Journal of Food Research; Vol. 5, No. 6; 2016
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Stability of Haskap Berry (*Lonicera Caerulea* L.) Anthocyanins at Different Storage and Processing Conditions

Rabie Khattab[1,2], Amyl Ghanem[2] & Marianne Su-Ling Brooks[2]

[1]Food Science Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria, Egypt

[2]Department of Process Engineering & Applied Science, Dalhousie University, B3H 4R2 Halifax, NS, Canada

Correspondence: Marianne Su-Ling Brooks. Department of Process Engineering & Applied Science, Dalhousie University, B3H 4R2 Halifax, NS, Canada. E-mail: Su-Ling.Brooks@dal.ca

Received: September 20, 2016 Accepted: October 8, 2016 Online Published: October 25, 2016

doi:10.5539/jfr.v5n6p67 URL: http://dx.doi.org/10.5539/jfr.v5n6p67

Abstract

The effect of freezing, frozen storage (–18 °C for 6 months), thawing, juice extraction, and hot-air drying on the anthocyanin profile of haskap berry (*Lonicera caerulea* L.) was investigated using RP-HPLC. Five anthocyanins (ANCs) were quantified: cyanidin 3,5-di-glucoside (4.27 % of the total ANCs), cyanidin 3-glucoside (89.39 %), cyanidin 3-rutinoside (2.07 %), pelargonidin 3-glucoside (0.83 %), and peonidin 3-*O*-glucoside (3.44 %). Freezing did not significantly affect the content of individual ANCs, while frozen storage resulted in significant reductions (16.00-24.50 %). Thawing the frozen berries in the microwave oven retained the highest content of different ANCs. The highest degradation, however, occurred while thawing at room temperature. Extracting juice from the berries significantly reduced the content of individual ANCs. Drying the berries to 25 % moisture content at 60, 100, and 140 ℃ reduced the individual ANCs by 73.85-76.19, 78.46-80.95 and 90.77-95.40 %, respectively. The overall stability of the five ANCs during storage and processing is summarized by the following trend (from most to least stable): peonidin 3-*O*-glucoside > pelargonidin 3-glucoside > cyanidin 3,5-diglucoside > cyanidin 3-rutinoside > cyanidin 3-glucoside.

Key words: haskap berry, storage, processing, anthocyanins, stability

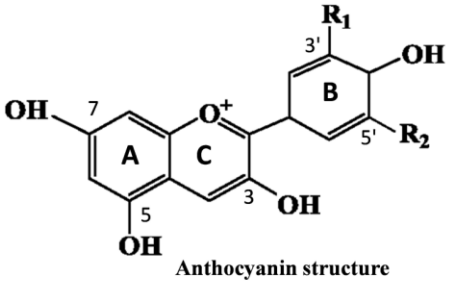
1. Introduction

The haskap berry (*Lonicera caerulea* L.) has been recently introduced as a commercial crop to the North American market. Some varieties and cultivars are currently available in Canada and USA (Bors *et al*., 2012). These berries are either consumed fresh or processed into juice, pastries, jams, ice cream, and dried berries (Celli *et al*., 2014). Haskap berries have attracted attention for their distinct profile of phenolic phytochemicals (Jurikova *et al*., 2012). They are particularly rich in anthocyanins (ANCs) with varied health benefits (Paredes-Lopez *et al*., 2010). The total anthocyanin content (TAC) of haskap berry was found to be up to 13.00 mg cyanidin 3-glucoside (C-3-G) equivalents per g fresh weight (FW) (Bakowska *et al*., 2007; Fan *et al*., 2011; Rupasinghe *et al*., 2012). This berry has much higher antioxidant potential than that reported for blueberry, blackberry, raspberry, bilberry, strawberry, sea buckthorn and black currant (Rop *et al*., 2011; Raudsepp *et al*., 2013; Celli *et al*., 2014).

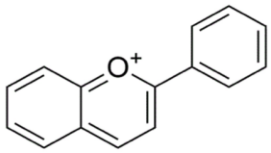
ANCs are the most important water-soluble phytochemicals in nature (Harborne, 1998). They are responsible for the distinguished colors of several fruits and vegetables. They are distinctive from the other flavonoids by their ability to form flavylium cations (Fig. 1) (Mazza, 2007). ANCs consist of an aglycon base or flavylium ring (anthocyanidin), sugars, and may contain acylating groups (Bueno *et al*., 2012). From the several anthocyanidins found in nature, only cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin (Fig. 1) are of importance in human nutrition (Harborne, 1998; Jaganath & Crozier, 2010; Bueno *et al*., 2012). Food ANCs play important roles in preventing various diseases including cancer, diabetes, cardiovascular diseases, and obesity. They are also associated with improving immunity and night vision, retarding aging and reducing the risk of degenerative disorders (Jing, 2006; Nikkhah *et al*., 2008). These beneficial effects of ANCs are attributed to their antioxidant, detoxification, anti-proliferation, anti-angiogenic, and anti-inflammatory activities (Miguel, 2011).

Upon seasonal harvest, haskap berries are frozen to be used all over the year for consumption or processing. The most economically-important haskap products are the juice and pressed berries, which are a by-product from

juice extraction and subsequently sold as a dried berry product. Despite the varied functions and health benefits of ANCs, they are very labile and undergo significant breakage and structural changes during storage and processing (Ochoa *et al*., 1999; Lohachoompol *et al*., 2004; Sadilova *et al*., 2006). Due to their highly reactive nature, ANCs readily degrade to colorless or brown compounds. Loss of ANCs is also accelerated by the presence of oxygen and enzymes, and during the high temperature processing (Jackman *et al*., 1987).



Anthocyanin structure



Flavylium cation

Anthocyanidin	R_1	R_2	Color
Peralgonidin	H	H	Orange
Cyanidin	OH	H	Orange-red
Delphinidin	OH	OH	Bluish-red
Peonidin	OCH_3	H	Orange-red
Petunidin	OCH_3	OH	Bluish-red
Malvidin	OCH_3	OCH_3	Bluish-red

Figure 1. Structures of major ANCs (adapted from Jing, 2006)

The effect of storage, thawing, extraction and drying conditions on the retention of haskap berry ANCs has been investigated in our laboratory. The total anthocyanin content (TAC) was reduced in different varieties by 39.31–59.24 and 36.87–56.57 % upon frozen storage for 6 months at-18 ℃ and-32 ℃, respectively (Khattab *et al*., 2015a). The reduction in TAC was 32.14–53.25, 28.55–51.45 and 18.92–47.22 % when the frozen fruits were thawed at room temperature (25±2 ℃), fridge (4 ℃) and in the microwave oven, respectively (Khattab *et al*., 2015b). In a recent study (Khattab *et al*., 2016a), we have investigated the effect of juice extraction and drying conditions on the stability of haskap berry ANCs. The juice extraction process significantly reduced the TAC by 48.18 % in the pressed residues as compared to that of the whole berries. Furthermore, the TAC decreased significantly at different drying temperatures with a strong positive correlation between the drying temperature and the degradation rate.

Our previous studies on haskap berries did not report on the levels of individual ANCs during storage and processing. It is important to determine the anthocyanin profiles for different haskap berry products and the effect of storage and processing conditions, as this will help develop strategies to improve the nutritional content of these products. Therefore, this study examines the effect of the processing chain including freezing, frozen storage, thawing, juice extraction, and drying conditions on the stability and retention of specific individual ANCs contained in haskap berry fractions.

