Effect of thermal treatments on phytochemicals in conventionally and organically grown berries

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Abstract

BACKGROUND: Consumer demand for organic foods is increasing despite a lack of conclusive evidence of nutritional superiority of organically grown foods. The objective of this investigation is to evaluate the effects of thermal treatments on phytochemicals of conventionally and organically grown berries. Two varieties of conventionally and organically grown red raspberries and blueberries were analyzed for the total anthocyanins, total phenolic contents, flavonoids and total antioxidant activity. Fresh berries were processed into cans and juice/puree with and without blanching, and the changes in phytochemicals were monitored.

RESULTS: The total anthocyanins and total phenolic contents in berries were not influenced by agricultural production system (conventional *versus* organic). The total antioxidant activity in berries was also not influenced by the production system but its level was affected significantly by cultivar. During canning the total anthocyanins decreased up to 44% while phenolic contents and antioxidant activity in both berries generally increased up to 50 and 53%, respectively. The level of changes in phytochemicals during berry puree processing was influenced by blanching and types of berries.

CONCLUSIONS: The phenolic contents and antioxidant activities in berries increased while total anthocyanins decreased during canning. The blanching treatment prior to puree processing improved the retention of phytochemicals in blueberries.

Keywords: Agricultural production system, blueberries, canning, puree processing, red raspberries

INTRODUCTION

National and global organic sectors are growing significantly due to increased consumer demands for organic foods. In the USA, the market for organic foods has increased significantly, and at the end of 2007 it was estimated to be worth over $15 billion[1]. Many surveys of consumer attitudes and characteristics are conducted to identify the reasons for this increased trend. The preference for organic food is associated with multiple factors that, in general, reflect an increased interest towards personal health, animal welfare, and environmental protection due to absence of contaminants in the production process[2,3].

The plant synthesis of phenolic compounds is well correlated to decreased damage from insect pests and pathogens, as well as from UV radiation, suggesting the protective role of these compounds[4]. Phenolic compounds also contribute to antioxidant and other health benefits of foods[5]; organically grown produce may therefore be more healthful by virtue of higher levels of phenolic compounds produced in response to insects and diseases. Many studies comparing the nutritional quality of organically and conventionally grown fruits and vegetables in terms of macronutrients, vitamins, minerals, and phytochemicals have been published. Several articles have presented critical reviews of results from published studies comparing the influence of organic and conventional production systems on phytochemical contents in fruits and vegetables[2,6-12]. The overall evidence seems in favor of enhancement of phytochemical content in organically grown produce, but there is a little systematic study of the salient factors that may contribute to an increased phytochemical content in organic crops[10]. Given several limitations in the majority of published studies, further research is necessary to elucidate the factors that may or may not contribute to enhanced phytochemical contents of organic produce.

The availability of fresh conventional and organic fruits and vegetables in temperate zones is limited during the winter months due to reduced agricultural production in the Northern Hemisphere. Some fruits and vegetables can be easily shipped from other regions of the world, but others, like berries are highly perishable products. Hence, some sort of processing, such as canning, freezing, juice processing or drying, is required to extend their shelf life. Processing/preservation methods invariably influence nutritional quality of fresh produce. Normal cooking at temperatures, 75 to 100°C and time 10 to 30min, is shown to affect phenolic content as well as anti-radical and anti-proliferative activities of juice from the majority of vegetables[13]. During jam processing, the total anthocyanin content per gram of fruit was reduced by 20 and 40% for ‘Heritage’ and ‘Zeva’ red raspberries, respectively[14]. Few studies have monitored the effects of post-harvest storage and processing on the phytochemical contents of organically versus conventionally grown fruits and vegetables[15,16]. There is a need to understand the mechanisms of degradation of phenolics in organic fruits and vegetables during common processing operations. The information on stability of these health-promoting components during processing will be useful to the organic food processing industry in maintaining important nutritional attributes.

The consumption of berry products is encouraged worldwide because of their possible health benefits. Red raspberries and blueberries contain higher amounts of anthocyanins, phenolic compounds, and thus have significant antioxidant activity. Berries are often processed into juice, juice concentrate or canned for subsequent use in beverages, syrups or other products[17]. However, various factors during the processing of berries may degrade anthocyanins, phenolic compounds, and other antioxidants. Blanching of fruits before thermal processing has shown to increase the radical-scavenging activity of the processed fruits due to higher recovery of anthocyanin pigments and phenolics[18]. Due to their possible beneficial roles as nutrients in food, it is critical that changes in anthocyanins, phenolic contents, and antioxidant activity during processing and manufacture be measured to better assess the dietary value of the processed products. The objectives of this investigation are to evaluate the effects of thermal treatment on anthocyanins, phenolic contents, and antioxidant activity of conventionally and organically grown red raspberries and blueberries.

MATERIALS AND METHODS

Fruit sampling

Red raspberry (*Rubus idaeus* Duch. cv. Meeker) and highbush blueberry (*Vacinium corymbosum* L. cvs. Duke and Reka) fruits were harvested at maturity from eight commercial farm fields in northwestern Washington State (48°20-30′N, 122°21-24′W) from 28 July to 1 Aug 2008. Paired fields were matched for crop, location and cultivar, with one field of each pair farmed as certified organic (ORG) and the other field farmed conventionally (CON). There were two matched ORG-CON pairs of ‘Meeker’ raspberries, with one pair (Meeker1-ORG and Meeker1-CON) mechanically harvested with an Over-Row Harvester (Littau Harvester, Stayton, Oregon) and the other pair hand harvested (Meeker2-ORG and Meeker2-CON), and one ORG-CON pair each of hand harvested ‘Duke’ (Duke-ORG and Duke-CON) and ‘Reka’ (Reka-ORG and Reka-CON) blueberries. Paired fields were either adjacent to one another (Reka-ORG and Reka-CON) or were within 5 km of each other. All fields were mature plantings, with soil texture classified either very fine sandy loam or silt loam. ORG fields were certified by the Washington State Department of Agriculture (WSDA Organic Food Program).

