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Anthocyanin Stability in Food Products made with Freeze-Dried Blueberry Powder

Samantha R. Findley

University of Arkansas, Fayetteville, srfindley@gmail.com

Luke Howard

University of Arkansas, lukeh@uark.edu

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Cover Page Footnote

Samantha R. Findley, Student, Food Science and Human Nutrition & Hospitality Innovation dual-major, University of Arkansas, Luke R. Howard Faculty Mentor, Professor, Food Chemistry, Department of Food Science, University of Arkansas This research was funded by a University of Arkansas honors college student research grant.

Anthocyanin stability in food products made with freeze-dried blueberry powder

Meet the Student-Author

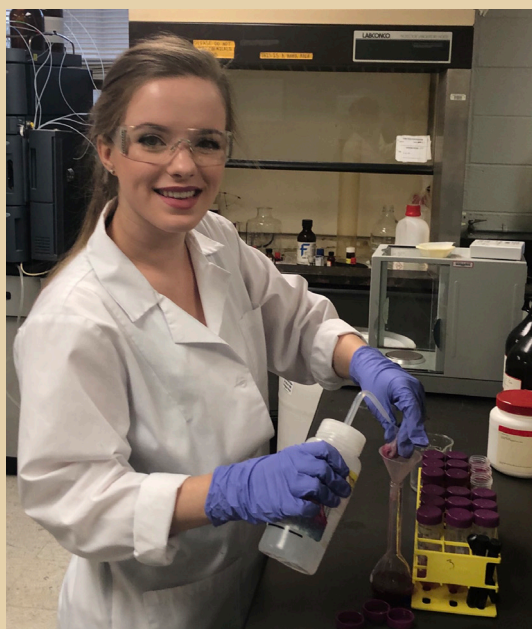


Samantha Findley

I am from The Woodlands, Texas. While at the University of Arkansas, I was a member of the Student Dietetic Association and served as Secretary my senior year. I also served as a Bumpers College Ambassador for two years. I graduated Magna Cum Laude with Honors Distinction with a degree in food science and a degree in human nutrition and hospitality innovation. Through studying nutrition and food science I have gained an interdisciplinary understanding of food, both in its complexity alone, and how it affects the human body. While conducting this research, I have employed techniques I learned about in courses but never thought I would get the chance to utilize and have truly had a hands-on experience. I am fortunate to have had guidance throughout the entire process. I thank Cindi Brownmiller for spending countless hours teaching me lab techniques, helping me analyze my thesis data and answering a myriad of questions. I also thank my mentor, Dr. Luke Howard, for his confidence in me, continuous support, and for always having a teaching heart; and to Drs. Jamie Baum and Han-Seok Seo for serving on my committee. I am thankful for the honors college research grant that supported my research as it showed a willingness to invest in me. After graduation, I will begin a combined master's degree and dietetic internship program in St. Louis and continue on my path to a career as a dietitian.

Research at a Glance

- This study evaluated the stability of six blueberry products (gummy, graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) prepared with freeze-dried wild blueberry powder.
- Food products were analyzed before and after processing and after two, four, six, and eight weeks of storage.
- The ice pop was the best product for shelf stability of chemical compounds found in blueberries in the food products evaluated.



Performing anthocyanin extractions on food products in Dr. Howard's lab in the Department of Food Science.

Anthocyanin stability in food products made with freeze-dried blueberry powder

Samantha Findley^{*} and *Luke Howard*[†]

Abstract

This study evaluated the stability of anthocyanins in six blueberry products (gummy, graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) prepared with freeze-dried wild blueberry powder during processing and over eight weeks storage. Total anthocyanins were determined by high-performance liquid chromatography (HPLC) before processing and at day 0 and 2, 4, 6, and 8 weeks of storage. Thermal processing of gummy and graham bar products resulted in losses of anthocyanins (50% and 31%, respectively). An eight-week storage time also resulted in a decrease in anthocyanins (7% to 51%) in products stored at ambient temperature. The ice pop, which was stored at -20 °C, was the best product for shelf-stability as it experienced no decline in total anthocyanins during processing or over the entire shelf-life study. Future research should be conducted to determine the differences in total anthocyanins in the products over time when they are stored under refrigeration. Additionally, polymeric color should be analyzed as this indicator has the potential to further explain the nature of the decrease in anthocyanins observed during storage.

