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

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Abstract

Basic blueberry processing includes juice processing or winemaking. By-products obtained from the juice and wine industry can be a source of new value-added products such as phenolic antioxidant supplements or ingredients for food processing. The phenolic compositions of products and by-products (pomaces) depend mainly on processing techniques such as duration of skin contact, crushing, pressing, and others. The present study was to evaluate the effects of fermentation type on retention of total anthocyanins, total phenolics, and antioxidant activity of blueberry by-products. Total phenolics (TPH), total anthocyanins (ACY), antioxidant activities (β-carotene bleaching assay and ferric thiocyanate assay), and antiradical activity (DPPH radical-scavenging assay) of rabbiteye blueberry (*Vaccinium ashei*) by-products (juice, wine, and vinegar pomaces) were determined. The wine pomace (WP) had higher TPH, antioxidant activities and antiradical activity. Vinegar pomace (VP) had the lowest ACY, TPH, antiradical activity, and antioxidant activities. The results indicate that the antioxidant and antiradical activities of blueberry by-products were not significantly affected by the wine making process. Acetification significantly decreased TPH, ACY, antioxidant activities, and antiradical activity. However, VP still maintained an important phenolics concentration and antioxidant activity.

Keywords: Blueberries; Antioxidant activity; Fermentation; Acetification; Pomaces

1. Introduction

Pomace is the press residue remaining when fruits are processed for juice, wine, or other products. The pomace consists of pressed skins, pulp residue, seeds and stems. The juice and wine industry produce very large amounts of pomace. By-products obtained from the juice and wine industry may be useful raw materials for creating new value-added products. There is increasing interest in finding new sources of dietary fibre with specific bioactive constituents. Many processes have been reported for utilization of this pomace, including production of anthocyanins, citric acid, ethanol, and grape seed oil (Hang, 1998; Mazza, 1995). However, pomaces are discarded or traditionally used as animal feed or fertilizer. Grape seeds and skins are rich in phenolic compounds. Even after contact with the wine, grape pomace has a high content of polyphenols with potential antioxidant activity (Larrauri, Ruperez, & Calixto, 1996). Phenolic compounds in pomaces can be extractable polyphenols or non-extractable polyphenols. Extractable polyphenols can be extracted from pomaces by using solvents, such as water, methanol, ethanol, acetone or their mixtures. Non-extractable polyphenols are mainly

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condensed tannins or high molecular weight polyphenols. Extractable polyphenols can be absorbed from the digestive tract and produce systemic effects, while non-extractable polyphenols are quantitatively recovered in feces (Bravo, Abia, & Saura-Calixto, 1994). Saura-Calixto (1998) reported that extractable polyphenols have a higher antioxidant capacity in grape pomace than in red wine.

Some berries, such as highbush, lowbush, and rabbiteye blueberry, have phenolic components mainly in the skin. Through common processing, only a minor part of the phenolic components is presented in products. Therefore, choice or improvement of processing methods is the way to produce good value-added products. Also, the pomaces can be processed into value-added products. A variety of products, including anthocyanins, food fibre, blueberry extract and citric acid, can be obtained from blueberry pomaces. It has been reported that grape pomace has a high content of polyphenols with potential high antioxidant activity (Larrauri, Ruperez, & Saura-Calixto, 1997). Like grape, blueberry pomaces should have high contents of polyphenols, especially anthocyanins, and potential antioxidant activity. Also, blueberry pomace can be a kind of antioxidant dietary fibre (Saura-Calixto, 1998) product as a potential food ingredient.

Drying may be an essential step of pomace processing. During the drying process, chemical and biochemical changes will take place. According to Hamama and Nawar (1991), phenolic antioxidants exhibit significant decomposition at high temperatures, giving rise to a number of breakdown products. Thus, the drying conditions should be well established. It has been found that polyphenolic content, colour, and antioxidant activity of red grape pomace peels were not significantly affected when dried at 60 °C. This suggests that antioxidant activities of grape pomace peels can be considered as fairly heat-stable. However, a drying temperature at 100 °C or above is not recommended because of the loss of antioxidant activity (Larrauri et al., 1997).

Many studies on antioxidant activity of fruits and vegetables, including blueberries (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002; Kanner, Frankel, Granit, German, & Kinsella, 1994; Kalt, Forney, Martin, & Prior, 1999), are focussed on grape products such as wines and pomaces (Fuhrman, Volkova, Suraski, & Aviram, 2001; Larrauri et al., 1997; Larrauri, Sanchez-Moreno, Ruperez, & Saura-Calixto, 1999; Mazza, 1995). However, there is no information on antioxidant activity of blueberry pomaces. The present study was to evaluate the effects of fermentation type on retention of total anthocyanins, total phenolics, and antioxidant activity of blueberry pomaces by extracting juice prior to fermentation (JP), by fermentation on the skin (WP), or acetification on the skin (VP). The samples studied were the pomaces derived from each process.

