Processing and Storage Effects on Blueberry (*Vaccinium corymbosum* L.) Polyphenolics

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Abstract

Blueberries are commonly consumed in processed forms, but limited information is available on how different processing methods and storage of processed products impact the polyphenolic content of the fruit. In this study, we determined how canning, puréeing and juicing of blueberries, as well as storage of processed products at 25°C, influenced the retention of chlorogenic acid, total flavonols, total anthocyanins, and total procyanidins. The retention of flavonols (57-99%) and chlorogenic acid (64-100%) was greater than that of anthocyanins (42-72%) and procyanidins (19-78%). The type of processing method impacted polyphenolic retention with canned products showing the greatest retention, followed by puréed products, and juices. Non-clarified juices retained higher levels of chlorogenic acid, total flavonols, and total anthocyanins than clarified juices, but clarified juices contained higher levels of total procyanidins. Significant losses of total anthocyanins and total procyanidins occurred in all processed products stored over six months at 25°C, which most likely was due to polymeric compounds formed as a result of condensation reactions among anthocyanins and procyanidins. In contrast, total flavonols and chlorogenic acid showed much greater retention during storage. Our results indicate that methods are needed to prevent polyphenolic losses during processing, especially in juices, and storage of processed products.

INTRODUCTION

Blueberries (*Vaccinium corymbosum* L.) contain a variety of polyphenolic compounds (anthocyanins, flavonols, procyanidins) in abundant quantities that are purported to have many health-promoting properties (Beattie et al., 2005). Blueberries, like other berries, are not only available fresh, but are available for consumption in several thermally processed forms (jellies, jams, juices, canned and purées). Several studies have investigated the effects of juice processing on blueberry polyphenolics (Skrede et al., 2000; Lee et al., 2002; Rossi et al., 2003; Srivastava et al., 2007), and the studies indicate that processing has a detrimental effect on polyphenolics. Besides juice processing, information is limited on how different processing methods and long term storage of processed products affect the nutritional quality of blueberries prepared from the same raw material. This information is needed for consumers who wish to incorporate higher levels of bioactive compounds into their diet, and processors who desire to retain, or possibly boost levels of bioactive compounds in their products. The objective of this study was to determine how different processing methods (juicing, canning, puréeing) and storage of processed products affected the retention of blueberry polyphenolics.

MATERIALS AND METHODS

Blueberry Samples

Blueberries (cv. Bluecrop) harvested at the fully ripe stage were obtained from a commercial grower in Fayetteville, AR in June 2005. The fruit was stored at −20°C for less than one month prior to processing.
Juice Processing

Frozen berries were simultaneously heated and mixed with a Mixco Batch mixer (Avon, NY) in a large steam kettle until the berry mash reached a temperature of 95°C. It was held at 95°C for 3 min and allowed to cool to 40°C. Depectinization of the mash was performed by adding 0.0827 ml/kg of Pectinex Smash® (Novozyme, Bagsvaerd, Denmark) and incubating the mash for 1 h at 40°C. Negative alcohol precipitation test was used as an indication of complete depectinization. Following enzymatic treatment, the mash was pressed in a 25-L Enrossi bladder press (Enoagricol Rossi s.r.l., Calzolaro, Italy), and the juice and presscake were isolated. Half of the juice was clarified by centrifugation for 10 min at 6000 x g in a model CRU-5000 centrifuge (Damon/IEC Division, Needham, MA), while the other half received no clarification treatment. Both clarified and non-clarified juices were filled into 6 oz glass bottles and heated in a steam box (American Sterilizer Company, Erie, PA) until the juice temperature reached 90°C. The bottle caps were tightened and the juices were allowed to cool overnight. Juice samples were stored in the dark at 25°C.

Canned-in-Water (CW) and Canned-in-Syrup (CS) Processing

Blueberries were canned by the Downing (1996) method. Frozen berries (278 g) were added to 303 x 406 cans. Syrup was prepared by adding Sweetose 4300 corn syrup (Tate and Lyle, London, UK) to boiling water to reach a final brix reading of 40°. Boiling syrup (for CS cans) or water (for CW cans) were added to the cans to the brim and cans were exhausted for 4 min in a steam box (American Sterilizer Company, Erie, PA) at 87.8-93.3°C. The cans were then sealed, immersed in boiling water for 15 min, and stored at 25°C.