Immediately after harvest, fruit were transported in commercial picking trays to Washington State University's Northwestern Washington Research and Extension Center in Mt. Vernon, where they were placed in refrigerated storage at 1.7°C. After all samples were collected, refrigerated fruit were transported in a chilled, insulated container to Washington State University Pullman campus, where they were immediately placed in refrigerated (5°C) storage. Fresh berries were immediately prepared for phytochemical analysis by grinding a sub-sample in liquid N_2 until a powder was formed. Powdered fruit tissue was stored in an ultra-low temperature freezer (-80°C) until analyzed. Another sub-sample of fruit were weighed fresh (12-15 raspberries and 15-18 blueberries x 6 replicates), dried in an oven at 80°C, then reweighed to obtain percent dry weight. The remaining fruit from each sample were either canned or juiced/pureed, as subsequently described.

Thermal treatments

Canning After cleaning and sorting, berries were steam blanched at 95°C for 2 min and then cooled to 38°C[19]. During steam blanching, leaching of some colored solids from the berries was observed. Blanched and non-blanched berries were filled into the cans (nr 303 × 406) and covered with 40°brix boiling sugar syrup, leaving a head space of 10 mm in order to provide effective steam flow closure[20]. Sugar syrup was prepared by mixing predetermined weight of sucrose to boiling water to achieve 40°brix. Cans filled with fruits and syrup were sealed using a high speed mechanical vacuum sealer (Model#DUV 8979, Dixie Canner Equipment, GA). Filled cans were immersed in boiling water (100°C) to increase the temperature at the center of the cans to at least about 88°C[20]. Can liquid temperature was measured at the geometric center of the cans using copper-constantan needle type thermocouple connected to a data acquisition system.

Thermocouple signals were recorded at 15s time intervals. The total times for processing of red raspberry and blueberry were 28 min and 22 min, respectively. Immediately after processing, cans were cooled down by sprinkling cold water on them until center temperature reached 30ºC.

Juice/Puree Blanched and unblanched raspberries and blueberries were processed into juice using a Fruit Juicer (The Champion Fruit Juicer, W. R. Laboratories, CA, USA). Crushing of berries was performed manually by pressing berries using wooden block into the juicer inlet; one of the outlets gave the fruit juice while another gave the pulp (containing raspberry seeds or skin in the case of blueberries). The juice was heated in a steel pan on a hot plate at about 92ºC for 2.0 minutes and pasteurized juice was then hot filled into bottles[21]. Juice filled bottles were kept upside down immediately after filling for sterilization of the cap and turned to their proper position after juice reached 23ºC. Juice prepared from non-blanched berries showed some thickening before pasteurization. This thickening of the juice might be a consequence of enzymes present in the berries. Thermally treated berries were immediately analyzed for retention of phytochemicals.

Phytochemicals analysis

Total antioxidant activity Antioxidant activity of hydrophilic and lipophilic fractions[22] of the berries was measuring by the end-point 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS)/hydrogen peroxide/peroxidase (Horseradish peroxidase, HRP, Type VI-A) method of Cano et al.[23], with modifications. Specifically, 0.2 g frozen fresh or canned fruit tissue or 0.5 mL N_2-gas dried (at 0ºC) canning syrup or juice/puree were extracted in 700 mL 50 mM MES (pH 6.0) and 700 mL ethyl acetate, vortexed for 30 sec, and centrifuged at 13000 rpm for 10 min at

4^{o}C. The organic (top) and aqueous (bottom) phases were separated with a pipette for measurement of hydrophilic and lipophilic antioxidant activities (HAA and LAA, respectively). For both fractions, 40 μL 1 mM H_2O_2, 100 μL 15 mM ABTS, and 10 μL 3.3 U μL^{-1} HRP were placed in quartz cuvettes and gently shaken for 10 sec, after which 50 mM phosphate buffer (pH 7.4) was added and mixed with a stir paddle. Absorbance was monitored at 734 nm on a UV-visible spectrophotometer (Model HP8453, Hewlett-Packard Co., Palo Alto, CA) until stable (<10 sec), then 5 μL (for HAA) or 40 μL (for LAA) extract was added for a total reaction volume of 1 mL, mixed with a stir paddle, and monitored at 734 nm until absorbance reached a minimum. HAA and LAA were calculated from the absorbance difference and expressed on the basis of Trolox equivalents from standard curves of 5 mM Trolox diluted in 50 mM MES buffer (pH 6.0) or 100% (v/v) ethyl acetate, respectively, and measured as described for the samples. HAA and TAA were summed to estimate total antioxidant activity (TAA).

Total Phenolics Total phenolic compounds in the berries were measured with the Folin-Ciocalteu (F-C) phenol reagent (2 N) according to revised methods of Singleton et al.[24]. Specifically, to 0.1 g frozen fresh or canned fruit tissue or 0.3 mL N_2-gas dried (at 0^{o}C) canning syrup or juice/puree, 1 mL 80% (v/v) methanol was added in microcentrifuge tubes. Samples were vortexed, allowed to extract 1 hr at room temperature and then overnight at -20^{o}C, followed by centrifugation at 14000 rpm for 20 min at 4^{o}C. The supernatants were removed and extraction of the pellet was repeated 2X as just described, with supernatants combined after each extraction and then made up to 4 mL with 80% (v/v) methanol after the final extraction. The assay was performed by adding 300 μL sample extract into two, 15-mL tubes containing 600 μL 80% (v/v) methanol, 5 mL 10% (v/v) F-C reagent, and either 4 mL saturated Na_2CO_3 (75 g L^{-1}) or 4 mL water. Tubes were thoroughly mixed and incubated at room temperature for 2 hr. One-milliliter aliquots from the sample tubes containing Na_2CO_3 or water were added to 1.5 mL plastic cuvettes and the absorbance of each was measured at 760 nm in the UV-visible spectrophotometer. Concentration of phenolic compounds was determined by subtracting absorbance of samples containing Na_2CO_3 from those containing water, and expressed as gallic acid (3,4,5-trihydroxybenzoic acid) equivalents.

Total Anthocyanins One-tenth gram frozen fresh or canned fruit tissue or 0.25 mL N_2-gas dried (at 0^oC) canning syrup or juice/puree were extracted in 1 mL 1% (v/v) HCl-methanol. After storage for 24 hr at -20^oC, sample tubes were centrifuged at 14000 rpm for 10 min at 4^oC. Similar extractions with HCl-methanol were repeated 2X for each sample. Following centrifugation on day four, supernatants were decanted into 15 mL plastic tubes and made up to 3-mL volumes with HCl-methanol. Total anthocyanins were determined by the pH differential absorbance method, using molar extinction coefficients of 37150 $M^{-1}cm^{-1}$ at 524 nm for cyanidin-3-sophoroside-5-glucoside in raspberries and 34300 $M^{-1}cm^{-1}$ at 530 nm for cyanidin-3-glucoside in blueberries[25].