2. Materials and Methods

2.1 Materials

2.1.1 Haskap Berries

Haskap berries (*Lonicera caerulea* L.); variety *Indigo Gem* (26 kilograms) were obtained from LaHave Natural Farms, Blockhouse, Nova Scotia, Canada. Upon receiving the fruits, they were analyzed for their anthocyanin profile. The fruits were then frozen and stored at –18 ℃. Half the berries were thawed and analyzed the next day to study the effect of the freezing process. The other half was kept frozen for 6 months to study the effect of frozen storage, thawing methods and drying conditions.

2.1.2 Chemicals and Phenolic Standards

All chemicals used for this research were of analytical and HPLC grades and were procured from Sigma Aldrich (Oakville, Ontario, Canada) and Fisher Scientific (Ottawa, Ontario, Canada). Authentic anthocyanin standards were obtained from Sigma Aldrich, Canada.

2.2 Experimental Procedures

2.2.1 Fruit Pressing and Juice Extraction

The juice extraction was carried out as previously described (Khattab *et al*., 2016b), using a lab-scale manual multi-fruit juice extractor (F. Dick 9060600, 6L; Friedrich DICK, Deizisau, Germany). In this study (Fig. 2) the frozen berries were allowed to thaw at different conditions including room temperature (25±2 °C for 12 h), and in the refrigerator (4 °C for 22 h) and microwave oven (1000 Watts for 0.29 h) according to Khattab *et al*. (2015b). The berries thawed at room temperature were loaded to the juice extractor (in 3 kg batches) and manually pressed. The pressing was done until obtaining 70 % of the original fruit weight as juice. The pressed berries were osmotically treated by mixing with sucrose (20 % of their weight), and left to infuse for 24 h. The mixture was then gently pressed using the juice extractor to drain the liquid part (syrup) without drastically affecting or rupturing the berries. The leftover berries were analyzed and dried.

2.2.2 Drying Process

The drying process was carried out according to Khattab *et al*. (2016a) using a lab-scale hot-air drying oven (Isotemp® Oven, Model 630F, Fisher Scientific, USA) at 60, 100, and 140 ℃. The drying continued up to 48 h and the time needed to reach 25 % moisture content was recorded.

2.2.3 Moisture Content

Moisture content of the haskap berry samples was determined using a hot-air drying oven (Isotemp® 630F, Fisher Scientific, USA) at 103 ± 2 ºC and atmospheric pressure until constant mass was reached (ISO, 2009).

2.2.4 HPLC Analysis of Anthocyanins from Haskap Berries

Samples were extracted with 80 % acidified methanol and prepared for HPLC analysis according to Khattab *et al*. (2015a). The anthocyanin profiles of fresh, frozen, pressed, and dried haskap berries were analyzed according to Khattab *et al*. (2015c) using the reversed-phase DAD-HPLC (Agilent 1100 Series, Agilent Technologies, Hewlett-Packard, Waldbronn, Germany). Chromatograms were acquired at 520 nm and data were analyzed using the Agilent ChemStation software (version A10.02).

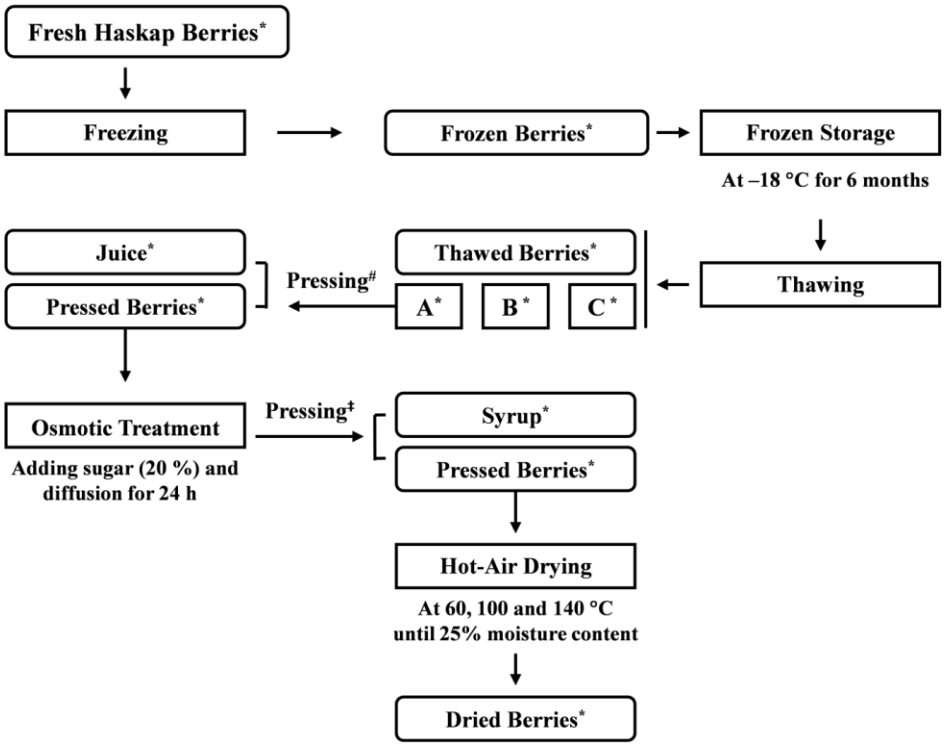


Figure 2. Schematic flowchart of the experimental work. Method of thawing is represented by A: Room temperature; B: Fridge temperature, C: Microwave oven. # Pressing until 70 % juice yield; ‡ Pressing to drain the remaining liquid; * HPLC profiling was conducted for these samples

2.2.5 Statistical Analysis

All experiments were done in triplicates and data were analyzed using a one factor analysis of variance (ANOVA). Tukey-Kramer mean separation tests were done for multiple comparisons with SigmaStat software (version 3.5). The significance was accepted at $p \leq 0.05$.

3. Results and Discussion

3.1 HPLC Profiling of Anthocyanins from Fresh Haskap Berries

The chromatogram of haskap berry extract from fresh haskap berries is illustrated in Fig. 3. The contents of the identified ANCs are shown in Table 1.

