

Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) fluid products as affected by fermentation

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Abstract

Frozen rabbiteye blueberries (*Vaccinium ashei*) were processed into juice (BJ), wines made without (BW1) or with (BW2) skin contact fermentation, and vinegars made from BW1 (JV), BW2 (WV) or blueberries (BV). Total phenolics, total anthocyanins, antioxidant activities (beta-carotene bleaching assay and ferric thiocyanate assay), and antiradical activity (DPPH radical-scavenging method) of these fluid products were determined. The differences in total anthocyanin contents of all blueberry products were significant. The BW2 had the highest content of anthocyanins and polyphenols and the highest beta-carotene bleaching activity and antiradical activity. Acetification decreased total anthocyanin content, total polyphenols and antioxidant activities. Correlations indicate that anthocyanins made significant contributions than did phenolics to antioxidant activities of products. The abilities of BJ, BW1 (wine from blueberry juice), and BW2 to inhibit linoleic acid peroxidation were high (~95%). The abilities of vinegar products to inhibit linoleic acid peroxidation were low. The results indicate that skin-contact fermentation is a better method for obtaining higher antioxidant activity of blueberry products. Also, acetification significantly decreased anthocyanins and antioxidant activities.

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1. Introduction

Farmers usually begin harvesting southern highbush blueberries (*Vaccinium corymbosum* L.) for the “fresh” market in May. By late June, rabbiteye blueberries (*V. ashei*) are harvested. By this time, the “fresh” market is beginning to be saturated with highbush blueberries from the northern states. Thus, Mississippi blueberries are processed for the frozen market. In this process, immature and other “cull” blueberries are discarded. They have the potential to be used in value-added products. The potential use of “processed” blueberries to produce vinegar and antioxidant products is an interesting approach that could develop new products with no agricultural residues. It is

further speculated that rabbiteye blueberry will have more antioxidant capacity than will highbush or lowbush. This might be due to their thicker skin and higher phenolics. The thicker skin may have higher concentrations of anthocyanins. According to Moyer, Hummer, Finn, Frei, and Wrolstad (2002), *V. ashei* from Florida and Georgia had higher levels of ACY than had many other fruits studied.

Blueberries are one of the richest sources of anthocyanins and exhibit one of the highest *in vitro* antioxidant capacities of various fruits and vegetables studied (Francis, 1989; Gao & Mazza, 1995; Wang, Cao, & Prior, 1996). Anthocyanins are widely distributed among fruits and vegetables. They are one of the main classes of water-soluble flavonoids and contribute significantly to the antioxidant activities of the flavonoids (Lapidot, Harel, Akiri, Granit, & Kanner, 1999). They are well known for their ability to give red, blue, and purple colours to plants. They are

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also free radical-scavengers and can potentially interact with biological systems, conferring enzyme-inhibiting, anti-bacterial, cardiovascular protection and antioxidant effects (Cowan, 1999; De Groot & Rauen, 1998; Parthasarathy, 1998; Serafini, Maiani, & Ferro-Luzzi, 1998). In addition to anthocyanins, blueberries are also a rich source of other polyphenolic compounds.

Phenolic compounds are secondary metabolites of plants. They are naturally present in fruits and vegetables. These compounds are a part of everyday diet and are also used as medicines or supplements. Researches have shown that fruits and vegetables contain antioxidant nutrients, in addition to vitamins C and E, and carotenoids, which significantly contribute to their total antioxidant capacity (Cao, Sofic, & Prior, 1996; Wang et al., 1996). Most antioxidant nutrients are polyphenolic compounds, that are components of fruits and vegetables having strong antioxidant capacity (Cao, Sofic, & Prior, 1997; Wang, Cao, & Prior, 1997).

In biological systems, lipid oxidation can produce toxic compounds and this initiates other harmful reactions. Phenolic compounds can act as antioxidants by many potential pathways such as free radical-scavenging, oxygen radical absorbance, and chelating of the metal ions (Halliwell, Aeschbach, Loliger, & Aruoma, 1995). These antioxidant activities of products can be impacted by many factors, such as types of products, varieties of products, maturity, storage and processing. The main purpose of food processing is to preserve foods. However, some nutrients will be lost during processing. Therefore, decrease of nutrient loss or even increase of nutritional value is a big issue of food processing.

