

Development and evaluation of kefir products made with aronia or elderberry juice: sensory and phytochemical characteristics

Du, X. and *Myracle, A. D.

5735 Hitchner Hall, University of Maine, Orono, Maine, U.S. 04469-5735

Article history

Received: 8 April 2017
Received in revised form:
24 May 2017
Accepted: 25 May 2017

Abstract

Aronia and elderberry are edible berries that are rich in anthocyanins and phenolic compounds. They are rarely consumed raw due to safety concerns and their unpalatable taste. Aronia and elderberry are not widely grown and they are not commonly used as food ingredients for commercial products, thus they are considered underutilized. Incorporating these berries into new products, such as kefir, provides diverse food choices and may increase the dietary consumption of these bioactive compounds. In this study, kefir containing either aronia or elderberry juice was developed using different sweeteners and levels of sweetness. Sensory tests were conducted to evaluate the consumer acceptability of the kefir products made with fresh aronia juice or commercial elderberry juice from Wyldewood Cellars® (Wichita, KS, U.S.). In the aronia kefir sensory test, the product sweetened with sucrose received the best overall acceptability (6.3) while the product sweetened with monk fruit extract was least favored (4.9). In the elderberry kefir sensory test, 5.7% sucrose-sweetened product was best accepted (6.6) followed by 4.3% sucrose-sweetened (6.1). Non-nutritive sweeteners (stevia and monk fruit extracts) were not well accepted in either tests. Phytochemical analyses showed that aronia kefir contained high amounts of total phenolic compounds and anthocyanins. Kefir made with commercial elderberry juice had a moderate amount of total phenolics and a low amount of anthocyanins. Antioxidant capacity was observed in all products indicating kefir with berries may benefit the decrease in oxidative stress. Kefir made with aronia or elderberry are acceptable functional foods which may contribute to the prevention of inflammation and chronic diseases when incorporated into a healthy diet.

© All Rights Reserved

Keywords

Sensory evaluation
Aronia berry
Elderberry
Kefir
Anthocyanins

Introduction

Epidemiological studies suggest that the consumption of anthocyanin-rich fruits may contribute to the decrease in the risk of type 2 diabetes (Wedick *et al.*, 2012). In addition, polyphenols contribute to the protection against oxidative-stress-related diseases due to their high antioxidant capacity as suggested by many studies (Zhang *et al.*, 2015; Chang *et al.*, 2016; Masisi *et al.*, 2016). Riso *et al.* (2013) indicate that blueberry extracts rich in polyphenols could decrease the levels of oxidized DNA bases in human subjects and attenuate DNA damage induced by H₂O₂. Anthocyanin intake (two levels, 40 and 200 mg/kg) is able to reduce high-fat-diet-induced oxidative stress in mice by boosting the activity of antioxidant enzymes (SOD and GPx). Systemic inflammation could be decreased with this range of intake by lowering the expression levels of inflammatory cytokines, such as IL-6 and TNF α (Wu *et al.*, 2014). *In vitro* studies reveal that anthocyanin-rich extracts could inhibit the formation of advanced glycation end products and consequently decrease the

risk of diabetic complications (Harris *et al.*, 2014).

Aronia melanocarpa (aronia) and *Sambucus nigra* subsp. *canadensis* (elderberry) are berries that contain high amount of anthocyanins and polyphenols (Kokotkiewicz *et al.*, 2010; Sidor and Gramza-Michałowska, 2015). They are not widely commercial cultivated thus they are underutilized. These berries exhibit high antioxidant capacity due to their phenolic compounds. Aronia and elderberry are known to reduce oxidative stress in humans (Youdim *et al.*, 2000; Denev *et al.*, 2012). Aronia and elderberry are popular in Europe where they are utilized as a functional food ingredient and color additive (Kokotkiewicz *et al.*, 2010; Szaloki-Dorko *et al.*, 2015); however, a market for these berries in the United States has not been well developed.