* Samantha Findley is an honors program May 2019 graduate with a major in food science, and human nutrition and hospitality innovation.

† Luke Howard, the faculty mentor, is a Professor in the Department of Food Science.

Introduction

Diet during childhood can influence bone mineral density and susceptibility to chronic disease later in life (Chen et al., 2010; Gilsanz and Wren, 2007). Population-based studies have shown fruit and vegetable intake is an independent predictor of bone size in children and may contribute to the building of bone mass (Novotny et al., 2004; Tylavsky et al., 2004; Lanham, 2006). Unfortunately, many children fail to consume the recommended amount of fruits and vegetables (Kuntz et al., 2015). The recent popularity of consuming fruits and vegetables in prepared forms is worth exploring as a means to increase the consumption of health-promoting compounds, like anthocyanins in blueberries (Kuntz et al., 2015). Additionally, anthocyanin-rich products are needed for human clinical trials that require subjects to consume large doses of anthocyanins each day.

Anthocyanins, a class of polyphenols, are responsible for the brilliant red, blue, purple and black colors of fruits and vegetables. The six major anthocyanins found in fruits and vegetables, delphinidin, cyanidin, petunidin, peonidin, pelargonidin, and malvidin vary according to the substituents (OH or OCH₃) attached at positions R₁ and R₃ on the B ring (Fig. 1). Anthocyanins in nature almost always have a sugar (glycoside) or multiple sugars attached at carbon three on the middle heterocyclic ring.

The anthocyanin content of berries varies distinctly from one type of berry to another (Lee et al., 2015). It

is thought that since the composition of anthocyanins in berries affects their bioavailability and antioxidant effects, different anthocyanin-containing berries may affect the same cells differently (Lee et al., 2015). Blueberries from various sources have been found to contain 20–27 different anthocyanins (Wu and Prior, 2005). Wild blueberries are an especially good source of the anthocyanins petunidin and malvidin, delivering 87.6 mg/100 g and 154.6 mg/100 g, respectively (Wu et al., 2006).

The average daily intake of raw blueberries has been estimated to be 0.93 g, yielding 3.39 mg of anthocyanins (Wu et al., 2006). Intake of greater than 100 mg of anthocyanins per day could easily be achieved with the regular consumption of blueberries (Wu et al., 2006). In an *in vitro* study, malvidin-3-glucoside, one of the major monoglucosides in wild blueberries, was found to be significantly more effective at inhibiting pro-inflammatory genes than epicatechin or chlorogenic acid (Esposito et al., 2014).

Degradation and loss of anthocyanins during processing is of great concern when developing processed blueberry products. Conditions during thermal processing must be considered to ensure the biological activity of anthocyanins is retained in a thermally processed product (Rodriguez-Mateos et al., 2014). Thermal processing has been found to result in total monomeric anthocyanin losses of 28% to 59% in canned, pureed, and juiced blueberry products (Brownmiller et al., 2008).

The objective of this study was to evaluate the stability of anthocyanins in six blueberry products (gummy,