Basic blueberry processing includes juice processing or winemaking. The phenolic compositions of products and pomaces depend mainly on processing techniques such as duration of skin contact, crushing and pressing. For example, higher antioxidant activity in red wines, in comparison to white wines, lies in their higher grape-skin-derived polyphenol content (Fuhrman, Volkova, Suraski, & Aviram, 2001). Processing white wine by imposing a short period of grape skin contact in the presence of alcohol showed that the antioxidant characteristics of the white wine are similar to those of red wine because of the extraction of grape skin polyphenols (Fuhrman et al., 2001). Other factors, such as oxygen, enzymes and temperature, will also impact the phenolic compositions and antioxidant activities of blueberries products. Phenolic compounds can act as antioxidants; they also can easily be oxidized. In order to retain or increase antioxidant activity of products, factors that impact the phenolic composition should be taken into account.

There are intense researches on antioxidant activity of fruits and vegetables, including blueberries. Many have focussed on grape products such as wines and pomaces. However, there is no information on antioxidant activity of blueberry wines, and vinegars. The present study was to evaluate effects of fermentation on retention of total

anthocyanins, total phenolics, and antioxidant activity of blueberry fluid products—juice (BJ), wines without (BW1) or with (BW2) skin contact fermentation, vinegars made from BW1 (JV), BW2 (WV) or blueberries (BV).

2. Materials and methods

2.1. Materials

Frozen rabbiteye blueberries (*V. ashei*) were obtained from a commercial processor in southern Mississippi. Red wine mother vinegar was obtained from a winemaking supplies company (Beer and Winemaking Supplies, Inc., Northampton, MA). All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Blueberry juice processing

Blueberries were crushed and then divided into three portions. One portion was processed into juice (BJ) and the other portions were used to make wine (BW2) and vinegar (BV). The juice was prepared, following crushing and pressing at 4 °C in a small basket press. The juice was filtered through four layers of fine cheesecloth and 0.22 µm filters. One portion of juice (BJ) was placed in a –25 °C freezer for further analysis.

2.3. Blueberry wine (BW1) processing

After pressing, the juice was placed in a 15 °C water bath to increase the temperature to fermentation temperature (15 °C) and then yeast (*Saccharomyces cerevisiae*) was introduced. The wine was fermented at 15 °C until the alcohol content reached 5–6%. First racking was performed by siphon to separate the wine from sediment that develops during fermentation. Second racking was completed when no bubbles were observed in the fermentation lock. Finished wine (BW1) was filtered through four layers of fine cheesecloth and 0.22 µm filters. The sample was placed in a –25 °C freezer for subsequent use and analysis.

2.4. Blueberry wine (BW2) processing

Blueberry must was placed in a 15 °C water bath to increase the temperature to fermentation temperature (15 °C) and then *S. cerevisiae* yeast was inoculated. The wine was fermented at 15 °C until the alcohol content reached 5–6%. After fermentation, a portion of the must was pressed and another portion of the must was blended (to be used as wine base for blueberry vinegar production). The wine and blended must were then transferred to two separate fermenters and fermented again at 15 °C until the alcohol contents reached 5–6%. Finished wine (BW2) was filtered through four layers of fine cheesecloth and 0.22 µm filters and placed in a –25 °C freezer for subsequent use and analysis.

2.5. Vinegar mother preparation

Red wine vinegar mother was inoculated into blueberry wine (8% alcohol content) and placed in a 21 flask equipped with a cheesecloth plug. To increase the surface area, the flask was just filled with inoculated wine. The flask was then placed in a 30 °C incubator until a bacterial film was formed. The bacterial film was used as inoculum for vinegar making.

2.6. Vinegar making

Wines used to make juice vinegar (JV), wine vinegar (WV), and blueberry vinegar (BV) were BW1, BW2, and the wine must (previously reserved from BW2 production), respectively. The procedures and equipment used were the same as for vinegar mother preparation except the inoculation method. The bacterial film previously made for the inoculum of vinegar making was cut into several pieces (30 × 30 mm). Each piece of bacterial film was placed on the top of a piece of wine bottle cork (30 × 30 × 5 mm). The wine bottle corks were boiled in water for several hours to remove undesired materials. Six pieces of wine bottle cork with bacterial film were placed on the surface of wine in a flask. The acidity was monitored daily until it was not changed. After fermentation, blueberry vinegar must was pressed to separate BV and vinegar pomace. All vinegars (JV, WV, and BV) were filtered through four layers of fine cheesecloth and 0.22 µm filters. Vinegars were placed in a –25 °C freezer for subsequent analysis.