Kefir is a fermented dairy beverage that originated in the Caucasus Mountains region over one hundred years ago (Satir and Guzel-Seydim, 2015; Bourrie *et al.*, 2016). Kefir grains consist of complex microbial communities and contain up to 30 species (KoŁakowski and Ozimkiewicz, 2012) where lactic acid bacteria is usually predominant, followed by

*Corresponding author.
Email: angela.myracle@maine.edu

yeast and acetic acid bacteria (de Oliveira Leite *et al.*, 2013). Several studies suggest that kefir has antimicrobial, anti-inflammatory, and anti-carcinogenic activities (De Vrese *et al.*, 1992; Rodrigues *et al.*, 2005; Lee *et al.*, 2007). Kefir is naturally lactose-free, this property makes kefir a good source of calcium and protein for lactose intolerant individuals (Sarkar, 2007). The fermentation process has the ability to increase the bioaccessibility and bioavailability of phenolic compounds due to the release of bound phenolic constituents by some lactic acid bacteria (Hunaefi *et al.*, 2013; Young *et al.*, 2015). The low acid environment of kefir is helpful to decrease the natural degradation of phenolic compounds (Friedman and Jurgens, 2000). Therefore, the combination of anthocyanin-rich berries and kefir may result in a value-added functional product. The objective of this study was to develop functional food products by incorporating either aronia or elderberry juice into a kefir beverage and evaluate their sensory and phytochemical characteristics. Different natural sweeteners (sucrose, stevia or monk fruit extract) were used to enhance the sweetness of the products. The acceptability of the products was evaluated via sensory tests. The aronia sensory test focused on the impact of sweetener variety on consumer acceptability. The elderberry kefir sensory test assessed the influence of sweetness levels. Bioactive constituents of the kefir products were analyzed in the laboratory.

Materials and Methods

Chemicals

Methanol, citric acid anhydrous, potassium chloride, hydrochloric acid, and sodium bicarbonate were obtained from Fisher Scientific (Waltham, MA, U.S.). 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin - Ciocalteu's phenol reagent and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.), and sodium acetate from Chem-Impex int'l inc. (Wood Dale, IL, U.S.). Ultrapure water was obtained from a Millipore water system (EMD Millipore, Billerica, MA, U.S.).

Dietary material

Aronia (*Aronia melanocarpa*, variety 'Viking') were obtained from the University of Connecticut (Storrs, CT, U.S.) from the 2014 growing season. Elderberry juice was a gift of Dr. John Brewer from Wyldewood Cellars® (Wichita, KS, U.S.). Elderberries (*Sambucus nigra*, subsp. *canadensis*) used in making samples for fresh juice phytochemical analyses were collected from the experimental garden at University

of Maine (Orono, ME, U.S.) during 2015 harvest season. Berries were harvested at full ripeness which was determined by a deep purple color and softness of the berry. They were de-stemmed and washed, then frozen at -20°C. The thawed berries were flash pasteurized (100°C, 5 minutes) in an aluminum sauce pan then juiced with a domestic juicer (Hamilton Beach, Southern Pines, NC, U.S.). Juice yield from 1kg berries averaged 360 g. Fresh juice was used immediately to make the kefir products. Commercial kefir culture (Yogourmet®, Lachute, QC, Canada) was used as the starter for the kefir. The viability of the lactic bacteria in the starter was determined by inoculation on MRS agar (Nedgen, Lansing, MI, U.S.) at 40°C for 48 hours. The viability of the yeast in the starter was measured by plating on antibiotic plate counting agar (Alpha Biosciences, Baltimore, MD, U.S.) at room temperature for 96 hours. Sucrose (Great Value®, Bentonville, AR, U.S.), stevia extract (Stevia in the Raw®, New York City, NY, U.S.), monk fruit extract (Monk Fruit in the Raw®, New York City, NY, U.S.) and 2% milk (Oakhurst®, Portland, ME, U.S.) were purchased from local supermarkets.