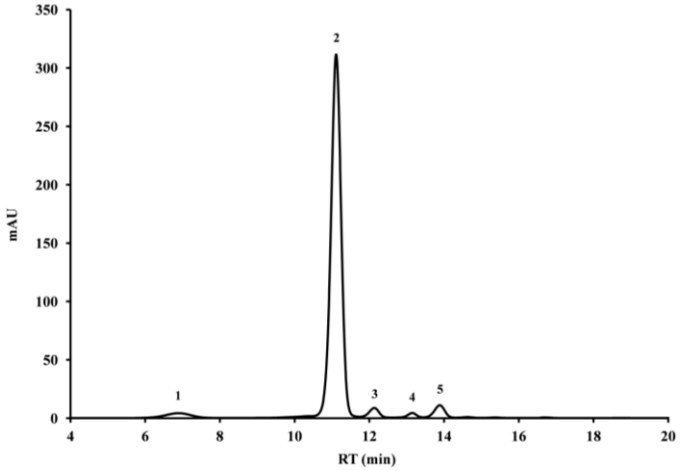


Figure 3. HPLC chromatogram of fresh haskap berries at 520 nm. 1: cyanidin 3,5-di-glucoside; 2: cyanidin 3-glucoside; 3: cyanidin 3-rutinoside; 4: pelargonidin 3-glucoside; 5: peonidin 3-*O*-glucoside

The TAC of the fresh fruit was 7.26 ± 0.24 mg/g FW. Five ANCs were identified in the whole fruit including cyanidin 3,5-di-glucoside (4.27 % of the TAC), cyanidin 3-glucoside or C-3-G (89.39 %), cyanidin 3-rutinoside (2.07 %), pelargonidin 3-glucoside (0.83 %), and peonidin 3-*O*-glucoside (3.44 %). The chemical structures of these ANCs are shown in Fig 4. These results are in agreement with those reported by Chaovanalikit *et al*. (2004) and Andersen & Jordheim (2007) where C-3-G was reported to dominate in berries of most *Lonicera* species. The distribution of ANCs in haskap berries was reported to be C-3-G (79–88 %), cyanidin-3-rutinoside (1–11 %), cyanidin-3,5-diglucoside (2.2–6.4 %), peonidin 3-*O*-glucoside (2.8–4.5 %), peonidin 3-rutinoside (0.3–1.3 %) and pelargonidin 3-glucoside (0.2–1.0 %) (Chaovanalikit *et al.,* 2004).

3.2 Effect of Freezing and Frozen Storage on the Anthocyanin Profile of Haskap Berries

The results of the present study (Table 1) indicate that the freezing process had no significant effect on the content of individual ANCs of haskap berries. The total content of all ANCs was reduced by only 1.79 % as compared to the fresh fruit. In the food industry, a storage temperature of –18 ℃ effectively reduces the chemical and biological spoilage of foods and extends their shelf life. However, freezing causes cell rupture and division allowing reactions between enzymes and their substrates (Tomás-Barberán & Espín, 2001).

Table 1. Effect of freezing and frozen storage (-18 °C for six months) on the anthocyanin profile of haskap berries

	Fresh	Frozen	Frozen stored	Reduction upon frozen storage (%)
ANC_1	0.31 ± 0.00^a	0.30 ± 0.00^a	0.25 ± 0.01^b	19.35
ANC_2	6.49 ± 0.23^a	6.37 ± 0.20^a	4.90 ± 0.08^b	24.50
ANC_3	0.15 ± 0.00^a	0.15 ± 0.00^a	0.12 ± 0.00^b	20.00
ANC_4	0.06 ± 0.00^a	0.07 ± 0.00^a	0.05 ± 0.00^b	16.67
ANC_5	0.25 ± 0.01^a	0.24 ± 0.00^a	0.21 ± 0.01^b	16.00

ANC_1: Cyanidin 3,5-di-glucoside; ANC_2: Cyanidin 3-glucoside; ANC_3: Cyanidin 3-rutinoside; ANC_4: Pelargonidin 3-glucoside; ANC_5: Peonidin 3-*O*-glucoside. Values are means of duplicate analyses ± standard deviation (SD). Values in the same row with similar superscript letters are not significantly different ($\rho \leq 0.05$).

Therefore, ANCs may be degraded during freezing and more extensively during thawing due to their interaction with oxidative enzymes. The effects of freezing and frozen storage on anthocyanin content and phenolic profile of different kinds of berries have been investigated by other researchers (Bushway *et al*., 1992; de Ancos *et al*., 2000; Häkkinen *et al*., 2000; Mullen *et al*., 2002). According to Selman (1992), the process of freezing itself does not alter the nutritive value of the product being frozen. Upon freezing raspberries at –30 ℃ within 3 hours of picking, Mullen *et al*. (2002) found no significant differences either in the levels of the individual ANCs or in the TAC of the fresh and frozen raspberries. This might be because ANCs in frozen fruits become more easily extractable due to degradation of cell structures during frozen storage over time. The enhanced extractability might have surmounted any ANCs degradation that might have occurred during the freezing process. In some cases, ANCs have been even reported to increase during freezing (de Ancos *et al*., 2000).

The effect of frozen storage at –18 ℃ for 6 months on the individual ANCs from haskap berries is shown in Table 1. The content of the five ANCs significantly decreased upon storage. The highest reduction (24.50 %) was recorded for C-3-G (the most abundant ANC), while the smallest decrease was that of peonidin 3-*O*-glucoside (16.00 %). This agrees with de Ancos *et al*. (2000) who found that C-3-G demonstrated a more significant degradation during frozen storage compared to the other ANCs found in raspberries. Structural analysis showed that less free hydroxyl groups and more methoxy groups in the B-ring of the aglycon improve anthocyanin stability (Liu *et al*., 2014).

Our results showed that cyanidins (C-3-G, cyanidin 3,5-diglucoside, and cyanidin 3-rutinoside) with two OH groups in the B-ring exhibited lower stability than pelargonidin 3-glucoside with one OH group. Peonidin 3-*O*-glucoside, with one hydroxyl group (OH) and one methoxy group (OCH_3) showed the highest stability among the five ANCs investigated. These results agree with other investigators. Chaovanalikit and Wrolstad (2004) reported that frozen storage at –23 °C for 6 months caused more than 75 % degradation in the ANC content of cherries, while storage at –70 °C resulted in better stability. The effect of frozen storage at –25 ℃ on the stability of individual ANCs from pomegranate juice was investigated by Mirsaeedghazi *et al*. (2014). The 5 major ANCs; C-3-G, cyanidin 3,5-diglucoside, delphinidin 3-glucoside, pelargonidin 3-glucoside and pelargonidin 3,5-diglucoside were decreased by 4.8, 3.5, 4.6, 6.0 and 3.4 %, respectively after 20 days of storage.

3.3 Effect of Thawing Conditions on the Anthocyanin Profile of Haskap Berries

Freezing techniques affect how the food thaws and its subsequent structural and compositional changes. Quick freezing retains cell integrity than slower freezing due to the smaller intracellular ice crystals formed. Cell integrity is further influenced by thawing regimes as quick thawing better retains fruit quality (Delgado & Rubiolo, 2005). The freezing and thawing chain ruptures the cells allowing reactions between enzymes and their substrates. Anthocyanins, therefore, may degrade during thawing due to their interaction with oxidative enzymes like polyphenol oxidases and peroxidases that have been reported to be active even at lower temperatures (Chisari *et al*., 2007).

In the present study, we compared the effect of thawing methods (room temperature, refrigerator, and microwave oven) on the anthocyanin profile of frozen-stored haskap berries. The content of individual ANCs of haskap berries as affected by thawing methods is shown in Table 2. All thawing methods reduced the ANCs of haskap berries with significant differences among them. In agreement with the present study, the quality of frozen food has been reported as being more affected by the thawing process than by the freezing itself (Kim *et al*., 2011). Thawing has a major impact on the food quality, as the compounds normally kept apart in the intact cell can mix and react with each other (Kmiecik *et al*. 1995).