2.7. Total phenolics

Total phenolics content (TPH) was measured by using the Folin–Ciocalteu method (Saura-Calixto, 1998). Results were expressed as mg gallic acid equivalents (GAE) per 100 ml of sample (mg GAE/100 ml).

2.8. Total anthocyanins

Total anthocyanins (ACY) was determined by using a differential pH method (Chiriboga & Francis, 1970). Results were expressed as mg of cyanidin-3-glucoside (c3g) equivalents per 100 ml of sample.

2.9. Beta-carotene bleaching assay

The method reported by Burda and Oleszek (2001) was used. In this study, 0.1 ml of each product was added to the assay mixture. Antioxidant activity (AA1) was calculated as percent inhibition of oxidation versus control (distilled water).

2.10. Ferric thiocyanate (FTC) assay

The FTC method (Larrauri, Ruperez, & Calixto, 1996) was used to determine the in vitro inhibition of linoleic acid

peroxidation. In this study, 0.1 ml of each product was added to the assay mixture. Antioxidant activity (AA2) was calculated as percent inhibition of linoleic acid peroxidation versus control.

2.11. Antiradical activity assay

The DPPH method (Burda & Oleszek, 2001) was used to determine free radical-scavenging potential of each sample. 0.05 ml of blueberry liquid products was added to 5 ml of DPPH solution (0.025 g/l). The absorbance was measured at 517 nm after 30 min of reaction at 25 °C. The antiradical activity was calculated as a percentage of DPPH decoloration versus control (methanol).

2.12. Statistical analysis

A completely randomized design was used. Six treatments with three replications were used. The data were analyzed using the general linear models (GLM) procedure. When significant, means were separated using the Fisher's protected least significant difference method. Correlation analysis was also conducted, by using the CORR procedure. The statistical analysis was conducted with SAS version 8.1 (SAS Inst. Inc., Cary, NC).

3. Results and discussion

3.1. Anthocyanins

The results (Table 1) show that the differences in ACY of all blueberry products were significant. The concentration of ACY varied from 0.97 to 9.96 mg/100 ml of c3g equivalents. The ACY content of blueberry products was very low compared to whole blueberry fruit (363 ± 6.7 mg/100 g of c3g equivalents). Our previous studies (Su & Silva, 2006) and studies performed by Lee, Durst, and Wrolstad (2002) indicate that presscake maintained an important ACY and TPH. The BW2 had the greatest amount of ACY, whereas JV had the lowest ACY. The difference was almost ten-fold. The ACYs of wines were higher than those of other products, especially vinegars. Acetification largely decreased ACY. For example, ACY of JV decreased 83% from BW1 and ACY of WV decreased 86% from BW2. According to Lukton, Chickester, and Mackinney (1956), anthocyanin breakdown is dependent on pH in the presence of oxygen and directly related to the level of pseudobase (colourless) and inversely related to the cation. Also, pH and temperature have been reported to be two critical factors that influence colour degradation in lowbush blueberry products (Kalt, McDonald, & Donner, 2000; Simard, Bourzeix, & Heredia, 1982). Simard et al. (1982) reported that the rate of anthocyanin monomer losses in lowbush blueberry juice increased with increasing temperature (Simard et al., 1982). These factors should be taken into consideration when making fermentation adjustments in vinegar making. According to the

Table 1

Total anthocyanin content (ACY), total phenolics (TPH), antiradical activity (AR%), and antioxidant activity (AA1, AA2%) of rabbiteye blueberry products

Products	ACY (mg/100 ml)	TPH (mg/100 ml)	AR%	AA1%	AA2%
BJ	4.52 ± 0.10c*	86.7 ± 1.56d	49.9 ± 1.18d	60.6 ± 1.99b	94.7 ± 0.34b
BW1	5.60 ± 0.14b	85.8 ± 1.54d	51.0 ± 4.42d	58.1 ± 1.42b	95.6 ± 0.30ab
BW2	9.96 ± 0.11a	115 ± 3.06a	78.0 ± 2.79a	69.9 ± 4.73a	96.5 ± 0.21a
JV	0.97 ± 0.06f	88.7 ± 1.54d	52.0 ± 1.21d	46.5 ± 1.50c	11.3 ± 0.86e
WV	1.37 ± 0.09e	111 ± 2.65b	65.6 ± 2.15b	58.1 ± 3.89b	32.3 ± 1.23c
BV	3.22 ± 0.13d	98.1 ± 1.66c	58.7 ± 2.43c	47.5 ± 3.19c	19.3 ± 0.74d

* Within the same column, means followed by different letters are significantly different at $P < 0.05$.

results of this study, the ACY of BW1 was higher than that of BJ. This could be due to the extraction and/or yeast release of anthocyanins from small pieces of skin that remained in the juice after filtration. The results also indicate that the winemaking process did not lower the ACY. Anthocyanin pigments were extracted from blueberry skins during fermentation. These pigments were largely responsible for the intense colour of BW2.