Kefir manufacture and formulas

All kefir products were prepared by the following method: 2% milk was heated to 82°C in a commercial size aluminum sauce pan, cooled to 26°C using an ice bath, and then transferred to a 4L pyrex glass bowl. The commercial starter was added to the milk (5g per quart), and the mixture was stirred for 5 minutes to ensure the starter was dissolved. Either aronia juice (~13%, w/w) or elderberry juice (~10%, w/w) was added to the mixture. A higher percentage of the juice was tested, but the mixture did not set up properly. The amount of non-nutritive sweetener used in each product was adjusted according to the instruction on the package to create equal sweetness to sucrose. After the addition of all ingredients, the mixture was covered with a cloth and left at room temperature to ferment for 24 hours. The kefir was homogenized with a Hamilton Beach immersion blender (Southern Pines, NC, U.S.). Each kefir product was divided into two glass containers with sealed lids and stored at 4°C. The kefir was allowed to chill at 4°C for 2 hours prior the sensory tests. The sensory tests were completed within 36 hours. Formulas are shown in Table 1. Kefir products were only formulated with juice and sweetening agents (sucrose, stevia extract, or monk fruit extracts); no additional modifiers were used. Low sucrose levels were selected for reducing calorie content and to ensure that the predominant flavor was from the berry juice.

An aliquot of the kefir prepared for the sensory

Table 1. Kefir products formulas

Product	Sweetener	Milk with starter (g)	Commercial elderberry juice (g)	Fresh elderberry juice (g)	Fresh aronia juice (g)	sucrose (g)	Stevia (g)	Monk fruit (g)
Aronia kefir	sucrose	83	---	---	13	4.0	---	---
	stevia	83	---	---	13	---	0.40	---
	monk fruit	83	---	---	13	---	---	0.80
Elderberry kefir (commercial juice)	low sucrose (4.3%)	90	10	---	---	4.5	---	---
	high sucrose (5.7%)	90	10	---	---	6.0	---	---
	low stevia (0.4%)	90	10	---	---	---	0.45	---
	high stevia (0.6%)	90	10	---	---	---	0.60	---
Elderberry kefir (Fresh juice)	low sucrose (4.3%)	90	---	10	---	4.5	---	---
	high sucrose (5.7%)	90	---	10	---	6.0	---	---
	low stevia (0.4%)	90	---	10	---	---	0.45	---
	high stevia (0.6%)	90	---	10	---	---	0.60	---

---- indicates the ingredient was not used in the formula

test was collected and stored at -20°C until laboratory analyses. The initial study design was to utilize fresh berry juices for the kefir products but due to crop failures in 2013 and 2014, fresh elderberries were not available. Commercial elderberry juice was used in this study. To better understand the difference between commercial and fresh elderberry juice, an additional set of elderberry kefir products made with fresh juice was prepared and analyzed in the laboratory at a later date.

Sensory analyses

All sensory tests were performed in the Sensory Testing Center at the University of Maine, Orono campus. Sensory tests were approved by the Institutional Review Board for the Protection of Human Subjects at the University of Maine (IRB). Tests were conducted in the afternoons, and 100 healthy participants were recruited from the community. Demographic information was collected, such as age and gender. Consumer familiarity with the berries and kefir was assessed. Consumer attitude toward purchasing healthy food was asked. Color, flavor, sweetness, texture and overall acceptability of all samples were evaluated using a 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely). Products received random 3-digit codes and samples were presented to the consumers in a randomized sequence. Samples were served

cold (4°C) in transparent 2 oz plastic cups and water was offered as a palate cleanser. Information was collected anonymously with computers using SIMS Sensory Software® (Berkeley Heights, NJ, U.S.). Each participant was compensated with \$2 for completion of the sensory test.