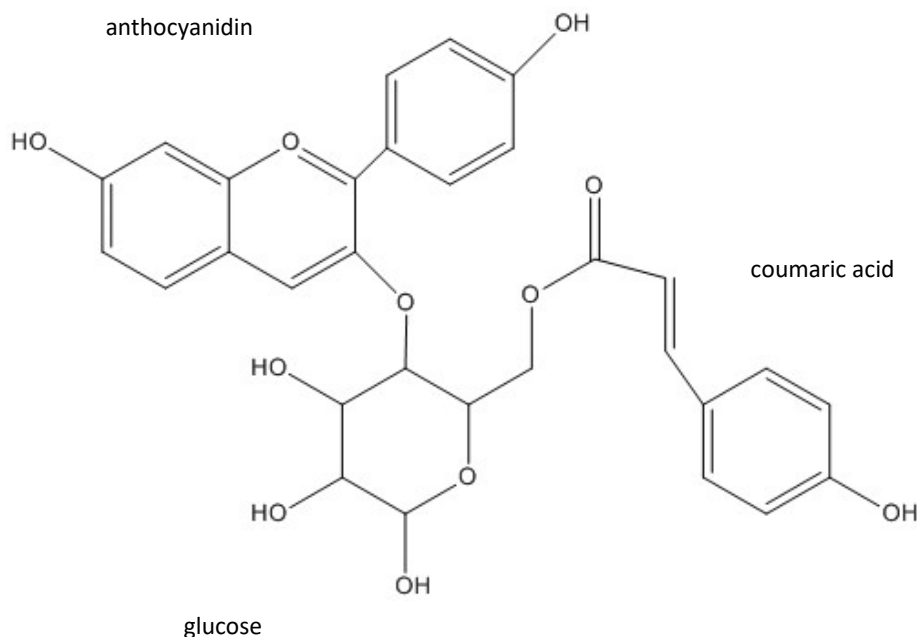


Fig. 1. The skeletal structure of an acylated anthocyanin.

graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) prepared with freeze-dried wild blueberry powder during processing and over eight weeks storage.

Materials and Methods

The freeze-dried powder used in the products and as a comparison was HiActives® North American wild blueberry powder 1.5% (FutureCeuticals, Momence, Illinois). Prepared blueberry products were previously formulated, produced, and packaged by others in the lab before the start of the shelf-life study. A 5-g sample for anthocyanin analysis was taken from each of three packages of each product (gummy, graham bar, oatmeal bar, rice krispy bar, ice pop, and juice) at each timepoint during the shelf-life study. Timepoints were: day 0 (immediately after processing) and after 2, 4, 6, and 8 weeks of storage at 21 °C (except for the ice pop which was frozen and stored at -20 °C).

Anthocyanin Extraction and Sample Preparation

Samples of each food product, gummy, graham bar, oatmeal bar, and rice krispy bar, excluding the ice pop and juice, were first homogenized with methanol/water/formic (60:37:3, v/v/v) acid solvent using a Euro Turrax T18 Tis-suemizer (Tekmar-Dohrman Corp., Mason, Ohio; Brown-miller et al., 2008). Homogenized samples were filtered through Miracloth (Calbiochem, La Jolla, Calif.) and the filtrates were adjusted to a volume of 150 mL in a volumetric flask (Brownmiller et al., 2008). The 150-mL filtrate solutions were poured into beakers to ensure adequate mixing of the filtrates and transferred from the beakers into labeled 50-mL test tubes. From the 50-mL test tubes, 5 mL was pipetted into separate labeled 50-mL test tubes. The test tubes containing 5 mL of filtrate solution were dried overnight in a Speed Vac® concentrator (ThermoSavant, Holbrook, New York) and reconstituted with 1 mL of aqueous formic acid solution (Cho et al., 2004).

Ice pop and juice samples did not require the same extraction method. Both pasteurized and unpasteurized juice samples were evaluated to measure the anthocyanin losses during thermal processing of the juice. The ice pop and juice samples were diluted (200 µL ice pop or juice + 800 µL 5% formic acid) in glass test tubes prior to high-performance liquid chromatography (HPLC) analysis. The reconstituted extracted solutions from the products and the diluted juice and ice pop samples were each passed through 25-mm syringe filters with 0.45 µm nylon membranes (VWR International™, Radnor, Pennsylvania) before analysis by HPLC.

High-Performance Liquid Chromatography

Chromatographic analysis was performed using an established HPLC method to measure the content of in-

dividual anthocyanin monoglycosides (Brownmiller et al., 2008). Anthocyanin analysis by HPLC was performed according to the method of Cho and coworkers with a 50-µL injection volume of samples using a Waters HPLC system (Waters Corp, Milford, Massachusetts) fitted with a model 600 pump, 717 Plus autosampler, and a model 996 photodiode array detector (Cho et al., 2004). For separation, a 4.6 mm × 250 mm Symmetry® C18 column (Waters Corp, Milford, Massachusetts) and a 3.9 mm × 20 mm Symmetry® C18 column (Waters Corp, Milford, Massachusetts) were utilized (Cho et al., 2004). A linear gradient of 5% formic acid and methanol was used for the mobile phase (Cho et al., 2004). Detection wavelength used for the anthocyanins was 510 nm (Cho et al., 2004). Identification of the anthocyanins in the freeze-dried blueberry powder was previously performed by HPLC-MS. Individual anthocyanin glycosides were quantified as delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents using external calibration curves of a mixture of the five anthocyanin glucosides. The concentrations of individual anthocyanins were summed and reported as total anthocyanins. Results are expressed as total milligrams anthocyanins per gram of blueberry powder in each product.