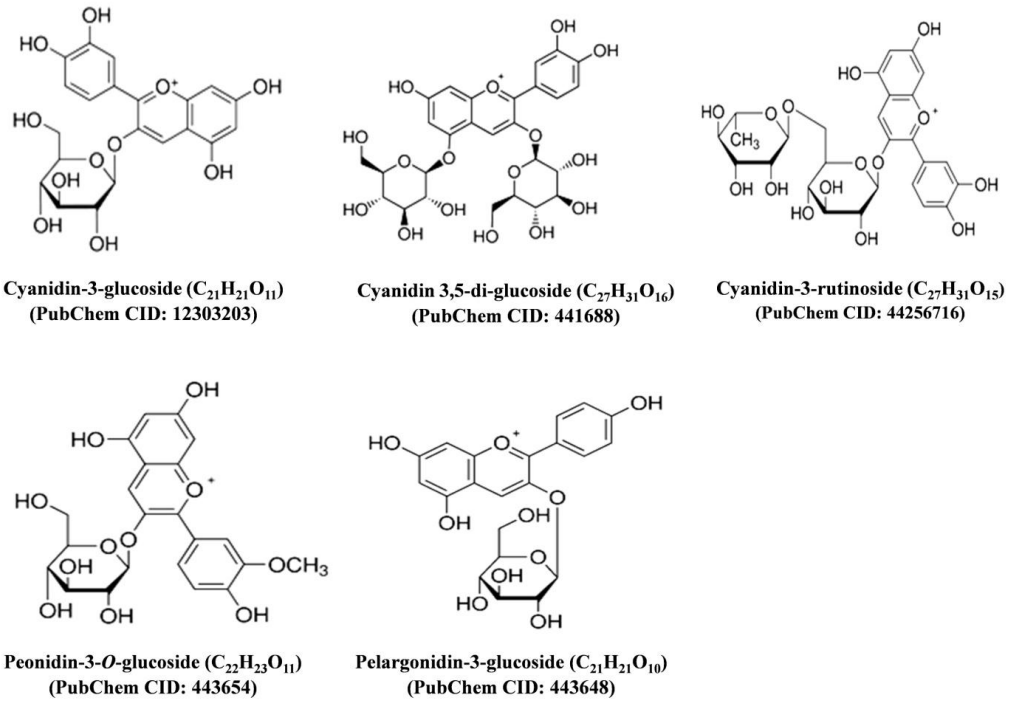


Figure 4. Chemical structures of haskap berry ANCs identified in this study

The reductions in Table 2 may be attributed to the hydrolytic reactions that convert anthocyanin glycosides to chalcones, which intuitively degrade into aldehydes and phenolic acids (Kamiloglu *et al*., 2015). It is also possible that enzymes might have played a role in the reduction of ANCs (Howard *et al*., 2010). The highest reduction occurred when thawing at room temperature followed by that in the refrigerator, while microwave thawing caused the least reduction. The higher ANCs retention during the microwave thawing might be attributed to the shorter time taken (17 min) as compared to 12 and 22 h in the room temperature and refrigerator thawing, respectively. Using microwave, thawing time was reduced by seven times compared to convective thawing at atmospheric temperature when appropriate conditions were used (Tong *et al*., 1993). Thawing at lower temperature (refrigerator), despite taking significantly longer time, retained more ANCs compared to room temperature thawing.

These results agree with Oszmiański *et al*. (2009) where considerable ANC losses were reported after thawing strawberries stored frozen for several months. Moreover, the ANC contents of frozen fruits were found to depend on their thawing methods. The differences of ANC contents between bilberries thawed at 2-4 ℃ and fruit thawed at room temperature (18-20 ℃) were approximately 10% (Kmiecik *et al*., 1995).

Table 2. Effect of thawing conditions on the content of individual anthocyanins (mg/g) in haskap berries

	Frozen-Stored berries	Thawed berries		
		Room temperature	Refrigerator temperature	Microwave oven
ANC[1]	0.25 ± 0.01^{a}	0.19 ± 0.00^{b}	0.21 ± 0.00^{b}	0.24 ± 0.00^{a}
ANC_2	4.90 ± 0.08^{a}	3.58 ± 0.00^{d}	4.08 ± 0.00^{c}	4.31 ± 0.29^{b}
ANC_3	0.12 ± 0.00^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.04^{a}
ANC_4	0.05 ± 0.00^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.08^{a}
ANC_5	0.21 ± 0.01^{a}	0.17 ± 0.01^{b}	0.19 ± 0.01^{a}	0.21 ± 0.08^{a}

ANC_1: Cyanidin 3,5-di-glucoside; ANC_2: Cyanidin 3-glucoside; ANC_3: Cyanidin 3-rutinoside; ANC_4: Pelargonidin 3-glucoside; ANC_5: Peonidin 3-*O*-glucoside. Values are means of duplicate analysis ± standard deviation (SD). Values in the same row with similar superscript letters are not significantly different ($\rho \leq 0.05$).

Our results showed that the reductions in individual ANCs were 19.05-26.94, 0.00-16.73 and 0.00-12.04 % after thawing at room, and using the refrigerator and microwave oven, respectively. A similar reduction trend was seen

as that observed for berries in frozen storage where C-3-G showed the highest reduction while the lowest reductions were noticed in peonidin 3-*O*-glucoside and pelargonidin 3-glucoside. This is supported by other studies that report that C-3-G is one of the most reactive ANCs during processing (Rommel *et al*., 1990; Boyles *et al*., 1993; Garcia-Viguera *et al*., 1998). Furthermore, Fleschhut *et al*. (2006) reported that an increase in hydroxyl groups in the B-ring of the anthocyanin nucleus results in reduced stability. They found that cyanins seemed to be less stable than petunins and peonidins indicating that methylation of hydroxyl groups in B-ring increases the stability of ANCs.

3.4 Effect of Juice Extraction on the Anthocyanin Profile of Haskap Berries

The results of the present study (Table 3) indicate that both juice and syrup showed significantly lower ANC content (1.61 and 1.00 mg/g FW, respectively) than that of the thawed berries (4.07 mg/g FW). The syrup showed significantly lower content than that found for the juice fraction, which might be attributed to high extractability of these water soluble compounds. These results are not surprising as it is known that ANCs suffer significant degradation and structural changes during processing (Sadilova *et al*., 2006). Extraction of fruit juice causes major ANC losses yielding significantly lower ANC contents in the obtained juice as compared to the corresponding fruit used.

Table 3. Effect of juice extraction (until 70 % juice yield) on the content of individual anthocyanins (mg/g) in haskap berry products

	Thawed berries*	Juice	Syrup	Pressed berries	
				FW	DW
ANC_1	0.19 ± 0.00^a	0.07 ± 0.00^b	0.04 ± 0.01^c	0.19 ± 0.01^a	0.66 ± 0.01
ANC_2	3.58 ± 0.00^a	1.43 ± 0.20^b	0.89 ± 0.08^c	3.97 ± 0.08^a	13.49 ± 0.18
ANC_3	0.09 ± 0.01^a	0.04 ± 0.00^b	0.02 ± 0.00^b	0.12 ± 0.00^a	0.37 ± 0.00
ANC_4	0.04 ± 0.01^a	ND	ND	ND	ND
ANC_5	0.17 ± 0.01^a	0.07 ± 0.00^b	0.05 ± 0.01^b	0.19 ± 0.01^a	0.65 ± 0.01

ANC_1: Cyanidin 3,5-di-glucoside; ANC_2: Cyanidin 3-glucoside; ANC_3: Cyanidin 3-rutinoside; ANC_4: Pelargonidin 3-glucoside; ANC_5: Peonidin 3-*O*-glucoside; FW: fresh weight; DW: dry weight. Values are means of duplicate analysis ± standard deviation (SD). * Thawed at room temperature. ** The content of the five ANCs of the thawed berries were 1.21, 25.91, 0.71, 0.28 and 1.08, respectively based on the DW base. Values in the same row with similar superscript letters are not significantly different ($\rho \leq 0.05$).