Purée Processing

Frozen berries were allowed to thaw and homogenized for 1 min on high speed using a commercial food processor. Blended berries were immediately added to the steam kettle and heated to a temperature of ~95°C. The purée was cooled and Sweetose 4300 corn syrup was added to the purée until 18° Brix was attained. The purée was subsequently heated to 92.8°C and added to 4 oz canning jars (Ball Corp., Muncie, IN). After sealing, the jars were immersed in boiling water for 15 min, cooled in cold water to 38°C, and stored in the dark at 25°C.

Extraction of Polyphenolics

Prior to the extraction of polyphenolics, the entire contents of canned samples (berries and cover solution) were blended for 1 min on high speed in a commercial food processor. Purée and juice samples required no pre-extraction step.

The extraction of anthocyanins, flavonols and chlorogenic acid was performed as described by Cho et al. (2004). The concentrated extracts were filtered through 0.45 µm PTFE syringe filters prior to HPLC analysis. Procyanidins were extracted and purified according to the method of Prior et al. (2001). The concentrated extracts were filtered through 0.45 µm PTFE syringe filters prior to HPLC analysis.

HPLC Analysis of Procyanidins

The procyanidin analysis by HPLC was performed according to an adapted method of Kelm et al. (2006) with a 250 X 4.6 mm i.d., 5 µm Develosil diol (Phenomenex, Torrance, CA). The mobile phase consisted of a binary gradient of acetonitrile/acetic acid (98:2 v/v) (A) and methanol/water/acetic acid (95:3:2 v/v/v) (B). The flow rate was 0.8 ml/min with a linear gradient as follows: 0-35 min, 0-40% B, 35-45 min, 40% B, 45-47 min 50% B, 47-49 min 60% B, 49-50 min 100% B, 50-52 min, 100% B, 53-60 min, 0% B, followed by 5 min re-equilibration time. The procyanidin peaks were quantified by fluorescence detection with excitation at 276 nm and emission at 316 nm using a Waters Model 474 fluorescence detector (Milford, MA). Individual procyanidins with degrees of polymerization (DP) from DP1 through DP8 were
quantified using external calibration curves of a composite procyanidin oligomer standard (DP1-DP10) purified from cacao. Total procyandins were calculated as the sum of individual procyandins with results expressed as mg per kg of original berry.

**HPLC Analysis of Anthocyanins, Flavonols and Chlorogenic Acid**

The polyphenolic analysis by HPLC was performed according to the method of Cho et al. (2004) with a 250 X 4.6 mm Symmetry C18® column (Waters Corporation, Milford, MA). The mobile phase consisted of a binary gradient of 5% formic acid (A) and 100% methanol (B). The flow rate was 1.0 ml/min with a linear gradient from 2% B to 60% B over 60 min. The chlorogenic acid, flavonol and anthocyanin peaks were quantified at 320, 360, and 510 nm, respectively, using a Waters Model 996 photodiode array detector (Milford, MA). Individual anthocyanin monoglycosides and acylated anthocyanin derivatives were quantified as delphinidin, cyanidin, petunidin, peonidin and malvidin glucoside equivalents using external calibration curves of a mixture of anthocyanin glucosides obtained from Polyphenols Laboratories AS (Sandnes, Norway). Total anthocyanins were calculated as the sum of individual anthocyanin monoglycosides and acylated anthocyanin derivatives. Chlorogenic acid was quantified using an external calibration curve of a standard obtained from Sigma Chemical Company (St. Louis, Mo.). Individual flavonols were quantified as rutin equivalents using an external calibration curve of the standard obtained from Sigma Chemical Company (St. Louis, Mo.). Polyphenolic results were expressed as mg per kg of original berry.

**Calculations**

For blended canned samples, juices, and purées, the chlorogenic acid, total anthocyanin, total flavonol, and total procyanidin values were converted to original berry weight using the following calculation:

\[ C_{\text{product}} \times R = C_{\text{berry}} \]

Where, 
- \( C_{\text{product}} \) = concentration of product
- \( R \) = ratio of the mass of product produced to the mass of the original berry
- \( C_{\text{berry}} \) = concentration based on original berry weight

This conversion allowed for concentration and dilution effects to be accounted for and all products to be compared on an equivalent basis.

**Statistical Analysis**

All data were reported as means ± standard error of five samples taken from each processed product at each sampling time.