Statistical Analysis

Statistical analysis was performed using JMP® software (SAS Institute, Inc., Cary, North Carolina). One-way analysis of variance with mean comparison by student's *t*-test was used to determine significant differences ($P < 0.05$) in average total milligrams anthocyanins per gram of blueberry powder in each product during the eight-week shelf-life study to measure anthocyanin stability over time. Significant differences ($P < 0.05$) in average total milligrams anthocyanins per gram of blueberry powder in each product at day 0 were also compared to average total milligrams anthocyanins per gram of blueberry powder to evaluate the stability of anthocyanins during processing of the products using a student's *t*-test.

Results and Discussion

Effects of Processing

Compared to the unprocessed blueberry powder, there was no difference ($P > 0.05$) in the average milligrams anthocyanins per gram of blueberry powder in any of the prepared products at day 0 (after processing), except for the graham bar and the gummy (Table 1). There was also no decrease for the juice ($P > 0.05$) in total anthocyanins during pasteurization (Table 1). The graham bar contained 31% fewer anthocyanins after processing and the gummy contained 50% fewer anthocyanins after processing (Table 1).

Shelf-life Study

For the gummy, there was a relatively linear decrease in total anthocyanins during the shelf-life study. Between day 0 and week 8, a 51% decrease in anthocyanins was measured (Fig. 2). The total anthocyanins in the graham bar remained relatively stable from day 0 to week 8 (Fig. 3). There was no decline in anthocyanins until week 6 and a 7% decrease in anthocyanins was measured between day 0 and week 8 (Fig. 3). Total anthocyanins in the rice krispy bar at day 0 and week 6 and 8 were different ($P < 0.05$) (Fig. 4). From day 0 to week 8 there was a 34% decrease in total anthocyanins (Fig. 4). Total anthocyanins in the oatmeal bar decreased gradually over the course of the shelf-life study (Fig. 5). From day 0 to week 8, a 28% decrease in total anthocyanins was observed (Fig. 5). No decrease ($P > 0.05$) in anthocyanins in the juice

was measured until week 6 of the shelf-life study (Fig. 6). Between day 0 and week 8, the pasteurized juice experienced a 47% decrease in total anthocyanins (Fig. 6). The anthocyanins in the ice pop were stable with no change ($P > 0.05$) observed between day 0 and week 8 (Fig. 7).

The anthocyanin contents of the prepared food products were compared to that of the freeze-dried blueberry powder used to prepare the products. Because there was no difference ($P > 0.05$) in the average milligrams anthocyanins per gram of blueberry powder in any of the products except for the graham bar and the gummy when compared to the unprocessed blueberry powder, it appeared the methods used to produce the oatmeal bar, rice krispy bar, ice pop, and juice (both pasteurized and unpasteurized) do not cause significant losses of anthocyanins (Table 1).

Table 1. Total anthocyanin content of prepared blueberry products.

Product	Average mg anthocyanins per g blueberry powder in product	Decrease in total anthocyanins measured (%)
Powder ^a	19.55 ± 1.6 ^b	Reference
Gummy	9.7 ± 0.7	50%
Graham bar	13.5 ± 0.7	31%
Rice krispy bar	16.9 ± 1.7	NS ^c
Oatmeal bar	19.2 ± 0.6	NS
Juice, NP ^d	21.81 ± 0.4	NS
Juice, P ^e	21.44 ± 1.3	NS
Ice pop	22.6 ± 0.3	NS

^a Blueberry powder used to prepare blueberry products.

^b Values represent means ($n = 3$) ± SEMs.

^c NS = no significant difference ($P > 0.05$).