The content of ANCs in the juices affects their storage stability and shelf life, for example, only 11–15% of the original ANCs were detected in the commercial juices at their expiry date, after storage for 35–49 weeks at room temperature (Hellstrom *et al*., 2013). It is known that manufacturing processes lead to anthocyanin degradation and color alteration in berries (Hager *et al*., 2008). Hellstrom *et al*. (2013) attributed the lower anthocyanin content in commercial berry juices to the severe production processes applied industrially. Furthermore, processing blueberries into purees caused 43 % loss in total ANCs, compared to the levels in fresh fruit (Brownmiller *et al*., 2008).

In other studies, it was found that the stability of individual ANCs in food systems depended greatly on their chemical structure (Jackman *et al*., 1987) and different ANCs had different degradation kinetics in juices (Hellstrom *et al*., 2013). Hydroxyl, methoxyl, sugar, and acylated sugar substituent groups have pronounced effects on the stability of the ACNs. Diglycosidic substitution gives more stability than monoglycosidic substitution (Mazza & Miniati 1993). Moreover, acylation of the ANC molecule improves its stability by preventing it from hydration (Brouillard, 1981). Pelargonidin 3-glucoside totally degraded and was not detected in the juice, syrup or pressed berries. This is might be due to its marginal content in the initial thawed berries. For the other ANCs, the same reduction trend was observed as seen during the storage and thawing where C-3-G suffered the highest reduction (47.94 %) followed by cyanidin 3-rutinoside (47.89 %) and cyanidin 3,5-di-glucoside (45.45 %). The least reduction, however, was observed for peonidin 3-*O*-glucoside (39.81 %).

3.5. Effect of Drying Conditions on the Anthocyanin Profile of Haskap Berries

The effect of hot-air drying at different temperatures is shown in Table 4. The drying time taken to reach a moisture content of 25 % was 16.0, 5.6 and 2.5 h at 60, 100 and 140 °C, respectively. Upon drying to this moisture content, the reductions in the individual ANCs of the dried berries were 73.85-79.99, 78.46-82.73 and 90.77-100.00 % at the three drying temperatures, respectively. The HPLC chromatograms of pressed and dried haskap berries at different temperatures are illustrated in Fig. 5. The TAC decreased by 71.85 and 88.30 % after 8 h

of drying at 60 and 100 °C, respectively. Even at lower temperature (60 °C), the degradation of ANCs continued with drying time to reach more than 95.00 % after 48 h where all ANCs were significantly degraded with pelargonidin 3-glucoside, cyanidin 3,5-di-glucoside and C-3-G being the most affected. However, ANCs were completely degraded after 32 h of drying at 100 °C.

Table 4. Anthocyanin profile of haskap berries dried at 60, 100 and 140 ℃ to 25 % moisture content

	Pressed berries	Dried berries		
		60 ℃	100 ℃	140 ℃
ANC_1	0.63 ± 0.01^a	0.15 ± 0.00^b	0.12 ± 0.00^c	ND
ANC_2	13.49 ± 0.18^a	2.70 ± 0.06^b	2.33 ± 0.09^c	0.62 ± 0.03^d
ANC_3	0.40 ± 0.00^a	0.09 ± 0.01^b	0.07 ± 0.01^b	0.02 ± 0.04^c
ANC_4	ND	ND	ND	ND
ANC_5	0.65 ± 0.01^a	0.17 ± 0.01^b	0.14 ± 0.01^b	0.06 ± 0.08^c

ANC_1: Cyanidin 3,5-di-glucoside; ANC_2: Cyanidin 3-glucoside; ANC_3: Cyanidin 3-rutinoside; ANC_4: Pelargonidin 3-glucoside; ANC_5: Peonidin 3-*O*-glucoside; ND: not detected; Values are means of duplicate analysis ± standard deviation (SD). Values in the same row with similar superscript letters are not significantly different ($\rho \leq 0.05$).

Logarithmic anthocyanin degradation with an arithmetic increase in temperature has been frequently reported (Drdak & Daucik, 1999; Rhim, 2002). The high temperatures blanching (95 ℃ for 3 min in combination with pasteurisation) involved in processing blueberries into purees resulted in 43 % loss in total ANCs (Brownmiller *et al*., 2008). In addition, ANCs were significantly decreased as a result of jam and marmalade processing of black carrots (Kamiloglu *et al*., 2015). After 20 weeks of jam and marmalade storage, ANCs were significantly higher at 4 ℃ that at 25 ℃.

Drying at 140 °C (Table 4), however, had a significant effect on the ANCs. Both pelargonidin 3-glucoside and cyanidin 3,5-diglucoside were completely degraded upon drying to 25% moisture content (2.5 h). The other ANCs were reduced by 90.77 to 95.40 % with C-3-G and peonidin 3-*O*-glucoside being the highest and least degraded ones, respectively. After 5 h of drying at this temperature, only C-3-G was detected in the samples but no other ANCs. These results agree with Drdak and Daucik (1999) and Rhim (2002). Garcia-Viguera *et al*. (1999) reported anthocyanin losses of 10 to 80 % during jam processing. Moreover, C-3-G and pelargonidin 3-glucoside in blackberry and strawberry puree were significantly reduced by thermal treatment at 70 °C for 2 min (Patras *et al*., 2009). Furthermore, the content of total ANCs of dehydrated potato flakes decreased by 23-45 % during the dehydration process at 100-150 ℃ (Nayak, 2011).

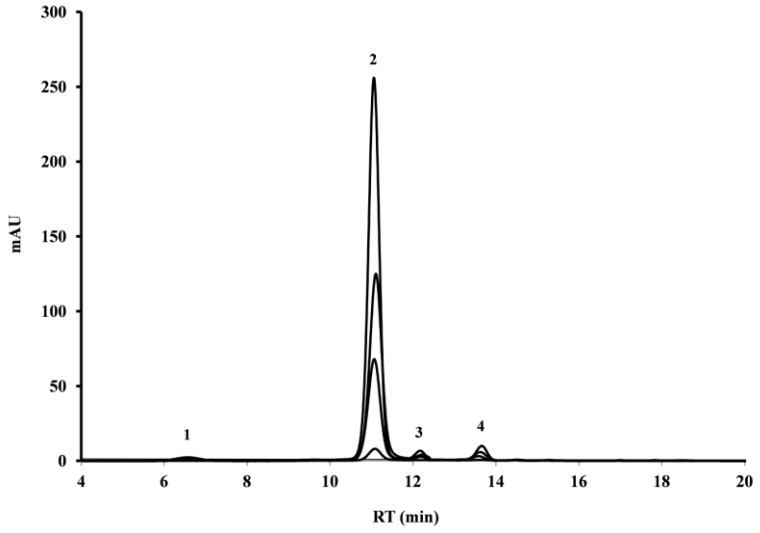


Figure 5. HPLC chromatograms of pressed and dried haskap berries (at different drying temperatures until 25 % moisture content). Chromatograms from top to bottom: pressed berries; dried berries at 60 ℃; dried berries at 100 ℃; dried berries at 140 ℃. 1: cyanidin 3,5-di-glucoside; 2: cyanidin 3-glucoside; 3: cyanidin 3-rutinoside; 4: peonidin 3-*O*-glucoside