Specific Polyphenolics Duplicate samples were ground with 1.5 mL methanol using a homogenizer (Talboys Engineering Corp., Montrose, PA, USA) for 1 min. The slurry was then transferred to Eppendorf tubes, and centrifuged at 5,000 rpm for 5 min at room temperature (25°C) (Beckman Microfuge, Beckman Coulter Inc., Fullerton, CA, USA). The supernatant was transferred to a new Eppendorf tube and dried under a stream of purified compressed nitrogen gas. Samples were prepared in duplicate with one of the replicates used for quantification of polyphenol aglycones without enzymatic hydrolysis (free samples). The second replicate was utilized for the indirect determination of polyphenol glycosides after enzymatic hydrolysis (total samples). Glycosides were indirectly quantified by taking a 150 µL aliquot of blueberry or raspberry extract and subjecting it to 1 mL HPLC-grade H_2O, 110 µL 0.78M sodium acetate acetic acid buffer (pH 4.8), 100 µL 0.1M ascorbic acid, and a 200 µL crude preparation of *H. pomatia* type HP-2 followed by incubation for 17–24 h at 37°C[26,27]. Subsequently, 25 µL of daidzein (internal standard) was added followed by 1 mL of cold acetonitrile to precipitate proteins. The mixture was vortexed for 1 min, and then centrifuged at 5000 rpm for 5 min. The supernatant was collected into 15 mL poly-propylene tubes and evaporated to dryness under a stream of compressed nitrogen gas. The residue was reconstituted with 400 µL mobile phase, vortexed for 1 min and centrifuged at 5000 rpm for 5 min. The supernatant was transferred to HPLC vials of 150 µL and injected into the HPLC system. *H. pomatia* Type-HP-2 is a β-glucuronidase that cleaves specifically the glycosylated sugar moiety of polyphenol glycosides as previously described[27]. Therefore, the samples without enzymatic hydrolysis (free samples or polyphenol aglycones) were utilized to determine the concentration of the aglycones, whereas the samples with enzymatic hydrolysis (total samples or polyphenol aglycones plus glycosides) were utilized to determine the concentration of the aglycones originally present plus the concentration of the glycosides converted to aglycones by the sugar moiety cleavage action of the enzyme. Finally, by subtracting the free sample concentration from the total sample, the concentration of glycoside in each sample was calculated.

Statistical analysis

Compiled data were presented as mean and standard deviation of the mean (mean ± standard deviation). The data were analyzed for statistical significance using SAS® 9.1 programme (SAS Institute, Inc., Cary, NC). Analysis of Variance (ANOVA) was employed for the samples with a value of $p < 0.05$ being considered statistically significant. Two treatments were considered at a time and independently analyzed to see the difference between each pair. The independent contrast and Fisher's LSD (Least Significant Difference) methods were used together for this purpose. The degree of freedom was one as two treatments were considered at each time.

RESULTS AND DISCUSSION

Influence of production system

Phytochemicals, such as anthocyanins and phenolics, and total antioxidant activity, including both hydrophilic and lipophilic components of conventionally and organically grown red raspberries and blueberries are shown in Table 1. Total anthocyanin concentrations in blueberries ranged from 1.0 to 1.9 g kg^{-1} of fresh weight while in red raspberries total anthocyanin ranged from 0.8 to 1.2 g kg^{-1} fresh weight. The production system (organic or conventional) and cultivar showed significant influence ($p<0.05$) on total anthocyanin content (Table 1), except that machine-harvested organic red raspberries (Meeker1-ORG) had anthocyanin content similar to conventionally grown red raspberries (Meeker1-CON). Total anthocyanin concentrations fell within the wide range of 0.3 to 5 g kg^{-1} for blueberries, but for most samples above the 0.3 to 1.0 g kg^{-1} for red raspberries reported by Mazza and Miniati[28] and de Ancos et al[29]. Skrede et al.[17] reported 1.0 g kg^{-1} of total anthocyanins in highbush blueberries.

Wang et al.[30] reported that total anthocyanin content of organically produced blueberries (1.3 g kg^{-1}) was higher than conventionally produced blueberries (0.82 g kg^{-1}). This variation was attributed to differences between cultivars and production systems. In contrast, total anthocyanin content of organically grown raspberries in this study was the same as or lower than that of conventionally grown raspberries; organically grown blueberries had lower total anthocyanins than their conventionally grown counterparts in one pairing, but had higher anthocyanins in the other pairing.

The concentration of total phenolic in blueberries varied from 2.2 to 2.6 g gallic acid equiv kg^{-1} whereas total phenolic in red raspberries varied from 1.9 to 2.3 g gallic acid equiv kg^{-1} (Table 1). The production system (organic or conventional) resulted in no significant differences in phenolics, except that machine-harvested organic red raspberries (Meeker1-ORG) had significantly higher phenolic levels than conventionally grown red raspberries (Meeker1-CON). Wang et al[30] found that an organic production system significantly increased the total phenolic content in blueberries. They reported that the mean phenolic contents were 1.9 and 3.2 g kg^{-1} for conventional and organic blueberries, respectively.

The cultivar and production system also influenced level of antioxidant activity in berries. In red raspberries, antioxidant activity ranged from 12.6 to 19.1 mol Trolox equiv kg^{-1} fresh weight whereas blueberries showed total antioxidant activity between 9.1 to 16.9 mol Trolox equiv kg^{-1} fresh weight. The hydrophilic and lipophilic antioxidant fractions in red raspberries varied from 11.0 to 16.9 and 1.61 to 2.18 mol Trolox equiv kg^{-1} fresh weight, respectively whereas hydrophilic and lipophilic antioxidant fractions in blueberries varied from 7.93 to 14.7 and 1.16 to 2.27 mol Trolox equiv kg^{-1} fresh weight, respectively. The mean antioxidant value of blueberries determined in this study were lower than the range of 27 to 55 mol Trolox equiv kg^{-1} fresh weight reported by Wang et al.[30], although they used a different cultivar and analytical method than in our study. The production system (organic or conventional) and cultivar both significantly affected total antioxidant activity, including the activities of both the hydrophilic and lipophilic fractions. For both berries, one pairing showed higher antioxidant activity in organically grown fruit while the trend reversed with the other pairing. Similar trends were reflected in hydrophilic and lipophilic fraction of antioxidant activity.