**RESULTS AND DISCUSSION**

**Chlorogenic Acid**

The percent retention of chlorogenic acid (compared to fresh berries) in processed blueberry products during storage is shown in Figure 1. Chlorogenic acid was well retained following pasteurization of non-clarified juices (97%), berries canned-in-water (100%), and berries canned-in-syrup (100%), but only 64% and 76% was retained in clarified juices and purées. The 97% retention of chlorogenic acid in non-clarified juices was much higher than the previously reported value (53%) for non-clarified blueberry juices (Skrede et al., 2000). The lower retention for chlorogenic acid in purées was most likely due to destruction of the compound by polyphenol oxidase (PPO) prior to the blanching treatment. Chlorogenic acid is an excellent substrate for PPO, and the enzyme isolated from blueberries is reported to cause oxidation of chlorogenic acid (Kader et al., 1997a,b). The much lower retention of chlorogenic acid in clarified juices compared to non-clarified juices was unexpected, since the only difference between the two processes was the clarification step. Presumably, the clarification step resulted in physical removal of the compound or removal of other polyphenolics that protected the compound during pasteurization of non-clarified juices.
After 6 months storage at 25°C, 83% and 80% of chlorogenic acid was retained in non-clarified juices and berries canned-in-syrup, 70% was retained in both berries canned-in-water and purées, while only 50% was retained in clarified juices. In comparing processing verses storage effects, processing had a greater effect on chlorogenic acid losses in clarified juices and purées, while storage had a greater effect on losses in non-clarified juices and berries canned in either water or syrup.

**Total Flavonols**

The percent retention of total flavonols in processed blueberry products (compared to fresh berries) during storage is shown in Figure 2. Following pasteurization, total flavonol levels were well retained in berries canned-in-water (99%) and purées (97%), moderately well retained in berries canned-in-syrup (84%) and non-clarified juices (79%), while only 57% was retained in clarified juices. The 79% retention of total flavonols in non-clarified juices was much higher than the previous reported values (35-47%) reported for non-clarified blueberry juice (Skrede et al., 2000; Lee et al., 2002). As previously mentioned for chlorogenic acid, the lower retention of flavonols in clarified juices compared to non-clarified juices may be attributed to physical removal of the flavonols, or removal of co-protectant compounds during the clarification step.

After 6 months storage at 25°C, 82% and 75% of total flavonols were retained in berries canned-in-syrup and non-clarified juices, 68% and 62% were retained in berries canned-in-water and purées, while only 44% was retained in clarified juices. In comparing processing verses storage effects, processing had a greater effect on total flavonol losses in clarified juices, non-clarified juices, and berries canned-in-syrup, while storage had a greater effect on total flavonol losses in berries canned-in-water, and purées.

**Total Anthocyanins**

The percent retention of total anthocyanins in processed blueberry products (compared to fresh berries) during storage is shown in Figure 3. Following processing, total anthocyanins were retained to a much lesser extent than chlorogenic acid and total flavonols. Total anthocyanins were retained to the greatest extent in berries canned-in-syrup and non-clarified juices (both 72%), while 66% and 57% were retained in berries canned-in-water and purées, and only 41% was retained in clarified juices. Other studies reported that only 11 to 34% of total anthocyanins were retained in non-clarified pasteurized blueberry juices (Skrede et al., 2000; Lee et al., 2002; Rossi et al., 2003; Srivastava et al., 2007). The marked differences in anthocyanin retention among our results and others may be attributed to differences in thawing procedures and blanch treatments. In our study, frozen berries were thawed and quickly blanched in a steam kettle; while in previous studies, fruit were thawed for 12hr at 5°C, followed by water (Srivastava et al., 2007), steam (Lee et al., 2002; Srivastava et al., 2007) or no blanch treatment (Skrede et al., 2000). We suspect that the rapid inactivation of polyphenol oxidase (PPO) by the steam treatment employed in our study resulted in a much higher retention of anthocyanins, as well as chlorogenic acid and flavonols. The type of process and number of processing steps appeared to play a major role in the variable losses of anthocyanins in the products. The complex steps involved in juice processing; blanching, mashing, enzyme treatment, pressing, clarification, and pasteurization resulted in the lowest retention of anthocyanins. Purées also incurred significant losses, which may be attributed to PPO catalyzed degradation of anthocyanins prior to pasteurization. The berries were not blanched prior to pureeing, which most likely created favorable conditions for enzymatic degradation. Interestingly, the purées also incurred significant losses of chlorogenic acid. Blueberry peroxidase is reported to cause degradation of anthocyanins in the presence of chlorogenic acid and hydrogen peroxide (Kader et al., 2002), and blueberry PPO has also been shown to degrade anthocyanins in the presence of chlorogenic acid via a similar chlorogenoquinone mediated reaction as peroxidase (Kader et al., 1997a,b). Our results suggest that a blanching treatment prior to pureeing is needed to rapidly inactivate enzymes that can cause destruction of anthocyanins and other
polyphenolics. Future studies will address the effects of rapid blanch treatments to ameliorate polyphenolic losses. The greater retention of anthocyanins in canned berries may be explained by the use of whole intact berries in the products, which most likely prevented enzymatic degradation due to compartmentalization of the enzymes and phenolic substrates.