The reduction of ANCs upon drying might be attributed to the heat labile nature of ANCs. The exposure to oxygen at higher temperatures might have also contributed to their degradation (Welcha *et al*., 2008). Sadilova *et al*. (2006) observed that only 50 % of ANCs were retained after heating elderberry for 3 h at 95 °C. Similar losses in raspberry purees were reported by Ochoa *et al*. (1999). This reduction might also be attributed to the osmotic treatment during which more ANCs might have leached out into the osmotic solution. Lohachoompol *et al*. (2004) found that the reduction in the TAC was 41 % in the dried blueberries and increased to 49 % when drying was preceded with osmotic treatment. The loss of ANCs was further attributed to several factors, including residual enzyme activity or condensation reactions of ANCs with other phenolics at higher temperatures (Jackman *et al*., 1987; Brownmiller *et al*., 2008). Fracassetti *et al*. (2013) found that storage of freeze-dried wild blueberry powder for 49 days at 25, 42, 60, and 80 ℃ reduced single and total ANCs at all temperatures. The reduction in ANCs depended on the temperature and occurred slowly up to 3% at day 14 at 25 and 42 ℃, whereas it was faster, reaching 60 and 85 % after three days at 60 and 80 ℃, respectively.

The stability of ANCs is influenced by the aglycon B-ring substituents and the presence of additional hydroxyl groups decreases the aglycon stability in neutral media. Furthermore, mono- and diglycosides derivatives are more stable than the non-glycosylated aglycons (Castañeda-Ovando *et al*., 2009). Our results showed that among the three cyanidins investigated in this study, cyanidin 3,5-diglucoside (with two sugar moieties) showed the highest stability followed by cyanidin 3-rutinoside and C-3-G.

The reductions in individual ANCs were 73.85-76.19, 78.46-80.95 and 90.77-95.40 % upon drying to 25 % moisture content at 60, 100 and 140 ℃, respectively. This excludes pelargonidin 3-glucoside (the least abundant anthocyanin in haskap berries) which was completely degraded at both 100 and 140 ℃. Cyanidin 3,5-diglucoside also disappeared in the samples dried at 140 ℃. Peonidin 3-*O*-glucoside, however, was the most stable anthocyanin at different temperatures. Drying haskap berries at 60 ℃ is recommended for better retention of ANCs. Drying at this temperature was recommended by Garba and Kaur (2014) who investigated the influence of hot air drying (40-60 ℃) on the TAC of black carrot and found that the optimum retention of ANCs was attained from drying at 60 ℃. In the study by Zoric *et al*. (2014), the effect of heating temperature (80-120 ℃) and processing time (5-50 min) on the stability of ANCs in freeze-dried sour cherry pastes was explored. They found that C-3-G was the most unstable among ANCs.

4. Conclusion

ANCs were significantly affected by frozen storage and subsequent thawing by different methods. Microwave thawing revealed the highest ANC retention. Drying significantly reduced ANCs and higher drying temperatures resulted in higher degradation. All ANCs were significantly reduced during the frozen storage, thawing, juice extraction and drying. Cyanidins were more degradable than pelargonidin than peonidin. Microwave thawing and lower-temperature storage and processing are recommended for better retention of haskap berry ANCs. Understanding the structure-stability relations and behavior of ANCs during storage and processing will help haskap and other berry processors to design high-quality berry products with improved nutritional/functional properties.

Acknowledgements

This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC). LaHave Natural Farms, Blockhouse, Nova Scotia, Canada is sincerely acknowledged for providing the haskap berries used in this study.

References

Andersen, M., & Jordheim, M. (2007). *The Anthocyanins*. *Chemistry, Biochemistry and Applications*, 4th ed., *CRC Press*: Boca Raton, FL, USA, 471-553.

Bakowska, A., Marianchuk, M., & Kolodziejczyk, P. (2007). Survey of bioactive components in Western Canadian berries. *Canadian Journal of Physiology and Pharmacology, 85,* 1139-1152.

Bors, B., Thomson, J., Sawchuk, E., Reimer, P., Sawatzky, R., & Sander, T. (2012). Haskap breeding and production-final report (pp. 1–142). Saskatchewan Agriculture: Regina.

Boyles, M. J., & Wrolstad, R. E. (1993). Anthocyanin composition of red raspberry juice: influence of cultivar, processing, and environmental factors. *Journal of Food Science, 58,* 1135-1141. http://dx.doi.org/10.1111/j.1365-2621.1993.tb06132.x

Brouillard, R. (1981). Origin of the exceptional color stability of the Zebrina anthocyanin. *Phytochemistry, 20,*

143-145. http://dx.doi.org/10.1016/0031-9422(81)85234-X

Brownmiller, C., Howard, L. R., Prior, R. L. (2008). Processing and storage effects on monomeric anthocyanins, percent polymeric colour, and antioxidant capacity of processed blueberry products. *Journal of Food Science*, H72-H79. http://dx.doi.org/10.1111/j.1750-3841.2008.00761.x

Bueno, J. M., Saez-Plaza, P., Ramos-Escudero, F., Jimenez, A. M., Fett, R. & Asuero, A. G. (2012). Analysis and antioxidant capacity of anthocyanin pigments. Part II: chemical structure, color, and intake of anthocyanins. *Critical Reviews in Analytical Chemistry, 42*, 126-151. http://dx.doi.org/10.1080/10408347.2011.632314

Bushway, A. A., Bushway, R. J., True, R. H., Work, T. M., Bergeron, D., Handley, D. T., & Perkins, L. B. (1992). Comparison of the physical, chemical and sensory characteristics of five rapsberry cultivars evaluated fresh and frozen. *Fruit Varieties Journal, 46*, 229-234.

Castañeda-Ovando, A., Pacheco-Hernández, M. L., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. (2009). Chemical studies of anthocyanins: a review. *Food Chemistry, 113*, 859-871. http://dx.doi.org/10.1016/j.foodchem.2008.09.001

Celli, G. B., Ghanem, A., & Brooks, M. S. (2014). Haskap Berries (*Lonicera caerulea* L.) - a Critical Review of Antioxidant Capacity and Health-Related Studies for Potential Value-Added Products. *Food and Bioprocess Technology, 7*, 1541-1554. http://dx.doi.org/10.1007/s11947-014-1301-2

Chaovanalikit, A, Thompson M. M., & Wrolstad, R. E (2004). Characterization and quantification of anthocyanins and polyphenolics in blue honeysuckle (*Lonicera caerulea* L.). *Journal of Agricultural & Food Chemistry, 52*, 848-852. http://dx.doi.org/10.1021/jf030509o

Chisari, M., Barbagallo, R. N., & Spagna, G. (2007). Characterisation of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry fruit. *Journal of Agricultural and Food Chemistry, 55*, 3469-3476. http://dx.doi.org/10.1021/jf063402k

De Ancos, B., Ibanez, E., Reglero, G., & Cano, M. P. (2000). Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit. *Journal of Agricultural and Food Chemistry, 48*, 873-879. http://dx.doi.org/10.1021/jf990747c

Delgado, A. E., & Rubiolo, A. C. (2005). Microstructural changes in strawberry after freezing and thawing processes. *Lebensmittelwissenschaft und -Technologie, 38*, 135-142. http://dx.doi.org/10.1016/j.lwt.2004.04.015

Drdak, M., & Daucik, P. (1999). Changes of elderberry (*Sambucus nigra*) pigments during the production of pigment concentrates. *Acta Alimentaria, 19*, 3-7.