Polyphenolics

A variety of different polyphenols, in particular flavonoids, were determined in blue berries and raspberries (Table 2). In raspberries, ellagic acid predominates for aglycones and taxifolin glycoside predominates for glycosides, but in blueberries, fisetin and hesperidin predominate for glycosides and ellagic acid predominates for aglycones. Although there were some apparent differences between farm pairs in terms of individual polyphenols, in both raspberries and blueberries analysis of organic versus conventional treatments and polyphenol content did not reveal any consistently discernable pattern of differences.

Influence of thermal treatment

Canning Red raspberries and blueberries are often thermally processed into juice, juice concentrate, and puree or whole berries in cans in order to reach a more widespread consumption. Because of their possible beneficial health effects, particular attention is paid to the changes that anthocyanins and phenolic compounds undergo with processing. In this study, berries were canned with 40^{o}brix sucrose syrup. During thermal processing some of the berry solids were leached out in the syrup. The total anthocyanins, total phenolics, and total antioxidant activity were determined in solid berries and canned syrup, and the phytonutrient values for liquid samples were converted to fresh weight basis for comparison to unprocessed berries (Tables 3-5). The total anthocyanin contents in canned berries generally decreased by 6 to 44% due to thermal processing (Table 3). The influence of production system on total anthocyanins degradation varied and was affected by type and cultivar of berries. For red raspberries, total anthocyanin degradation of organic fruit was higher than conventional fruit whereas no significant difference was found in total anthocyanin content for second pairing. In the case of 'Reka' blueberries, total anthocyanins during thermal processing decreased by 14% in conventional berries and increased by 16% in organic berries (Reka-ORG). However, total anthocyanins decreased in 'Duke' variety of both conventional and organic berries (Table 3). The total phenolic content in canned berries was 11 to 50% higher than the total phenolics in fresh berries (Table 4). This trend was consistent across both berry type and cultivar, and production system. The increase in total phenolics content in conventionally grown 'Duke' blueberries (Duke-CON) was higher than in organically grown (Duke-ORG), while this trend was reversed for 'Reka' blueberries (Table 4). This is could be due to two factors: result of the complete inactivation of native enzymes, and a greater extraction yield linked to the increase of fruit caused by the heat treatment[31]. The change in total phenolics in blueberries was also reflected in total antioxidant activity. The total antioxidant activity in canned red raspberries decreased up to 45%, while it increased up to 53% in canned blueberries (Table 5). Berry cultivar and production system had mixed effects on total antioxidant activity. In all cases, the canned syrup contained much less anthocyanins and phenolics, and lower antioxidant activity than the canned berries (Tables 3-5).

Puree/juice concentrates Total anthocyanins, phenolics, and antioxidant activity in both raspberries and blueberries generally decreased with pureeing, except for anthocyanins in raspberries (Meeker1 and Meeker2) and phenolic content in Meeker-CON (Tables 1-3). The significant losses in anthocyanin and phenolic contents, and antioxidant activity occurred in both conventionally and organically grown blueberries. The losses of anthocyanin and phenolic contents during pureeing/juicing were probably due to native polyphenol oxidase[32,33]. Skrede et al.[17] showed that substantial losses of anthocyanins and polyphenolics occurred when blueberries were processed into juice and concentrate, and that different classes of compounds had varying susceptibility to degradation with different processing operations. These results need to be taken into consideration when evaluating possible health benefits of processed berry products.

Influence of blanching

Blanching before canning of berries had varying degrees of influence on degradation of total anthocyanins, phenolics, and antioxidant activity. The degradation in total anthocyanin content in canned blueberries was higher due to blanching while the total anthocyanin content increased in red raspberries that were blanched prior to canning (Table 6). This tendency of decrease in total anthocyanins in blueberries and increase in raspberries can also be due to interaction of anthocyanin molecules with the major sugars fructose and glucose present in the berries[34]. During blanching, the exposure to high temperature steam can cause tissue disruption and release of anthocyanins from cellular structure. The extent of tissue damage may depend on heat tolerance of fruit structure. The types of anthocyanins present in raspberries and blueberries may differ and bonds between these anthocyanins and the cell structure may also differ. These variations can result different degree of breakage in these bonds resulting in different responses to heat sensitivity of anthocyanins in these berries[35]. Faller and Fialho[34] also observed that increase and decrease of polyphenols during domestic cooking depended on the types of vegetable. Leaching of the anthocyanins from canned berries to can syrup increased due to blanching. Blanching treatment had no influence on retention of phenolic compounds and antioxidant activities in berries during canning except in canned syrup of blueberries where phenolic content and antioxidant activity increased significantly ($p<0.001$) increased (Table 6). The influence of blanching treatment on the retention/degradation of anthocyanins, phenolics, and antioxidant activity in puree/juice was consistent with type of berry and production system. The blanching treatment significantly improved the retention of anthocyanins, phenolics, and antioxidant activity only in blueberry puree/juice (Figures 1-3). Specifically, the retention of total anthocyanin in blueberry puree/juice improved significantly when blanched. Rossi et al.[18] also found that blanching prior to blueberry juice processing resulted in higher retention of anthocyanins in juice. Kader et al.[32,33] reported that the native polyphenol oxidase in blueberries was responsible for destruction of anthocyanins in crushed blueberries. We also observed that blanching treatment was able to retain total antioxidant activity in both 'Duke' and 'Reka' blueberry puree/juice. In contrast, blanching had no influence on the retention of anthocyanins, phenolics, and antioxidant activity in raspberry puree/juice (Figures 1-3).

Influence of harvesting system and cultivar

Red raspberries and blueberries have a very low mechanical resistance and are commonly hand harvested. The hand harvesting of berries used for processing become expensive and mechanical harvesting is one way to reduce costs. However, the mechanical harvesting may have influence on physical quality and phytochemicals contents of berries especially intended for further processing. The harvesting system influenced the phytochemical contents in organically grown raspberries (Meeker2). Mechanically harvested organic red raspberries contained higher amount of phytochemicals than hand harvested raspberries (Tables 7-9), while in case of conventionally grown red raspberries, only total antioxidant activity was significantly higher ($p<0.05$) in mechanically harvested berries (Table 9). The phytochemicals content in mechanically harvested red raspberries was higher than hand harvested berries even after thermal treatments.