pH, titratable acidity, total soluble solids and color measurements

pH, titratable acidity (TA), and total soluble solids (°Brix) were evaluated following the methods reported by Mena *et al.* (2011) with minor modification. Briefly, pH was measured using a Sartorius pH meter (Bohemia, NY, U.S.). TA was determined by titrating 5 g of kefir with 0.1M NaOH solution. The results were expressed as % lactic acid (Temiz and Kezer, 2015) in kefir and % citric acid in juice. Total soluble solids were tested using a PAL-3 refractometer by Atago (Tokyo, Japan) and values were expressed as °Brix. The color was measured using a LabScan XE spectrophotometer manufactured by HunterLab (Reston, VA, US) and was recorded as L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness). Hue angle and Chroma values were calculated by the following formulas (McLellan *et al.*, 1995; Maria Salinas-Hernandez *et al.*, 2015).

$$\text{Hue} = \text{Arc tan}(b^*/a^*) \text{ for the first quadrant (+a, +b)}$$

$$\text{Hue} = 360 + \text{Arc tan}(b^*/a^*) \text{ for the fourth quadrant (+a, -b)}$$

$$\text{Chroma} = \sqrt{(a^{*2} + b^{*2})}$$

pH and °Brix were measured in triplicate, TA was measured in duplicate due to limited sample availability. Color was measured in five independent tests to confirm the uniformity of the sample and obtain representative results.

Extraction

Phenolic compounds were extracted following the method reported by Ścibisz *et al.*, (2012) with modification. After adding acidified 80% methanol (1% citric acid, w/v, 1:10, sample:solvent) to the kefir matrix, the mixture was vortexed then sonicated in a Branson 5510 sonicator (Danbury, CT, U.S.) for 1 hour. Samples were centrifuged at $16639 \times g$ (Eppendorf 5804R, Hamburg, Germany) for 30 minutes at 4°C. The supernatant was collected in a clear tube. This process was repeated three times. The combined supernatant was evaporated under a vacuum (Eppendorf Vacufuge plus, Hamburg, Germany) at room temperature. Dried samples were re-suspended using acidified 100% methanol (1%, citric acid), and kept at -20°C for one hour to precipitate the protein. The slurry was then

centrifuged at 16639×g for 30 minutes at 0°C and the supernatant was collected. Supernatant was dried under a vacuum and re-suspended with 80% acidified methanol (1%, w/v). The extract samples were kept at -20°C until analyses.

Total phenolic (TP) content

TP content was determined using the Folin-Ciocalteu method as described by Velioglu et al., (1998) with minor modifications. Briefly, after mixing extract (20 µl) and Folin-Ciocalteu reagent (90µl), the plate was left at room temperature for 5 minutes and then sodium bicarbonate (6g/100 ml, 90 µl) was added. The plate was covered and incubated at room temperature in the dark for 90 minutes. The absorbance was read at 750 nm with a Biotek plate reader (ELx 800, Winooski, VT, U.S.). All samples were measured in triplicate, and the results are expressed as gallic acid equivalents (GAE).

Total monomeric anthocyanin (ANC) content

ANC content was determined using the pH differential method developed by Lee *et al.* (2005) with modifications to fit a 96-well plate format. Briefly, the extract (20 µl) was diluted with 180µl of pH 1.0 buffer (0.025 M, potassium chloride) and 180µl pH 4.5 buffer (0.4 M, sodium acetate) separately. The mixture was incubated for 20 minutes at room temperature in the dark. The absorbance (abs) was read at 520 nm and 690 nm using the plate reader, and the ANC content was calculated by the following formula and expressed as cyanidin-3-glucoside (C3G) equivalents.

$$\text{Anthocyanin (cyanidin-3-glucoside equivalents, mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times L}$$

Where: A = (abs520 – abs700nm) pH 1.0 – (abs520nm – abs700nm) pH 4.5;

MW (molecular weight) = 449.2g/mol for cyanidin-3-glucoside;

DF = dilution factor;

L = pathlength in cm;

ε = 26900L/mol·cm, for cyanidin-3-glucoside;

10³ = factor for conversion from g to mg.