Fan, Z., Wang, Z., & Liu, J. (2011). Cold-field fruit extracts exert different antioxidant and antiproliferative activities in vitro. *Food Chemistry, 129*, 402-407. http://dx.doi.org/10.1016/j.foodchem.2011.04.091

Fleschhut, J., Kratzer, F., Rechkemmer, G., & Kulling, S. (2006). Stability and biotransformation of various dietary anthocyanins *in vitro*. *European Journal of Nutrition, 45*, 7-18. http://dx.doi.org/10.1007/s00394-005-0557-8

Fracassetti, D., Del Bo, C., Simonetti, P., Gardana, C., Klimis-Zacas, D., & Ciappellano, S. (2013). Effect of Time and Storage Temperature on Anthocyanin Decay and Antioxidant Activity in Wild Blueberry (*Vaccinium angustifolium*) Powder. *Journal of Agricultural and Food Chemistry, 61*, 2999-3005. http://dx.doi.org/10.1021/jf3048884

Garba, U., & Kaur, S. (2014). Effect of drying and pretreatment on anthocyanins, flavenoids and ascorbic acid content of black carrot (*Daucus carrota* L.). *Journal of Global Biosciences, 3*, 772-777.

Garcia-Viguera, C., Zafrilla, P., Artés, F., Romero, F., Abellán, P., & Tomas-Barberan, F. A. (1998). Colour and anthocyanin stability of red raspberry jam. *Journal of the Science of Food and Agriculture, 78*, 565-573. http://dx.doi.org/10.1002/(SICI)1097-0010(199812)78:4<565::AID-JSFA154>3.0.CO;2-P

Garcia-Viguera, C., Zafrilla, P., Romero, P., Abellan, P., Artes, F., & Tomas-Barberan, F. A. (1999). Color stability of strawberry jam as affected by cultivar and storage temperature. *Journal of Food Science, 64*, 243-247. http://dx.doi.org/10.1111/j.1365-2621.1999.tb15874.x

Hager, T. J., Howard, L. R., & Prior, R. L. (2008). Processing and storage effects on monomeric ANCs, percent polymeric color, and antioxidant capacity of processed blackberry products. *Journal of Agricultural and Food Chemistry, 56*, 689-695. http://dx.doi.org/10.1021/jf071994g

Häkkinen, S. H., Kärenlampi, S. O., Mykkänen, H. M., & Törrönen, A. R. (2000). Influence of domestic processing and storage on flavonol contents in berries. *Journal of Agricultural and Food Chemistry, 48*, 2960-2965. http://dx.doi.org/10.1021/jf991274c

Harborne, J. B. (1998). Phenolic Compounds in Phytochemical Methods-a Guide to Modern Techniques of Plant Analysis, 3rd ed., *Chapman & Hall*, New York, pp. 66-74.

Hellstrom, J., Mattila, P., & Karjalainen, R. (2013). Stability of anthocyanins in berry juices stored at different temperatures. *Journal of Food Composition and Analysis, 31*, 12-19. http://dx.doi.org/10.1016/j.jfca.2013.02.010

Howard, L. R., Castrodale, C., Brownmiller, C., & Mauromoustakos, A. (2010). Jam processing and storage effects on blueberry polyphenolics and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 58*, 4022-4029. http://dx.doi.org/10.1021/jf902850h

International Organization of Standardization (ISO) (2009). Cereals and cereal products - determination of moisture content - reference method. ISO 712.

Jackman, R. L., Yada, R. Y., Tung, M. A., & Speers, R.A. (1987). Anthocyanins as food colorants-a review. *Journal of Food Biochemistry, 11*, 201-247. http://dx.doi.org/10.1111/j.1745-4514.1987.tb00123.x

Jaganath I. B. & Crozier A. (2010). Dietary flavonoids and phenolic compounds. In Plant Phenolics and Human Health: Biochemistry, Nutrition, and Pharmacology (edited by Cesar G. Fraga). *John Wiley & Sons, Inc*., Hoboken, New Jersey.

Jing, P. (2006). Purple corn anthocyanins: chemical structure, chemopreventive activity and structure/function relationships. PhD thesis; The Ohio State University, U.S.A., pp. 5-90.

Jurikova, T., Rop, O., Mlcek, J., Sochor, J., Balla, S., Szekeres, L., Hegedusova, A., Hubalek, J., Adam, V., & Kizek, R. (2012). Phenolic Profile of Edible Honeysuckle Berries (*Genus Lonicera*) and Their Biological Effects-a review. *Molecules, 17*, 61-79. http://dx.doi.org/10.3390/molecules17010061

Kamiloglu, S., Pasli, A. A., Ozcelik, B., Camp, J. V., & Capanoglu, E. (2015). Colour retention, anthocyanin stability and antioxidant capacity in black carrot (*Daucus carota*) jams and marmalades: Effect of processing, storage conditions and in vitro gastrointestinal digestion. *Journal of Functional Foods, 13*, 1-10. http://dx.doi.org/10.1016/j.jff.2014.12.021

Khattab, R., Celli, G. B., Ghanem, A., & Brooks, M. S. (2015a) Effect of frozen storage on polyphenol content and antioxidant activity of haskap berries (*Lonicera caerulea* L.). *Journal of Berry Research, 5*, 231-242. http://dx.doi.org/10.3233/JBR-150105

Khattab, R., Celli, G. B., Ghanem, A., & Brooks, M. S. (2015b). Effect of thawing conditions on polyphenol content and antioxidant activity of frozen haskap berries (*Lonicera caerulea* L.). *Current Nutrition & Food Science, 11*, 223-230. http://dx.doi.org/10.2174/1573401311666150519235845

Khattab, R., Brooks, M. S., & Ghanem, A. (2015c). Phenolic analyses of haskap berries (*Lonicera caerulea* L.): spectrophotometry versus high performance liquid chromatography. *International Journal of Food Properties, 19*, 1708-1725. http://dx.doi.org/10.1080/10942912.2015.1084316

Khattab, R., Ghanem, A., & Brooks, M. S. (2016a). Quality of Dried Haskap Berries (*Lonicera caerulea* L.) as Affected by Prior Juice Extraction, Osmotic Treatment and Drying Conditions. *Drying Technology*, http://dx.doi.org/10.1080/07373937.2016.1175472

Khattab, R., Ghanem, A., & Brooks, M. S. (2016b). Effect of Juice Extraction Methods on the Physicochemical Characteristics of Haskap Berry (*Lonicera caerulea* L.) Products. *Current Nutrition & Food Science, 12*, 220-229. http://dx.doi.org/10.2174/1573401312666160608122248

Kim, T. H., Choi, J. H., Choi, Y. S., Kim, H. Y., Kim, S. Y., Kim, H. W., & Kim, C. J. (2011). Physicochemical properties of thawed chicken breast as affected by microwave power levels. *Food Science and Biotechnology, 20*, 971-977. http://dx.doi.org/10.1007/s10068-011-0134-2

Kmiecik, W., Jaworska, G., & Budnik A. (1995). Influence of thawing methods of frozen berries fruits on their quality. *Rocz. PZH* 46, 2, 135-143 [in Polish].