The influence of cultivar on phytochemical contents of fruit was analyzed with blueberries. The phytochemicals content of Duke was consistently higher than Reka in conventionally grown blueberries before and after thermal treatments (Tables 7-9). In organically grown blueberries, the total anthocyanins and antioxidant activity was higher in Duke than Reka blueberries while no significant difference was found in total phenolics of both cultivars (Tables 7-9). The trend in phytochemical contents of both blueberry cultivars was similar after thermal treatments.

CONCLUSIONS

Total anthocyanins, phenolic compounds, and antioxidant activity in fresh red raspberries and blueberries were not influenced by agricultural production systems (conventional *versus* organic). The total antioxidant activity in berries was different between each of the paired fields, but was not consistently higher or lower for fields grown organically. The phytochemicals content of mechanically harvested organic red raspberries was higher than hand harvested raspberries. Duke blueberries contained higher level of phytochemicals than Reka blueberries.

Thermal treatments caused significant changes in phytochemicals in both conventionally and organically grown berries. The phenolic contents and antioxidant activities in berries increased while total anthocyanins decreased during canning. The level of changes in phytochemicals during puree/juice processing of berries depended on blanching and type of berry. The puree/juice obtained from blanched blueberries retained higher levels of total anthocyanins, phenolics, and antioxidant activity due to the positive effects of thermal treatment. The higher recovery of phenolic compounds resulted in a significant increase in antioxidant activity of the puree/juice. The addition of a blanching step prior to blueberry puree/juice processing may be considered a very important factor when assessing blueberry purees and juices for their possible health benefits.

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Table 1. Total anthocyanin and phenolic concentrations, and hydrophilic, lipophilic, and total antioxidant activities of conventionally (CON) and organically (ORG) grown red raspberries and blueberries at harvest

Field code	Total anthocyanins (g Cyd-3-Soph-5-Glu[1] or Cyd-3-Glu[2] kg^{-1} FW)	Total phenolics (g gallic acid equiv kg^{-1} FW	Hydrophilic antioxidant activity (mol Trolox equiv kg^{-1} FW)	Lipophilic antioxidant activity (mol Trolox equiv kg^{-1} FW)	Total antioxidant activity (mol Trolox equiv kg^{-1} FW)
Meeker1-CON	1.22 (0.18)	1.98** (0.002)	12.9 (0.39)***	1.24 (0.024)***	14.2*** (0.42)
Meeker1-ORG	1.24 (0.12)	2.25** (0.080)	16.9 (0.36)***	2.18 (0.11)***	19.1*** (0.47)
Meeker2-CON	1.17** (0.10)	1.92 (0.14)	13.6 (0.54)**	1.78 (0.088)*	15.3*** (0.50)
Meeker2-ORG	0.83** (0.05)	1.95 (0.056)	11.0 (0.61)**	1.61 (0.019)*	12.6*** (0.60)
Duke-CON	1.86* (0.11)	2.43 (0.10)	12.5 (0.86)*	1.77 (0.091)**	14.3* (0.95)
Duke-ORG	1.68* (0.063)	2.59 (0.040)	14.7 (0.70)*	2.27 (0.13)**	16.9* (0.79)
Reka-CON	0.997* (0.020)	2.17 (0.038)	9.56 (0.27)**	1.33 (0.075)*	10.9** (0.33)
Reka-ORG	1.19* (0.13)	2.28 (0.222)	7.93 (0.25)**	1.16 (0.043)*	9.1** (0.29)

The values in brackets are standard deviation of 3 replicates

[1] Cyanidin-3-sophoroside-5-glucoside @ 524nm for raspberries

[2] Cyanidin-3-glucoside @ 530nm for blueberries

Statistical significance between CON and ORG in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 2. Polyphenolic compounds (glycosides and aglycones) of conventionally (CON) and organically (ORG) grown red raspberries and blueberries

(mg kg^{-1})		Meeker1-CON	Meeker1-ORG	Meeker2-CON	Meeker2-ORG	Duke-CON	Duke-ORG	Reka-CON	Reka-ORG
Ellagic acid	Glycosides	3.28	0.216	0	0.027	2.49	0	0	42.5
	Aglycones	80.2	90.2	100.2	110.2	120.2	130.2	140.2	150.2
Taxifolin	Glycosides	1180	853	1140	0	0	0	0	0
	Aglycones	11.25	3.31	6.84	0	0	0	0	0
Myricetin	Glycosides	0	0	0	0	135	64.2	0.764	0
	Aglycones	0	0	0	0	0.013	13.1	0.370	0
Fisetin	Glycosides	314	245	261	275	248	174	417	199
	Aglycones	14.3	0.466	0.582	0.054	0	0	0.291	0.357
Quercetin	Glycosides	13.1	02.13	12.3	20	73.1	124	141	64.9
	Aglycones	11.7	11.4	0	0	11.8	11.3	12.0	0
Phloridzin	Glycosides	030.4	0.123	7.91	0.654	8.33	7.94	0.502	8.83
	Aglycones	07.79	7.66	0	7.74	0	0	8.17	0
R-Naringenin	Glycosides	0.299	0.279	21.9	1.35	0	0.693	1.24	1.24
	Aglycones	22.1	22.0	0	21.3	0	21.2	22.3	22.3
S-Naringenin	Glycosides	0.258	0.091	11.7	3.46	0	14.0	3.18	3.12
	Aglycones	11.6	11.76	0	11.2	0	0	11.6	11.9
Hesperidin	Glycosides	0	189	190	182	206	191	208.3	237
	Aglycones	0	0	0	0.55	0	1.41	0	0
Kaempferol	Glycosides	20.7	0	11.1	10.9	02.73	7.70	9.23	15.1
	Aglycones	0	0	0.155	0	0	0	0	0

Table 3. Total anthocyanin contents in fresh, canned, and pureed/juiced conventionally (CON) and organically (ORG) grown red raspberries and blueberries

Field code	Total anthocyanins in fresh berries (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)	Total anthocyanins in canned berries and syrup (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)		Total anthocyanins in puree/juice (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)
		In berries	In syrup	
Meeker1-CON	1.22 (0.18)	0.867 (0.080)	0.279*** (0.003)	1.39 (0.11)
Meeker1-ORG	1.24 (0.12)	0.682 (0.053)	0.230*** (0.017)	1.40 (0.104)