^d NP = not pasteurized.

^e P = pasteurized.

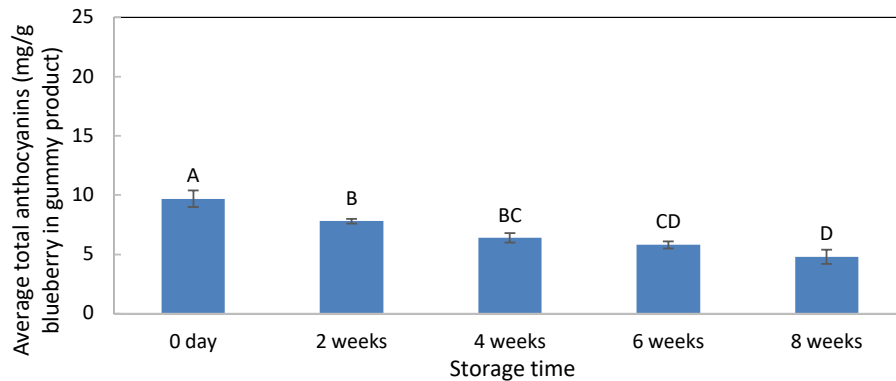


Fig. 2. Average total anthocyanins in gummy during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product ± standard error of the mean. Bars with different letters are significantly different ($P < 0.05$)

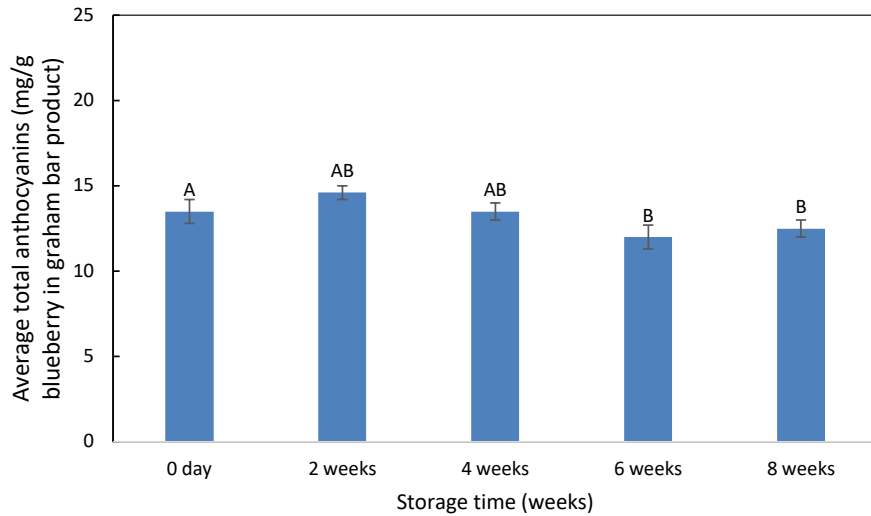


Fig. 3. Average total anthocyanins in graham bar during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$). The total anthocyanins in the graham bar remained relatively stable from day 0 to week 8. There was no significant decline in anthocyanins until week 6 and a 7% decrease in anthocyanins was measured between day 0 and week 8.

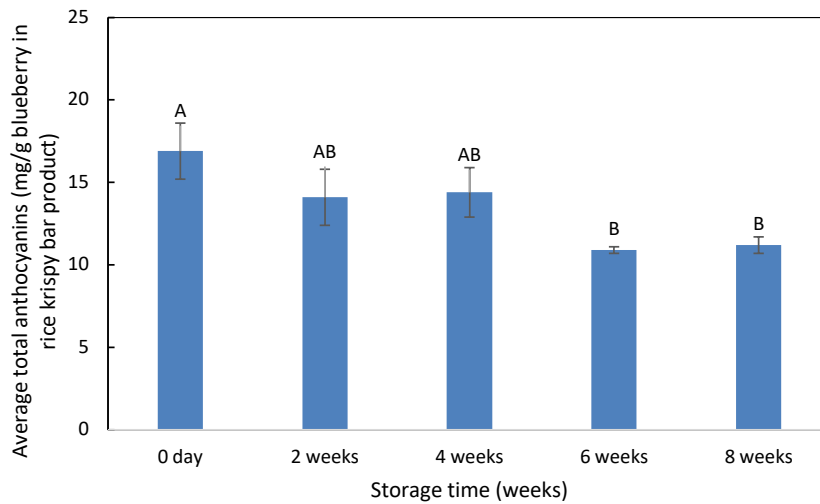


Fig. 4. Average total anthocyanins in rice krispy bar during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$).