Antioxidant capacity by DPPH scavenging methods

Antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method by Duymus *et al.* (2014) with minor modifications. 150 µl of DPPH solution (0.3 mM) were added to 150 µl serially diluted extracts, the mixture was incubated in the dark at room temperature for 30 minutes and read at 515nm. The inhibition rate was calculated by the following

formula:

$$\% \text{inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

Inhibition (%) was plotted against extract concentration, and the IC₅₀ (the concentration to scavenge fifty percent of DPPH free radical) was calculated. Gallic acid was used as a positive control.

Statistical analysis

Data are shown as mean ± standard deviation (SD). Sensory data was analyzed with one-way analysis of variance using JMP 12 software by SAS Institute Inc. (Cary, NC, U.S.). Tukey's Honest Significant Difference (HSD) test was used for mean comparisons. Pearson test was used to determine correlations. A significance level was set at α = 0.05.

Results and Discussion

Aronia kefir sensory test

Three aronia kefir products containing different sweeteners were evaluated in this test. Since the only difference among aronia kefir products was the type of the sweetener, participants' perception about different sweeteners could be assessed. Demographic results demonstrated that participants in this test were balanced in gender (54 female and 46 male), and the general age range was 18-34 years old. A question about the importance of purchasing food with potential health benefits was addressed and 94% of participants responded positively. Thus, functional food products, like aronia kefir, comply with consumers purchasing trends. Sensory attributes and overall acceptability results are shown in Figure 1. The results show that the participants accepted the color of all the products equally; however, significant differences in acceptability were detected for other attributes. Based on the ratings for sweetness, the participants liked the sucrose-sweetened sample best followed by stevia and monk fruit extract was the least favored. Similar results were obtained by another study where chocolate milk sweetened with sucrose received better acceptability compared to stevia or monk fruit extracts (Li, Lopetcharat and Drake, 2015). A study by Cardello, Da Silva and Damasio (1999) demonstrate that many non-nutritive sweeteners, such as stevia and aspartame have a residual bitter taste which is not well accepted by the consumers. In our test, consumers noted that kefir products sweetened with non-nutritive sweeteners (stevia and monk fruit extract) had a "bad", "longer aftertaste", or "unpleasant aftertaste" which lowered consumer liking of the products. Flavor was best

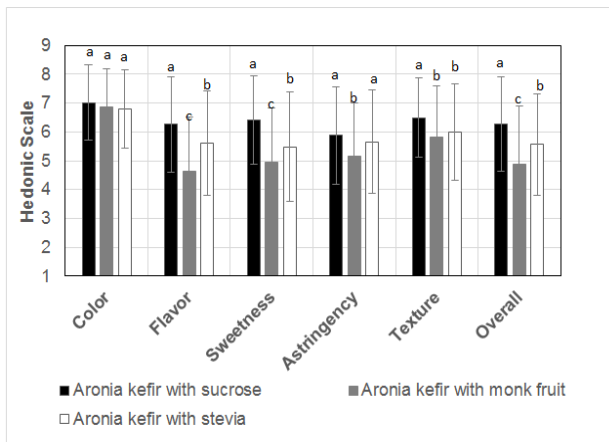


Figure 1. Consumer acceptability of aronia kefir made with different sweeteners.

Data are shown as means \pm standard deviation. n=100.

Different letters indicate significant differences among means within each attribute, $p < 0.05$.