Meeker2-CON	1.17^{**} (0.10)	NA	NA	1.21^{***} (0.13)
Meeker2-ORG	0.83^{**} (0.05)	NA	NA	0.93^{***} (0.052)
Duke-CON	1.86^{*} (0.11)	1.09 (0.13)	0.166^{***} (0.006)	0.08 (0.000)
Duke-ORG	1.68^{*} (0.063)	0.903 (0.080)	0.120^{***} (0.005)	0.12 (0.003)
Reka-CON	1.00^{*} (0.020)	0.732^{***}(0.018)	0.125 (0.006)	0.02 (0.003)
Reka-ORG	1.19^{*} (0.13)	1.27^{***} (0.19)	0.122 (0.003)	0.11 (0.008)

The values in brackets are standard deviation of 3 replicates

*Cyanidin-3-sophoroside-5-glucoside @ 524nm for raspberries

**Cyanidin-3-glucoside @ 530nm for blueberries

Statistical significance between CON and ORG in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 4. Total phenolics content in fresh, canned, and pureed/juiced conventionally (CON) and organically (ORG) grown red raspberries and blueberries

Field code	Total phenolics in fresh berries (g gallic acid equiv kg^{-1} FW)	Total phenolics in canned berries and syrup (g gallic acid equiv kg^{-1} FW)		Total phenolics in puree/juice (g gallic acid equiv kg^{-1} FW)
		In berries	In syrup	
Meeker1-CON	1.98^{**} (0.0023)	2.60 (0.69)	0.36 (0.025)	1.11^{***} (0.063)
Meeker1-ORG	2.25^{**} (0.080)	2.12 (0.14)	0.38 (0.12)	1.43^{***} (0.073)
Meeker2-CON	1.92 (0.14)	NA	NA	1.07^{*} (0.076)
Meeker2-ORG	1.95 (0.056)	NA	NA	0.93^{*} (0.050)
Duke-CON	2.43 (0.10)	2.71 (0.55)	0.35^{**} (0.008)	0.73 (0.055)
Duke-ORG	2.59 (0.040)	2.66 (0.62)	0.30^{**} (0.011)	0.66 (0.088)
Reka-CON	2.17 (0.037)	2.35 (0.38)	0.31 (0.018)	0.62 (0.065)
Reka-ORG	2.28 (0.22)	2.93 (0.75)	0.30 (0.015)	0.69 (0.087)

The values in brackets are standard deviation of 3 replicates

Statistical significance between CON and ORG in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 5. Total antioxidant activity of fresh, canned, and pureed/juiced conventionally (CON) and organically (ORG) grown red raspberries and blueberries

Field code	Total antioxidant activity in fresh berries (mol Trolox equiv kg^{-1} FW)	Total antioxidant activity in canned berries and syrup (mol Trolox equiv kg^{-1} FW)		Total antioxidant activity of puree/juice (mol Trolox equiv kg^{-1} FW)
		In berries	In syrup	
Meeker1-CON	14.2^{***} (0.42)	8.80 (1.36)	3.34 (0.72)	16.6 (3.00)
Meeker1-ORG	19.1^{***} (0.47)	7.43 (1.02)	2.80 (0.39)	15.1 (3.60)
Meeker2-CON	15.3^{***} (0.50)	NA	NA	10.7 (2.24)
Meeker2-ORG	12.6^{***} (0.60)	NA	NA	10.7 (1.88)
Duke-CON	14.3^{**} (0.95)	14.0 (1.53)	3.57 (0.23)	5.21 (0.36)
Duke-ORG	16.9^{**} (0.79)	15.2 (0.99)	3.18 (0.73)	6.07 (1.21)
Reka-CON	10.9^{**} (0.33)	9.78 (0.29)	2.87 (0.52)	3.46^{*} (0.25)
Reka-ORG	9.1^{**} (0.29)	11.1 (0.65)	2.83 (0.28)	5.55^{*} (1.35)

The values in brackets are standard deviation of 3 replicates

Statistical significance between CON and ORG in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 6. Effect of blanching on total phenolic and anthocyanin contents and total antioxidant activity in canned raspberries and blueberries

Field code	Blanching treatment	Total phenolics contents in processed berries (g gallic acid equiv kg^{-1} FW		Total anthocyanins contents in processed berries (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)		Total antioxidant activity after processing (mol Trolox equiv kg^{-1} FW)	
		In berries	In syrup	In berries	In syrup	In berries	In syrup
Meeker1-CON	No	2.60 (0.69)	0.364 (0.025)	0.867^{***} (0.080)	0.279^{*} (0.003)	8.80 (1.36)	3.34 (0.72)
Meeker1-CON	Yes	2.46 (0.54)	0.402 (0.030)	1.14^{***} (0.14)	0.318^{*} (0.017)	11.2 (1.80)	3.74 (0.31)
Duke-ORG	No	2.66 (0.62)	0.302^{***} (0.011)	0.903^{**} (0.080)	0.120^{***} (0.005)	15.2 (0.99)	3.18^{***} (0.73)
Duke-ORG	Yes	2.26 (0.35)	0.498^{***} (0.041)	0.692^{**} (0.063)	0.244^{***} (0.003)	14.1 (2.78)	6.38^{***} (1.56)

The values in brackets are standard deviation of 3 replicates

*Cyanidin-3-sophoroside-5-glucoside @ 524nm for raspberries

**Cyanidin-3-glucoside @ 530nm for blueberries

Statistical significance between blanched and nonblanched each crop ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 7. Influence of harvesting system (mechanical vs. hand) and cultivar (Duke vs. Reka) on total anthocyanin content of fresh and processed berries

Field code	Harvesting system	Total anthocyanins in fresh berries (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)	Total anthocyanins in canned berries and syrup (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)		Total anthocyanins in juice (g Cyd-3-Soph-5-Glu* or Cyd-3-Glu** kg^{-1} FW)
			In berries	In syrup	
Meeker1-CON	Mechanical	1.22 (0.18)	0.867 (0.080)	0.279 (0.003)	1.39 (0.11)
Meeker2-CON	Hand	1.17 (0.10)	NA	NA	1.21 (0.13)
Meeker1-ORG	Mechanical	1.24^{**} (0.12)	0.682 (0.053)	0.230 (0.017)	1.40^{**} (0.104)
Meeker2-ORG	Hand	0.83^{**} (0.05)	NA	NA	0.93^{**} (0.052)
Duke-CON	Hand	1.86^{***} (0.11)	1.09^{**} (0.13)	0.166^{***} (0.006)	0.08^{***} (0.000)
Reka-CON	Hand	1.00^{***}(0.020)	0.732^{**}(0.018)	0.125^{***} (0.006)	0.02^{***} (0.003)
Duke-ORG	Hand	1.68^{**} (0.063)	0.903^{*} (0.080)	0.120 (0.005)	0.12 (0.003)
Reka-ORG	Hand	1.19^{**} (0.13)	1.27^{*} (0.19)	0.122 (0.003)	0.11 (0.008)