3.2. Total phenolics

The TPH of blueberry products (Table 1) ranged from 85.8 ± 1.54 (BW1) to 115 ± 3.06 (BW2) mg/100 ml GAE. The BW2 had the greatest amount of TPH, whereas BW1, BJ, and JV were the lowest in TPH. There was no significant difference between BJ and BW1, indicating that the TPH of semi-fermented wine (5–6% alcohol content; 3–5 days fermentation) was not affected by off-skin fermentation. However, on-skin fermentation largely influenced TPH and ACY. White wines, similar to BW1 in this study, are usually made from the free running and processed juice, having no contact with the skins. This is thought to be the main reason for the relatively low phenolics content. Alcohol concentration and skin contact time were reported to increase polyphenol content of white wine (Fuhrman et al., 2001). In this study, TPH increased 35% because of skin contact. This is lower than the results of Fuhrman et al. (2001), probably because of the low alcohol content (6%) and different kinds of raw materials used.

Studies on phenolic contents of fruit products have mainly focussed on juices and wines (Arnous, Makris, & Kefalas, 2001; Burns et al., 2001; Landrault et al., 2001; Skrede, Wrolstad, & Durst, 2000). Little is known about changes of phenolic profiles and total phenolic content by the acetification process. In this study, the TPH of blueberry vinegars ranged from 88.7 ± 1.54 (JV) to 111 ± 2.65 (w/v) mg/100 ml GAE. TPH significantly decreased in WV, but not in JV, as compared to its wine. The TPH of BV (98.1 ± 1.66 mg/100 ml GAE) was significantly higher than those of BJ and BW1. These results suggest that the extraction effects still played their role during the acetification process. Oxidation and other factors can alter phenolic content during the acetification process. However, these factors apparently did not have practical impact on this study. These findings were also reported by Morales,

Tesfaye, Garcia-Parrilla, Casas, and Troncoso (2002). According to Andlauer, Stumpf, and Furst (2000), one of the few studies investigating the alterations of total phenolic content by the acetification process, the results of changes in TPH of different vinegars were different. For example, the decrease in total phenol content was highest for cider vinegar (40%) and lower for red wine vinegar (13%) and white wine vinegar (2–14%). The results of this study are somewhat in agreement with their findings (Andlauer et al., 2000).

3.3. Beta-carotene bleaching assay

The BW2 had the highest antioxidant activity (69.9 ± 4.73). There was no significant difference between BJ and BW1. JV and BV had lower antioxidant activities among blueberries products (Table 1).

Correlation between AA1 and ACY of all products in this study was good and significant ($r = 0.77$, $P \leq 0.01$). However, it was low and insignificant between AA1 and TPH ($r = 0.45$). The results indicate that ACY made more significant contributions than did TPH to antioxidant activities of products. This finding is in agreement with the result of studies conducted by Kalt et al. (2001) and Kalt et al. (2000). For TPH, the low correlation with AA1 could be due to the different phenolic profiles of different products. For the same kind of product, correlation between AA1 and TPH or AA1 and ACY of wine products in this study was greater than 0.85 ($P \leq 0.05$). Correlations between AA1 and TPH and between AA1 and ACY of vinegar products in this study were 0.81 ($P \leq 0.01$) and -0.27, respectively. Because of low anthocyanin and high phenolic contents in vinegar products, especially JV (TPH/ACY = 91.4), the contributions to antioxidant activities were mainly attributed to TPH. Therefore, an insignificant, negative correlation was obtained.

3.4. Ferric thiocyanate (FTC) assay

Blueberry juice and wine products showed a high capacity to inhibit linoleic acid peroxidation when the FTC assay was performed (Table 1). Similar to the beta-carotene bleaching assay, BW2 had highest antioxidant abilities (96.5 ± 0.21 %). Also, there was no significant difference between BJ and BW1. The abilities of vinegar products to

inhibit linoleic acid peroxidation were very poor. The decrease in antioxidant activity because of acetification process was up to 88 % when the FTC assay was performed.