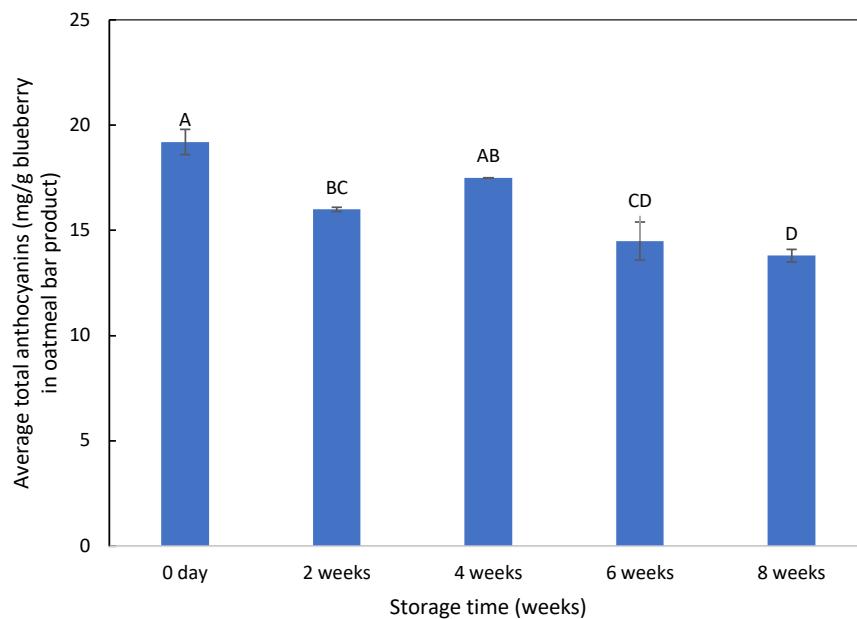


Fig. 5. Average total anthocyanins in oatmeal bar during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$).

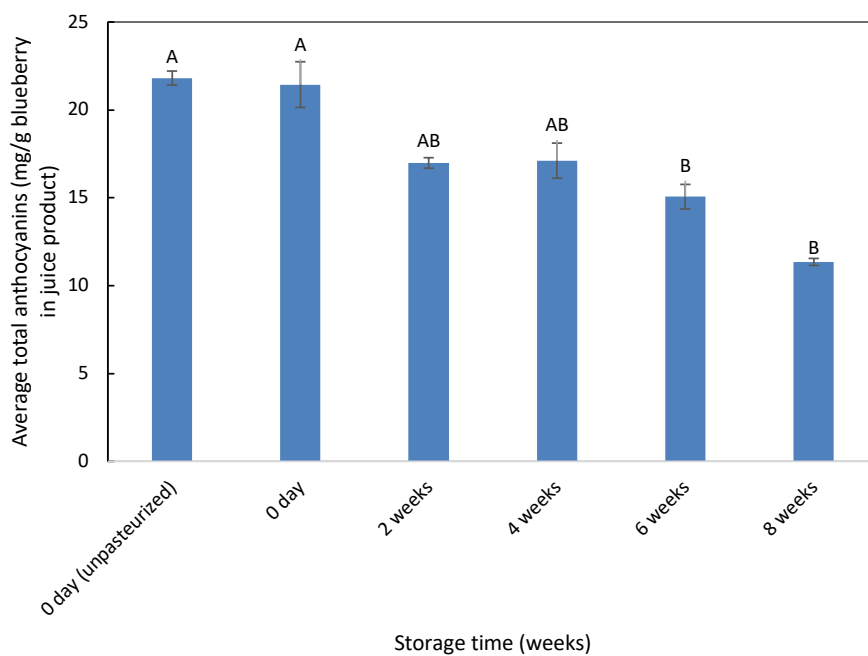


Fig. 6. Average total anthocyanins in juice during an eight-week shelf-life study. Juice from day 0 to week 8 was pasteurized. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. Bars with different letters are significantly different ($P < 0.05$).