The values in brackets are standard deviation of 3 replicates

*Cyanidin-3-sophoroside-5-glucoside @ 524nm for raspberries

**Cyanidin-3-glucoside @ 530nm for blueberries

Statistical significance between mechanical and hand harvesting or Duke and Reka cultivar in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 8. Influence of harvesting system (mechanical vs. hand) and cultivar (Duke vs. Reka) on total phenolics content of fresh and processed berries

Field code	Harvesting system	Total phenolics in fresh berries (g gallic acid equiv kg^{-1} FW)	Total phenolics in canned berries and syrup (g gallic acid equiv kg^{-1} FW)		Total phenolics in juice (g gallic acid equiv kg^{-1} FW)
			In berries	In syrup	
Meeker1-CON	Mechanical	1.98 (0.0023)	2.60 (0.69)	0.36 (0.025)	1.11 (0.063)
Meeker2-CON	Hand	1.92 (0.14)	NA	NA	1.07 (0.076)
Meeker1-ORG	Mechanical	2.25** (0.080)	2.12 (0.14)	0.38 (0.12)	1.43** (0.073)
Meeker2-ORG	Hand	1.95** (0.056)	NA	NA	0.93** (0.050)
Duke-CON	Hand	2.43* (0.10)	2.71 (0.55)	0.35* (0.008)	0.73 (0.055)
Reka-CON	Hand	2.17* (0.037)	2.35 (0.38)	0.31* (0.018)	0.62 (0.065)
Duke-ORG	Hand	2.59 (0.040)	2.66 (0.62)	0.30 (0.011)	0.66 (0.088)
Reka-ORG	Hand	2.28 (0.22)	2.93 (0.75)	0.30 (0.015)	0.69 (0.087)

The values in brackets are standard deviation of 3 replicates

Statistical significance between mechanical and hand harvesting or Duke and Reka cultivar in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

Table 9. Influence of harvesting system (mechanical vs. hand) and cultivar (Duke vs. Reka) on total antioxidant activity of fresh and processed berries

Field code	Harvesting system	Total antioxidant activity in fresh berries (mol Trolox equiv kg^{-1} FW)	Total antioxidant activity in canned berries and syrup (mol Trolox equiv g kg^{-1} FW)		Total antioxidant activity of juice (mol Trolox equiv kg^{-1} FW)
			In berries	In syrup	
Meeker1-CON	Mechanical	14.2* (0.42)	8.80 (1.36)	3.34 (0.72)	16.6* (3.00)
Meeker2-CON	Hand	15.3* (0.50)	NA	NA	10.7* (2.24)
Meeker1-ORG	Mechanical	19.1*** (0.47)	7.43 (1.02)	2.80 (0.39)	15.1 (3.60)
Meeker2-ORG	Hand	12.6*** (0.60)	NA	NA	10.7 (1.88)
Duke-CON	Hand	14.3** (0.95)	14.0** (1.53)	3.57 (0.23)	5.21** (0.36)
Reka-CON	Hand	10.9** (0.33)	9.78** (0.29)	2.87 (0.52)	3.46** (0.25)
Duke-ORG	Duke	16.9*** (0.79)	15.2** (0.99)	3.18 (0.73)	6.07 (1.21)
Reka-ORG	Hand	9.1*** (0.29)	11.1** (0.65)	2.83 (0.28)	5.55 (1.35)

The values in brackets are standard deviation of 3 replicates

Statistical significance between mechanical and hand harvesting or Duke and Reka cultivar in each crop pair ($^{*}p$-Value < 0.05, $^{**}p$-value < 0.01, $^{***}p$-value < 0.001)

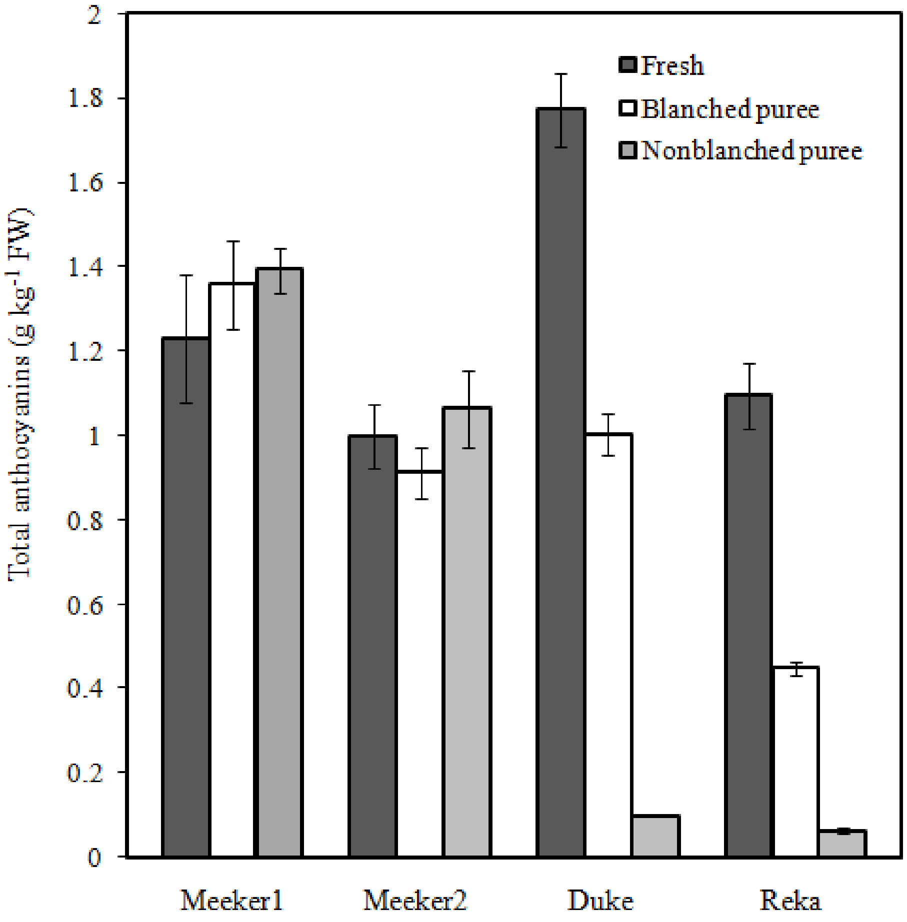

Figure 1. Total anthocyanin contents in fresh and puree/juice of blanched or nonblanched raspberries and blueberries. Error bars indicate standard deviation of replicates.