received in the sucrose-sweetened aronia product. Kefir has a unique flavor due to the lactic acid and carbon dioxide (Arslan, 2015). In this study, aronia juice and sweeteners were added to the kefir and contributed to the overall flavor profile. Aftertaste of non-nutritive sweeteners may be a reason for the low hedonic scores of the products in the flavor attribute. The best texture was received in the sucrose-sweetened product where a thicker consistency was noticed. Cardoso and Bolini (2008) indicated that sucrose contributed a more viscous texture to a beverage product compared to non-nutritive sweeteners. The unique foamy texture of kefir generated by carbonation during fermentation (Altay *et al.*, 2013) may be novel to the American palate and may have an impact on the acceptability. Consumer preference of the texture may be influenced by the viscosity and the carbonation of the product. The best overall acceptability (6.3) was received in aronia kefir made with sucrose. Aronia kefir made with monk fruit extract, by contrast, received the lowest hedonic score (4.9). In addition to the influence by sweeteners, consumers' unfamiliarity with the kefir and/or aronia berry may be another reason which had an impact on the overall acceptability of the products. Over half of the participants (57%) were naive to kefir (never consumed kefir previously) and 78% were not familiar with aronia. Orjuela-Palacio *et al.* (2014) proved that repeated exposure to a high-polyphenol beverage increased the consumer acceptance. This indicates that the acceptability of the kefir products in this study could be higher if our participants were more familiar with either kefir or aronia. Generally, overall acceptability of each aronia kefir product was better received by participants who were previously familiar with kefir compared to the kefir-naive participants (data not shown).

Aronia kefir quality parameters

pH, TA, °Brix and color results of aronia kefir are shown in Table 2. The pH values of aronia kefir were in the acidic range as expected and this acidic environment is necessary to maintain the integrity of the phenolic compounds (Friedman and Jurgens, 2000). TA is representative of the sour taste in the product. Lactic acid produced by the kefir culture and citric acid in aronia juice contributed to TA values. No correlation was observed between TA and pH ($r = -0.13$, $p = 0.73$). The highest °Brix value was observed in the sucrose-sweetened product as expected. Several factors contribute to the °Brix of the product. They include the added sucrose, fructose in aronia berries, lactose in milk and the breakdown products of the disaccharides by the living culture. Concentrations of viable lactic acid bacteria and yeasts in the commercial kefir starter were 9.83×10^8 CFU/g and 5.3×10^4 CFU/g, respectively.

Chroma values indicate the saturation or the intensity of the color. High Chroma values of the aronia kefir products indicated the color was saturated. Based on the similar Chroma values among the aronia kefir products, there was no difference in the color intensity. Hue angle values close to 0 (360) indicated reddish color. The hue angle values of aronia kefir indicate that the products displayed a reddish color. High L^* values of the aronia kefir show the products had a bright color. Positive a^* values and negative b^* values of the products indicate the products presented a bluish-red color. Anthocyanins present a red to a blue color when the environment changes from acidic to alkaline. The bluish-red color was expected in the products due to the acidic environment of kefir.

Aronia kefir phytochemical analyses

The results of the phytochemical analyses of aronia kefir samples are shown in Table 3. Aronia kefir products had high ANC contents (16.57-17.22mg C3G/100 g kefir). A typical serving size of kefir is 8oz. One serving of the aronia kefir product would provide more than 39 mg ANC. Average ANC intake in the United States is 12.5 mg/day/person (Wu *et al.*, 2006). Our products provided three times more than the average intake for ANC. An epidemiologic study shows that in a population with an intake of 22.3-24.3 mg/day of ANC there was a lower incidence of type 2 diabetes compared to a population with only 2.0-2.3 mg/day (Wedick *et al.*, 2012). In addition, Jennings *et al.* (2014) indicate that an ANC intake of 39.9mg/day was associated with lower inflammation levels and improved insulin resistance compared to an ANC consumption of only 3.54mg/day. According to Seymour *et al.* (2014), consumption of cherries

containing 25.83 mg ANC increased the plasma antioxidant capacity for 12 hours in healthy humans. This evidence confirms that products developed with aronia juice could increase anthocyanin intake in a normal diet and contribute to a decrease in inflammation and prevent type 2 diabetes.