The gummy was notably the least stable of all the products analyzed, experiencing a 50% decrease in total anthocyanins during processing (Table 1) and then another 51% decrease by week 8 of the shelf-life study (Fig. 2). With the gummy only appearing to contain approximately 25% of the anthocyanins present in the blueberry powder used to formulate it, it does not seem to be as viable of an option for consistent delivery of anthocyanins compared to the other products. Additional research is needed to determine why the anthocyanins were unstable during gummy processing and storage.

The graham bar also experienced a 31% decrease in total anthocyanins during processing (Table 1). Unlike the gummy, the graham bar remained relatively stable over time with no decrease until week 6 and only a 7% decrease in total anthocyanins during the entire shelf-life study (Fig. 3). While the rice krispy bar did not experience a decrease in anthocyanins during processing (Table 1), it experienced a 34% decrease in anthocyanins during the shelf-life study (Fig. 4). The oatmeal bar also experienced a moderate 28% decrease in anthocyanins from day 0 to week 8 (Fig. 5).

While the thermal processing (pasteurization) of the juice did not decrease the total anthocyanins measured in the product (Table 1), the anthocyanins declined by 47% over 8 weeks of storage (Fig. 6). The ice pop was by

far the most stable of the products evaluated. There was no decline in total anthocyanins during processing (Table 1) or the entire shelf-life study (Fig. 7). The stability of the anthocyanins is likely due to the fact the ice pop was stored frozen rather than at ambient temperature like the other products. Freezing presumably suppressed the mechanism(s) responsible for loss of anthocyanins observed in products stored at 21 °C.

While a decrease in total anthocyanins was measured in several of the products during processing and storage, the question remains about whether the decrease was due to actual losses as a result of thermal degradation or conversion of anthocyanins into polymerized forms. Previous research found that, while some of the decreases in anthocyanins in blueberry products is due to thermal degradation during processing and storage, polymerization of anthocyanins with other anthocyanins and phenolic compounds also occurs (Brownmiller et al., 2008). Furthermore, the ability of the blueberry products to scavenge peroxy radicals (hydrophilic antioxidant capacity) was not found to decline as significantly as total anthocyanin content over storage (Brownmiller et al., 2008). This suggests these blueberry products likely do not experience as significant of a decline in bioactivity as the decrease in measured total anthocyanins (Brownmiller et al., 2008). The authors postulated that antho-

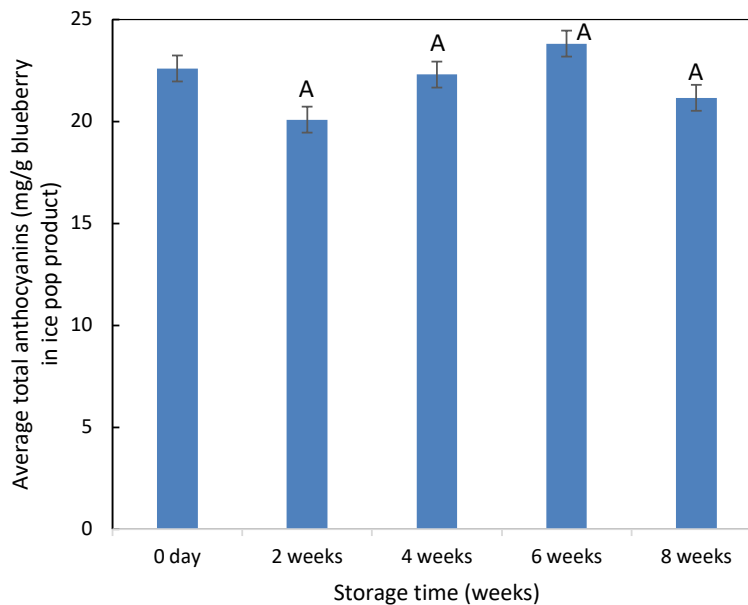


Fig. 7. Average total anthocyanins in ice pop during an eight-week shelf-life study. Values are mean total anthocyanins in mg/g blueberry in product \pm standard error of the mean. No means were significantly different ($P > 0.05$).

cyanins formed polymeric structures with procyanidins during storage, and these polymeric compounds retained potent hydrophilic antioxidant capacity (Brownmiller et al., 2008).