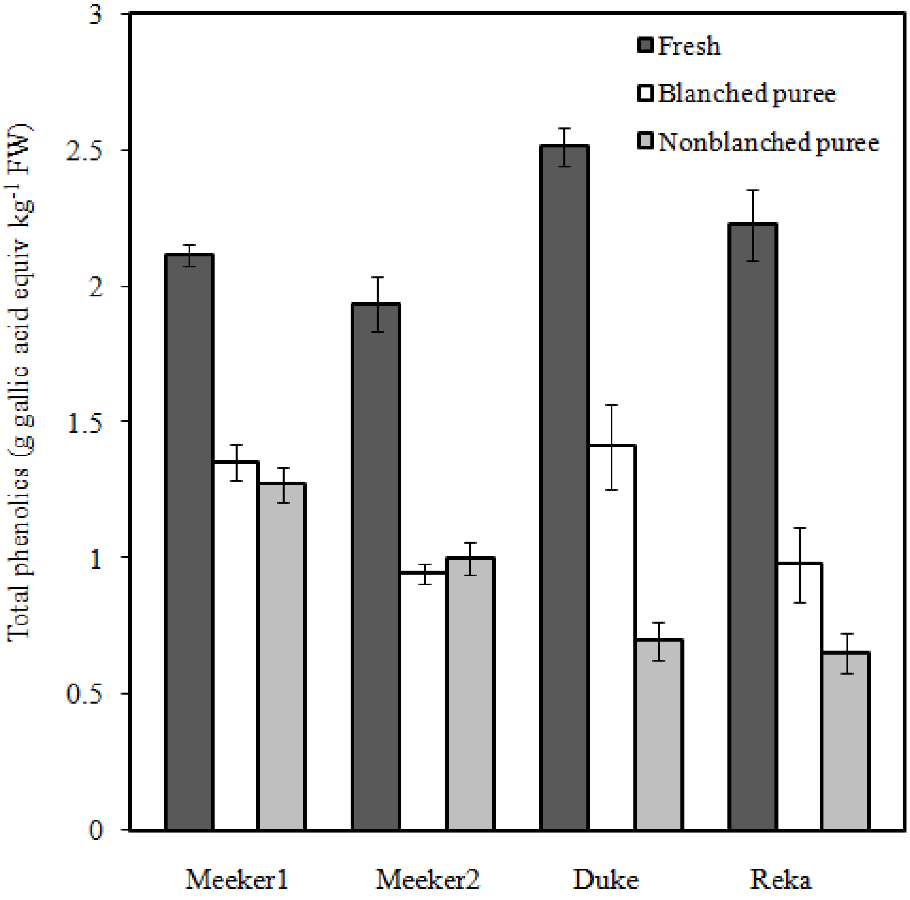

Figure 2. Total phenolic contents in fresh and puree/juice of blanched or nonblanched raspberries and blueberries. Error bars indicate standard deviation of replicates.

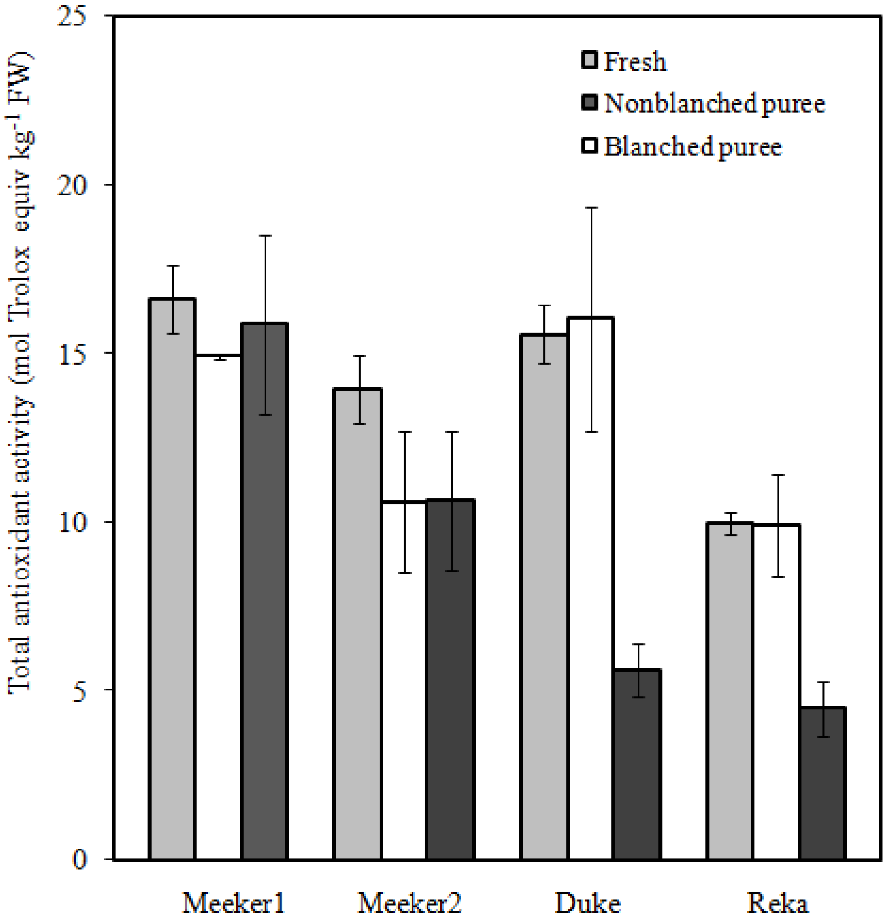

Figure 3. Total antioxidant activity in fresh and puree/juice of blanched or nonblanched raspberries and blueberries. Error bars indicate standard deviation of replicates.