost the same. On the basis of data from Savolainen and Kuuis (*43*), it is reasonable to assume that under the same conditions pure pigments would have suffered a greater degradation and would therefore make betalain compounds not viable for food products that require heat treatment. It would be necessary to add a "stabilizer", such as EDTA and vitamin C, to help reduce the rate of pigment degradation (*43*). The reduction−oxidation cycle imparted by various phytochemicals already embedded in the beet matrix may still preserve the antioxidant activity of betalains even though beet pigments were reduced in the thermal processing. The loss of pigmentation does not necessarily translate to a loss in antioxidant activity. Our results clearly showed that overall antioxidant activity was unchanged regardless of heat or time treatment.

Dietary folate and its synthetic counterpart, folic acid, is a vitamin B complex that plays an important role in cardiovascular diseases and cancer prevention (*13−15*). According to the TOSC assay, folic acid has an antioxidant activity value (EC_{50}) of 13.31 ± 1.39 μM, which is twice the activity of vitamin C. Because beets are an excellent plant source of dietary folate, it was interesting to see the effects of thermal processing had on dietary folate content. The estimated $D_{115°C}$ value for free dietary folate in canned beets was approximately 219 min. It has been reported that minor folate loss was noted in closed systems, whereas blanching steps may extract considerable amounts of mono-glutamate folate out of the system (*44*). In contrast, dietary folate in canned green beans, which was initially 5-fold lower than that in beets, was not affected by thermal processing. This phenomenon parallels the effects we saw with vitamin C content earlier and perhaps could be similarly explained. Packing and processing in water could have created a reducing environment that could prolong the degradation or initiate the recycling of already free folate. Conjugated forms of dietary folate may have been released during thermal processing and can contribute to the leveling affect seen with the canned green beans. This result was not seen in the canned beets because folate concentration could not be recycled in the absence of the reducing environ-

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ment. Like vitamin C, dietary folate constitutes a small portion of phytochemicals in both beets and green beans; therefore, loss or conserved folate content might not significantly impact total antioxidant activity of the product.

We adapted the method used by Singleton et al. (*28*) to analyze for total phenolic contents. The phenolic compounds of beet were found in the free, bound ethyl acetate, and bound water-soluble extracts, whereas the phenolic compounds of green beans were found in free and bound ethyl acetate extracts. Our results showed that for canned beets, heat treatment slightly increased the free and bound water-soluble phenolic compounds. There was neither a positive nor negative affect on bound ethyl acetate-soluble phenolic content. Half of total phenolic compounds were from free acetone-soluble extracts, 25% from the bound ethyl acetate extracts and the remaining 25% from the bound water-soluble extracts. Under various holding times of 15, 30, and 45 at 115 °C, there was an initial 5% loss of total phenolic content followed by a 5 and 17% increase. Treatments at temperatures of 105, 115, and 125 °C for 30 min showed a profile of free phenolic compounds similar to that of the previous treatment group. The total phenolic content of processed beets processed at the highest temperature (125 °C) showed a 14% increase in total phenolic compounds compared to the raw control. Unlike the canned beets, the distribution of phenolics in canned green beans was 100% from free acetone-extracts for the unprocessed control, 90% from free acetone-extracts, and 10% from bound ethyl acetate-extracts for the latter heat treatments. Green beans processed at 10, 20, and 40 min at 115 °C showed an increase in total phenolic content approximately by 10 percent, but this still represented a 36, 32, and 30% decrease from the control. Treatments at temperatures of 100, 115, and 121 °C for 20 min resulted in a slightly increasing profile for the heat treatment group, an effect not seen with the previous treatment groups. The total phenolic content of the 100, 115, and 121 °C group was 40, 32, and 23% lower, respectively, than that of the unprocessed group.

In general, phenolic compounds found in fruits and vegetables are linked covalently to amine functional groups and are esterified to glycosides (*45*). Heat treatment has been shown to significantly increase the phenolic content of sweet corn (*7*). It has been suggested that colonic digestion of fruits and vegetables by intestinal microflora may release the bound phytochemicals and induce a site-specific antioxidant re-enforcement (*46*, *47*). On the basis of our results, canned beets and green beans would contribute very little to this site-specific re-enforcement of antioxidants. The positive effects of thermal processing on phenolics of sweet corn and tomatoes were not exhibited with canned beets and green beans, which should counter the increase in antioxidant activities of these two vegetables as compared to those seen previously.

Flavonoids have very potent antioxidants and anticancer activity. The total flavonoid content of canned beets was considerably lower than phenolic content, which was expected. At best, total flavonoid content for both vegetables made up about half of phenolic content. The profiling of flavonoids closely resembled the distribution of phenolic compounds. For beets, there was an overall increase of 32, 41, and 47% for the 15, 30, and 45 min treatments at 115 °C. The constant time set also had increasing values of 37, 41, and 50% in total flavonoid content due to increasing temperature of thermal processing. The increasing release of flavonoids is a promising sign that antioxidant activities of canned beets might be in positive balance or at least remain unchanged. The similar profiling of flavonoids to phenolics was also seen in canned green beans.

A 63, 58, and 62% decrease in total flavonoid content from the control was measured for the 10, 20, and 40 min at 115 °C group—a set of values closely resembling those of its total phenolic content. Processed green beans at 100, 115, and 121 °C for 20 min showed a decrease of 65, 58, and 55% of total flavonoid as compared to the control. Again, this pattern closely resembled the phenolic data, which suggests that there will be a drop in antioxidant activity of canned green beans.

The TOSC assay quantifies the overall additive and/or synergistic antioxidant activities of phytochemicals (*3*). The thermal processing of beets and green beans showed a consistent free antioxidant activity for both treatment groups, which closely followed its free phenolic and flavonoid contents as expected. Processing of beets did not change the antioxidant activity, and processing decreased the activity in green beans. This differs from our previous results (*6*, *7*), where thermal processing increased antioxidant activities in tomatoes and sweet corn.

Our results did not show a consistent trend of effects of thermal processing on phenolic, flavonoid, vitamin C, or folate content or total antioxidant activities in processed beets and green beans when compared to the thermally processed tomatoes and sweet corn (*6*, *7*). This suggests that the effect of thermal processing on phenolic, flavonoid, vitamin C, or folate content or total antioxidant activities will be different in different produce and is worth further investigation. In addition, differences in processing methods used may have impacted results; others have shown that different methods of cooking vegetables (steaming, boiling, pressure cooking, microwaving) can have distinctly different effects on vitamin C and organic acid content. Others have shown that with green beans, during processing, flavonoids are not degraded but leach into the cooking water (*21*). We can only conclude at this time that, depending upon the particular produce, and processing parameters and methods, thermal processing may enhance, reduce, or cause no change in total antioxidant activity from that of fresh produce. This reinforces the concept that consumers may obtain optimal health benefits when they obtain their diet from a complementary variety of natural sources (fruits, vegetables, and whole grains), which are prepared in a variety of ways. Our findings, in combination with previous works, defy the notion that processed fruits and vegetables offer lower nutritional quality. However, it also suggests that different foods offer different benefits and strengthens the need to incorporate variety into our diet.

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