Liu, Y., Zhang, D., Wu, Y., Wang, D., Wei, Y., Wu, J., & Ji, B. (2014). Stability and absorption of anthocyanins from blueberries subjected to a simulated digestion process. *International Journal of Food Science & Nutrition, 65*, 440-448. http://dx.doi.org/10.3109/09637486.2013.869798

Lohachoompol, V., Srzednicki, G., & Craske, J. (2004). The change of total ANCs in blueberries and their antioxidant effect after drying and freezing. *Journal of Biomedicine & Biotechnology, 5*, 248-252. http://dx.doi.org/10.1155/S1110724304406123

Mazza, G. J. (2007). Anthocyanins and heart health. *Annali dell'Istituto Superiore di Sanità, 43*, 369-374.

Mazza, G., & Miniati, E. (1993). Anthocyanins in Fruits, Vegetables and Grains. Boca Raton, FL: CRC Press, Inc.

Miguel, M. G. (2011). Anthocyanins: Antioxidant and/or anti-inflammatory activities. *Journal of Applied Pharmaceutical Science, 1*, 7-15.

Mirsaeedghazi, H., Emam-Djomeh, Z., & Ahmadkhaniha, R. (2014). The effect of frozen storage on the anthocyanins and polyphenolic components of pomegranate juice. *Journal of Food Science and Technology, 51*, 382-386. http://dx.doi.org/10.1007/s13197-011-0504-z

Mullen, W., Stewart, A. J., Lean, M. E. J., Gardner, P., Duthie, G. G., & Crozier, A. (2002). Effect of freezing and storage on the phenolics, ellagitannins, flavonoids, and antioxidant capacity of red raspberries. *Journal of Agricultural and Food Chemistry, 50*, 5197-5201. http://dx.doi.org/10.1021/jf020141f

Nayak, B. (2011). Effect of Thermal Processing on the Phenolic Antioxidants of Colored Potatoes. Academic dissertation, Department of Biological Systems Engineering, Washington State University.

Nikkhah, E., Khayami, M., & Heidari, R. (2008). In vitro screening for antioxidant activity and cancer suppressive effect on blackberry (*Morus nigra*). *Iranian Journal of Cancer Prevention, 1*, 167-172.

Ochoa, M. R., Kesseler, A. G., Vullioud, M. B., & Lozano, J. E. (1999). Physical and chemical characteristics of raspberry pulp: storage effect on composition and color. *LWT–Food Science and Technology, 149*, 149-153. http://dx.doi.org/10.1006/fstl.1998.0518

Oszmianski, J., Wojdylo, A., & Kolniak, J. (2009). Effect of L-ascorbic acid, sugar, pectin and freeze-thaw treatment on polyphenol content of frozen strawberries. *LWT - Food Science and Technology, 42*, 581-586. http://dx.doi.org/10.1016/j.lwt.2008.07.009

Paredes-Lopez, O., Cervantes-Ceja, M. L., Vigna-Perez, M., & Hernandez-Perez, T. (2010). Berries: improving human health and healthy aging, and promoting quality life-a review. *Plant Foods for Human Nutrition, 65*, 299-308. http://dx.doi.org/10.1007/s11130-010-0177-1

Patras, A., Brunton, N. P., Gormely, T. R., & Butler, F. (2009). Impact of high pressure processing on antioxidant activity, ascorbic acid, ANCs and instrumental colour of blackberry and strawberry puree. *Innovative Food Science and Emerging Technologies, 10*, 308-313. http://dx.doi.org/10.1016/j.ifset.2008.12.004

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Pedastsaar, P. & Mäesaar, M. (2013). The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite. *Food Control, 31*, 129-135. http://dx.doi.org/10.1016/j.foodcont.2012.10.007

Raudsepp, P., Anton, D., Roasto, M., Meremäe, K., Pedastsaar, P. & Mäesaar, M. (2013). The antioxidative and antimicrobial properties of the blue honeysuckle (*Lonicera caerulea* L.), Siberian rhubarb (*Rheum rhaponticum* L.) and some other plants, compared to ascorbic acid and sodium nitrite. *Food Control, 31*, 129-135. http://dx.doi.org/10.1016/j.foodcont.2012.10.007

Rein, M. (2005). Copigmentation Reactions and Color Stability of Berry Anthocyanins. Academic dissertation, Department of Applied Chemistry and Microbiology, University of Helsinki.

Rhim, J. W. (2002). Kinetics of thermal degradation of anthocyanin pigment solutions driven from red flower cabbage. *Food Science and Biotechnology, 11*, 361-364.

Rommel, A., Heatherbell, D. A., & Wrolstad, R. E. (1990). Red raspberry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. *Journal of Food Science, 55*, 1011-1017. http://dx.doi.org/10.1111/j.1365-2621.1990.tb01586.x

Rop, O., Řezníček, V., Mlček, J., Jurıková, T., Balık, J., & Sochor, J. (2011). Antioxidant and radical oxygen species scavenging activities of 12 cultivars of blue honeysuckle fruit. *Horticultural Science, 38*, 63-70.

Rupasinghe, H. P. V., Yu, L. J., Bhullar, K. S., & Bors, B. (2012). Haskap (Lonicera caerulea): A new berry crop with high antioxidant capacity. *Canadian Journal of Plant Science, 92*, 1311-1317. http://dx.doi.org/10.4141/cjps2012-073

Sadilova, E., Stintzing, F. C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated ANCs. *Journal of Food Science, 71*, C504-C512. http://dx.doi.org/10.1111/j.1750-3841.2006.00148.x

Selman, J. D. (1992). Vitamin retention during blanching of vegetables. Presented at the Royal Society of Chemistry Symposium 'Vitamin Retention in Cooking and Food Processing', 24 November, London, UK: 137-147.

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture, 81*, 853-876. http://dx.doi.org/10.1002/jsfa.885

Tong, C. H., Lentz, R. R., & Lund, D. B. (1993). A microwave oven with variable continuous power and a feedback temperature controller. *Biotechnology Progress, 9*, 488-496. http://dx.doi.org/10.1021/bp00023a600

Welcha, C. R., Wub, Q. & James E. Simonb, J. E. (2008). Recent Advances in Anthocyanin Analysis and Characterization. *Current Analytical Chemistry, 4*, 75-101. http://dx.doi.org/10.2174/157341108784587795

Zoric, Z., Dragovic-Uzelac, V., Pedisic, S., Kurtanjek, Z., Garofulic, I. E. (2014). Heat-Induced Degradation of Antioxidants in Marasca Paste. *Food Technology & Biotechnology, 52*, 101-108.