2. Materials and methods

2.1. Materials

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

2.2. Blueberry juice processing

Blueberries were crushed and then divided into two portions. One portion was processed into juice and another portion was used to make wine and vinegar. The juice was prepared, following crushing and pressing at 4 °C, in a small basket press. Juice pomace (JP) was collected and placed in a −25 °C freezer for further analysis.

2.3. Blueberry wine-making

Blueberry must 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%. After fermentation, a portion of the must was pressed and another potion of the must was blended (to be used as wine base of blueberry vinegar production). The wine must was then transferred to a fermenter and fermented again at 15 °C until the alcohol contents reached 5–6%. The wine pomace (WP) was collected and placed in a −25 °C freezer for further analysis.

2.4. Vinegar mother preparation

Red wine vinegar mother was inoculated into blueberry wine (8% alcohol content) and placed in a 2 litre flask equipped with a cheesecloth plug. To increase the surface area, the flask was just filled with inoculated wine to 5 cm high. 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.

2.5. Vinegar-making

The procedures and equipments used were the same as for vinegar mother preparation, except the inoculation method. The bacterial film previously made for inoculum in vinegar making was cut into several pieces (30 mm × 30 mm). Each piece of bacteria film was placed on the top of a piece of wine bottle cork (30 mm × 30 mm × 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 the wine must (previously reserved from wine-making) in a flask. The acidity was monitored daily until the acidity was not changed. After fermentation, the must was pressed to separate vinegar and vinegar pomace (VP). VP was collected and placed in a −25 °C freezer for further analysis.

2.6. Pomaces dehydration

Forced air-drying of pomaces (Larrauri et al., 1997) was used. The dehydration process was performed at 60 °C for 8 h. The dry pomaces were frozen in liquid nitrogen and powdered under liquid nitrogen. The powdered dry pomaces were then placed in a −25 °C freezer for further analysis.

2.7. Pomace extracts preparation

Each powdered pomace sample (500 mg) was extracted with 15 ml of solvent (1% HCl in methanol) at room temperature for 60 min. After centrifugation at 2500*g* for 15 min at 4 °C, the supernatant was collected. These procedures were thrice performed. The supernatants were pooled and adjusted to pH 3.0. The solution was then made up to 50 ml with methanol.

2.8. 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 gramme of sample (mg GAE/g).

2.9. Total anthocyanins

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

2.10. β-Carotene bleaching assay

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

2.11. Ferric thiocyanate (FTC) assay

The FTC method (Larrauri et al., 1997) was used to determine the in vitro inhibition of linoleic acid peroxidation. In this study, 0.1 ml of each extract was added to the assay mixture. Antioxidant activity (AA2) was calculated as percent inhibition of linoleic acid peroxidation versus control.

2.12. DPPH radical-scavenging assay

The DPPH method (Burda & Oleszek, 2001) was used to determine free radical-scavenging potential of each sample. 0.05 ml of each extract 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).

2.13. Statistical analysis

A completely randomized design was used. Three treatments with three replications were used. The data were analyzed using the General Linear Models (GLM) procedure and CORR procedure. The statistical analysis was conducted with SAS version 8.1 (SAS Inst. Inc., Cary, NC, USA).

3. Results and discussion

3.1. Anthocyanins

The results are shown in Table 1. All values were expressed on a dry basis. The differences in ACY of all blueberry by-products were significant. The JP had the highest ACY (11.9 ± 0.03 mg/g c3g equivalent), followed by WP (10.9 ± 0.03 mg/g c3g equivalent), and VP had the lowest (2.3 ± 0.01 mg/g c3g equivalent). The difference between JP and VP was 9.6 mg/g c3g equivalent. The results indicate that extraction effects existed during fermentation, while anthocyanins were very sensitive to the acetification process. One of the factors that caused the large anthocyanins loss of VP was polyphenol oxidase (Sakamura, Watanase, & Obata, 1965). Other factors, such as oxygen, temperature and enzymes, produced by microorganisms during acetification, might cause the loss of anthocyanins.

3.2. Total phenolics content

The TPH of blueberry products (Table 1) ranged from 29.2 ± 0.58 (JP) to 20.7 ± 0.18 (VP) mg/100 ml GAE. The JP had the greatest amount of TPH, followed by WP (27.8 ± 0.52 mg/g GAE), and VP. All values were expressed on dry basis. The differences in TPH of all blueberry by-products were also significant. Like anthocyanins content, VP had the lowest TPH. However, the TPH loss (30%) due to the acetification process was smaller than that of ACY (80%). This might be explained by the fact that one part of THP is low in oxidizable phenolics (Andlauer, Stumpf, & Furst, 2000).