The results showed a good correlation between AA2 and ACY ($r = 0.8$, $P \leq 0.01$) or between AA2 and AA1 ($r = 0.79$, $P \leq 0.01$) of all products. Correlation between AA2 and TPH or between AA2 and ACY of wine products was greater than 0.86 ($P \leq 0.05$). Correlation between AA2 and TPH of vinegar products was greater than 0.97 ($P \leq 0.01$). The results also show a high correlation between AA2 and AA1 of vinegar products ($r = 0.85$, $P \leq 0.01$) or between AA2 and AA1 of wine products ($r = 0.92$, $P \leq 0.01$).

For rate of hydroperoxide production (Fig. 1), the results show that juice and wine products had almost the same low rate (0.0006–0.0009 $\Delta A/h$); whereas vinegar products had 10-fold higher rate (0.008–0.011 $\Delta A/h$) and 1.5–2-fold lower rate than control (0.016 $\Delta A/h$).

3.5. Antiradical activity assay

BW2, like other assays, showed the highest free radical scavenging effect ($78.0 \pm 2.79\%$), followed by WV ($65.6 \pm 2.15\%$). Antiradical activity depends on the structural conformation of phenolic compounds (Bors, Heller, Michel, & Saran, 1990; Bors, Michel, & Stettmaier, 1997; Larrauri et al., 1996). Therefore, antiradical activity is greatly influenced by the phenolic composition of the sample. In this study, large changes of ACY were observed due to the acetification process. Thus, significant difference of antiradical activity between BW2 and WV was obtained. However, no significant difference was found between BW1 and JV because of, as stated previously, the high TPH/ACY ratio of JV and insignificant difference of TPH between BW1 and JV. Through oxidative phenolic coupling reactions, phenolic dimers and oligomers are formed. Each dimer or oligomer may retain its original

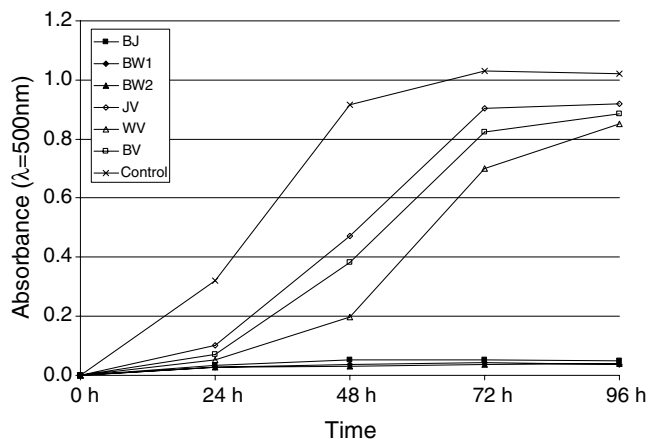


Fig. 1. Antioxidant activities of rabbiteye blueberry product assessed by the FTC method. BJ: blueberry juice; BW1: wine from juice fermentation; BW2: wine from on-skin fermentation; JV: vinegar from BW1; WV: vinegar from BW2; BV: vinegar from on-skin acetification.

number of reactive hydroxyl groups. This will enhance antioxidant and/or antiradical activity, if it is still soluble. During acetification, these reactions might occur and enhance the antiradical activities of vinegar products. In this study, the antiradical activities of WV and BV were significantly higher than those of BJ and BW1, respectively.

Correlation between AR and ACY of all products in this study was low but significant ($r = 0.54$, $P \leq 0.05$). Almost the same correlation was found between AR and AA1 of all products ($r = 0.55$, $P \leq 0.05$). However, AR and TPH of all products were highly correlated with each other. For the same kind of product, correlation between AR and TPH or AR and ACY of wine products was high ($r = 0.98$, $P \leq 0.01$). Correlation between AR and TPH of vinegar products, in this study, was 0.94 ($P \leq 0.01$). The AR and ACY of vinegar products were not correlated. These results are similar to those obtained by β -carotene bleaching assays.

4. Conclusion

The BW2 had the highest content of anthocyanins and polyphenols and the highest AA1 and AR. Total anthocyanin content, total polyphenol content, antiradical activity, and antioxidant activities of vinegars decreased. The acetification process largely decreased ACY. However, it did not have a practical influence on TPH.

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