The anthocyanins were further degraded during storage for six months at 25°C. After six months, berries canned-in-water and berries canned-in-syrup retained only 38% and 29% of total anthocyanins, while non-clarified juices, purées, and clarified juices retained only 23%, 20%, and 15%, respectively. The significant decreases in anthocyanins during storage were accompanied by large increases in polymeric color values (data not shown). We suspect that the anthocyanins were extensively polymerized during storage and were not detectible by HPLC analysis. Anthocyanins are reported to undergo condensation reactions with other phenolics including catechins (Reed et al., 2005), flavonols (Rein et al., 2005), and phenolic acids (Rein et al., 2005). In comparing processing verses storage effects, processing had a greater effect on total anthocyanin losses in all processed products, excluding berries canned-in-water, and non-clarified juices.

**Total Procyanidins**

The percent retention of total procyanidins (DP1-DP8) in processed blueberry products (compared to fresh berries) during storage is shown in Figure 4. Following pasteurization, 78% of the total procyanidins were retained in berries canned-in-water, but only 66%, 42%, 36%, and 23% were retained in berries canned-in-syrup, purées, clarified juices, and non-clarified juices, respectively. The 36% retention of total procyanidins in non-clarified juices was lower than the value of 43% reported for non-clarified blueberry juice (Skrede et al., 2000). Similar to anthocyanins, the fate of procyanidins appears to be affected by the type of process (use of macerated vs whole fruit) and number of processing steps. Significant losses of procyanidins most likely occurred during juice processing as a result of physical removal of the seeds during pressing. The much higher retention of procyanidins in berries canned-in-water verses berries canned-in-syrup suggests that the corn syrup promoted the degradation of procyanidins during pasteurization, but additional research is needed to confirm this finding.

The procyanidins were further degraded during storage for six months at 25°C. After 6 months, berries canned-in-water and berries canned-in-syrup retained only 32% and 22%, respectively, of the total procyanidins, while both clarified juices, and non-clarified juices retained only 9%, and purées retained only 7%. In comparing processing verses storage effects, processing had a greater effect on total procyanidin losses in all processed products, except for berries canned in either syrup or water, which experienced greater losses during storage. The marked losses of procyanidins during processing and storage paralleled the losses of anthocyanins, indicating that the two types of flavonoids may react in condensation reactions. There are reports of condensation reactions of anthocyanins with flavan-3-ols or polyflavan-3-ols (Reed et al., 2005), that can be mediated by acetaldehyde (Es-Safi et al., 1999) and furfural (Es-Safi et al., 2000) or occur via direct anthocyanin-tannin reactions (Remy et al., 2000). Additional work is needed to identify the polymeric compounds formed in response to processing and storage.

**CONCLUSIONS**

Flavonols and chlorogenic acid were retained to a much greater extent than anthocyanins and procyanidins in all thermally processed products. The type of processing method also impacted polyphenolic losses, with juice processing resulting in the greatest losses, followed by purée processing, and canning. Significant losses of anthocyanins and procyanidins also occurred over six months storage at 25°C, which most likely was due to polymeric compounds formed as a result of condensation reactions among anthocyanins and procyanidins. In contrast, the flavonols and chlorogenic acid showed much greater retention during storage. Our results indicate that methods are
needed to prevent polyphenolic losses during processing and storage of processed products. Future studies should focus on 1) the development of methods to rapidly inactivate polyphenol oxidase and peroxidase, 2) the development of methods to recover polyphenolics from juice by-products, and 3) the development of methods to prevent the formation of polymeric compounds during storage.

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**Literature Cited**


**Figures**

Fig. 1. Percent retention of chlorogenic acid in processed blueberry products as affected by storage at 25°C. Bars represent standard error of the mean (n=5).

Fig. 2. Percent retention of flavonols in processed blueberry products as affected by storage at 25°C. Bars represent standard error of the mean (n=5).
Fig. 3. Percent retention of anthocyanins in processed blueberry products as affected by storage at 25°C. Bars represent standard error of the mean (n=5).

Fig. 4. Percent retention of procyanidins in processed blueberry products as affected by storage at 25°C. Bars represent standard error of the mean (n=5).