Table 1
Total anthocyanins content (ACY), total phenolics (TPH), antiradical activity (AR), and antioxidant activities (AA1, AA2) of rabbiteye blueberry by-products

Products	ACY (mg/g)	TPH (mg/g)	AR (%)	AA1 (%)	AA2 (%)
JP	11.9 ± 0.03a*	29.2 ± 0.58a	64.3 ± 0.66a	59.9 ± 2.57a	87.5 ± 0.35a
WP	10.9 ± 0.03b	27.8 ± 0.52b	62.0 ± 2.51a	60.5 ± 2.38a	92.2 ± 0.77b
VP	2.3 ± 0.01c	20.7 ± 0.18c	41.2 ± 1.31b	47.4 ± 0.82b	73.4 ± 0.79c

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

According to Larrauri et al. (1997), the bioactive constituents of white grape pomace peels extract are more heat-resistant than those from red grape pomace peels. In this study, some of the phenolics are anthocyanins which are more sensitive to processing.

3.3. Antioxidant activities of blueberry by-products

JP and WP had higher ability to minimize the loss of β-carotene during the oxidation of linoleic acid, to inhibit linoleic acid peroxidation, and to scavenge DPPH-radicals (Table 1). Correlations between AA1 and TPH and between AA1 and ACY were 0.93 ($P \leqslant 0.01$) and 0.95 ($P \leqslant 0.01$), respectively. The AA2 of by-products also showed high correlations between ACY ($r = 0.94$, $P \leqslant 0.01$), TPH ($r = 0.92$, $P \leqslant 0.01$), and AA1 ($r = 0.94$, $P \leqslant 0.01$). Antiradical activities of blueberry by-products were highly correlated with ACY ($r = 0.99$, $P \leqslant 0.01$) and TPH ($r = 0.98$, $P \leqslant 0.01$). Correlations between AR and AA1 ($r = 0.97$, $P \leqslant 0.01$) and between AR and AA2 ($r = 0.94$, $P \leqslant 0.01$) of blueberry by-products were also high. Correlation analyses indicate that the antioxidant activities of blueberry by-products were well correlated with ACY and TPH. It is reported that there is a significant linear relationship between ORAC and the total anthocyanin or total phenolic content in different maturities and varieties of *Vaccinium* spp. (Prior et al., 1998). Simonetti, Pietta, and Testolin (1997) also reported that the total antioxidant activities of 13 typical Italian wines were well correlated with phenol ($r = 0.9902$) and flavanol ($r = 0.9270$) contents.

The TPH/ACY ratios of JP, WP, and VP were 2.5, 2.6, and 8.9, respectively. These indicate that the contributions to antioxidant activities were by both TPH and ACY. The results also show that the acetification process was accompanied significant decreases in the total phenolics content, total anthocyanins content and antioxidant activity (AA1). According to some studies, free radical-scavenging activity depends on the structural conformation of phenolic compounds (Bors, Heller, Michel, & Saran, 1990; Bors, Michel, & Stettmaier, 1997; Larrauri et al., 1996). Thus, free radical-scavenging activity is greatly influenced by the phenolic composition of the sample. In this study, there was no significant difference between JP and WP. This indicates that the winemaking process did not significantly change antiradical activity of pomaces.

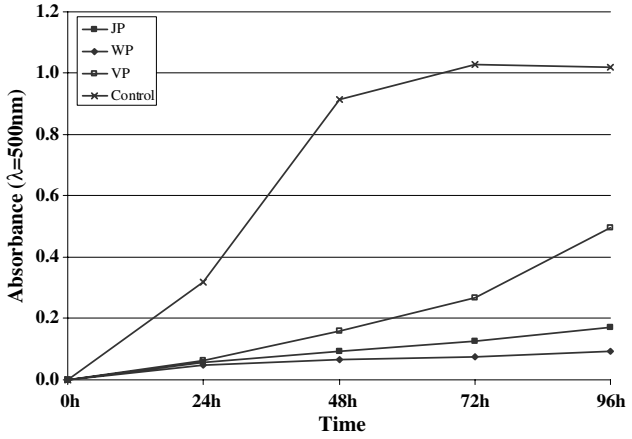

Fig. 1. Antioxidant activities of rabbiteye blueberry by-products as assessed by the FTC method. JP: juice pomace; WP: wine pomace; VP: vinegar pomace.

After 24 h of the experiment, all three by-products showed a good inhibition of linoleic acid peroxidation (Fig. 1). As shown in Fig. 1, there was no prooxidative behaviour observed in any by-products. For hydroperoxide production, the results show that JP and WP had low rates (0.0019 and 0.0012 ΔA/h), whereas VP had a higher rate (0.0035 ΔA/h). The AA2 of by-products showed high correlations between ACY ($r = 0.94$, $P \leqslant 0.01$), TPH ($r = 0.92$, $P \leqslant 0.01$) and AA1 ($r = 0.94$, $P \leqslant 0.01$).

In conclusion, the wine-making process significantly lowered total anthocyanin content and total polyphenol content of by-products, but did not significantly affect antiradical activity or antioxidant activities. The results also show that acetification significantly decreased total anthocyanin content, total polyphenols and antioxidant activities. This study demonstrates that these by-products still retained important phenolic concentrations and antioxidant activities. Especially, JP and WP were good phenolic and antioxidant sources.

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