Conclusions

Thermal processing of gummy and graham bar products resulted in significant losses of anthocyanins (50% and 31%, respectively). An 8-week storage time also resulted in a significant decrease in anthocyanins (7% to 51%) in products stored at ambient temperature, except for the ice pop, which was stored at -20 °C. The ice pop was the best product for shelf-stability as it experienced no significant decline in total anthocyanins during thermal processing or the entire shelf-life study. Future research should be conducted to determine the differences in total anthocyanins in the products over time when they are stored under refrigeration. Additionally, polymeric color should be analyzed as this indicator has the potential to further explain the nature of the decreases in anthocyanins that were observed.

Literature Cited

- Brownmiller, C., L.R. Howard, and R. Prior. 2008. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. *J. Food Sci.*, 73(5):H72-H79.
- Chen, J., O.P. Lazarenko, X. Wu, J. Kang, M.L. Blackburn, K. Shankar, T.M. Badger, and M.J. Ronis. 2010. Dietary-induced serum phenolic acids promote bone growth via p38 MAPK/ β -catenin canonical Wnt signaling. *J. Bone Miner. Res.*, 25(11):2399-2411.
- Cho, M.J., L.R. Howard, R.L. Prior, and J.R. Clark. 2004. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric.*, 84(13):1771-1782.
- Esposito, D., A. Chen, M.H. Grace, S. Komarnytsky, and M.A. Lila. 2014. Inhibitory effects of wild blueberry anthocyanins and other flavonoids on biomarkers of acute and chronic inflammation in vitro. *J. Agric. Food Chem.*, 62(29):7022-7028.
- Gilsanz, V. and T. Wren. 2007. Assessment of bone acquisition in childhood and adolescence. *Pediatrics*, (Supplement): S145-S149.
- Kuntz, S., S. Rudloff, H. Asseburg, C. Borsch, B. Frohling, F. Unger, S. Dold, B. Spengler, A. Rompp, and C. Kunz. 2015. Uptake and bioavailability of anthocyanins and phenolic acids from grape/blueberry juice and smoothie in vitro and in vivo. *Br. J. Nutr.*, 113(7):1044-1055.
- Lanham-New, S. 2006. Fruit and vegetables: The unexpected natural answer to the question of osteoporosis prevention? *Am. J. Clin. Nutr.*, 83(6):1254-1255.
- Lee, S. G., B. Kim, D.Y. Soung, T. Vance, J.S. Lee, J. Lee, S.I. Koo, D. Kim, H. Drissi, and O.K. Chun. 2015. Relationship between oxidative stress and bone mass in obesity and effects of berry supplementation on bone remodeling in obese male mice: An exploratory study. *J. Med. Food*, 18(4):476.
- Novotny, R., Y.G. Daida, J. Grove, S. Acharya, T.M. Vogt, and D. Paperny. 2004. Adolescent dairy consumption and physical activity associated with bone mass. *Prev. Med.*, 39:355-360.
- Rodriguez-Mateos, A., R.D. Pino-Garcia, T.W. George, A. Vidal-Diez, C. Heiss, and J.P. Spencer. 2014. Impact of processing on the bioavailability and vascular effects of blueberry (poly)phenols. *Mol. Nutr. Food Res.*, 58(10):1952-1961.
- Tylavsky, F., K. Holliday, R. Danish, C. Womack, J. Norwood, and L. Carbone. 2004. Fruit and vegetable intakes are an independent predictor of bone size in early pubertal children. *Am J. Clin. Nutr.*, 79(2):311-317.
- Wu, X., G. Beecher, J. Holden, D. Haytowitz, S. Gebhardt, and R. Prior. 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem.*, 54(11):4069-4075.
- Wu, X. and R. Prior. 2005. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: Fruits and berries. *J. Agric. Food Chem.*, 53(7):2589-2599.