TP content in aronia product was 40.32 – 43.04mg GAE/100g kefir. An 8oz serving provides more than 95mg GAE of phenolics to the consumers. This equals about one-fifth of the average daily consumption of TP (450 mg GAE/person) in the American diet (Chun *et al.*, 2005). Dall'Asta *et al.* (2015) prove that dietary polyphenols could stimulate insulin secretion and protect β cells from the damage induced by oxidative stress. Aronia kefir products could add to the overall consumption of total phenolic in the diet and contribute to the prevention of diabetes. DPPH IC_{50} values of aronia kefir products were between 27.59 to 28.84mg kefir/ml. These results showed that the products had the ability to sequester free radicals. The antioxidant capacity of aronia kefir products is associated with the amount of ANC and TP. Kefir is a live culture and the environment is dynamic. Monomeric ANC and TP are being actively metabolized which helps explain the low recovery rates. Recovery rates of ANC and TP were about 45% and 55% respectively. ANC ingested from fruit and vegetables are usually conjugated and have low bioavailability (Fernandes *et al.*, 2015). Using a food matrix like kefir that contains live cultures may enhance the bioaccessibility of the aronia bioactive compounds by increasing monomeric ANC due to the de-conjugation of the anthocyanins.

Elderberry kefir sensory test

Four elderberry kefir products sweetened with either sucrose or stevia were evaluated in this test. In a previous study, monk fruit extract was not well accepted and it was eliminated. The products were sweetened to two levels to test the impact of sweetness. Commercial products usually have about 20% added sucrose. The °Brix values of the products in this study without added sweetener were approximately 9% (data not shown). Thus 4.3% or 5.7% sucrose was used to sweeten elderberry kefir to keep the total °Brix at least 5% lower than commercial products. 0.4% and 0.6% stevia extracts were used to create equal sweetness to the sucrose products respectively. Demographic results showed that participants in this test were balanced for gender (55 female and 45 male) and the major age range was 18-34 years old. All participants in this test responded positively that purchasing health-beneficial food was important.

The consumer acceptability test (Figure 2)

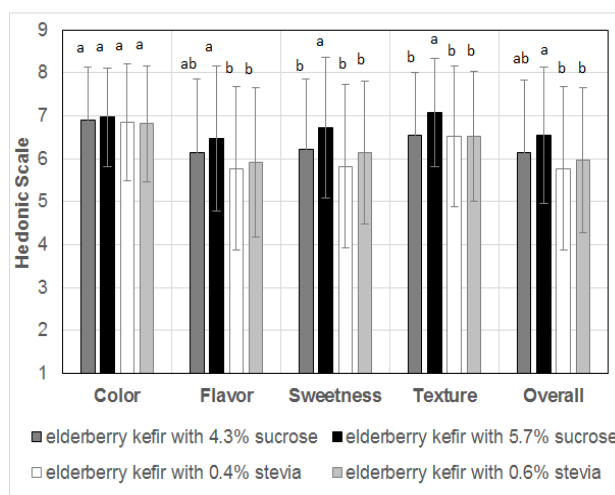


Figure 2. Consumer acceptability of elderberry kefir made with different Sweeteners.

Data are shown as means \pm standard deviation. n=100.

Different letters indicate significant differences among means within each attribute, $p < 0.05$.

demonstrated that the products were accepted equally for color. The best acceptability in sweetness was observed in elderberry kefir sweetened with higher sucrose content (5.7%). Significantly lower ratings were observed in the two products sweetened with stevia extract. This is potentially driven by the unpleasant aftertaste of stevia extract based on the consumer comments. Sweeter products (5.7% sucrose and 0.6% stevia) were accepted better compared to less sweet products (4.3% sucrose and 0.4% stevia). There was no significant difference between two sucrose-sweetened products in this study. A similar trend was observed by Johansen *et al.* (2010). Their study indicated that yogurt sweetened with 13% sucrose significantly increased consumer preference compared to a product sweetened with 9% sucrose. Flavor was rated best in elderberry kefir sweetened with higher sucrose content (5.7%). The acid profile in kefir matrix, added sweetener and elderberry juice contributed to the complex flavor of the elderberry products. Since fermented dairy products have the distinctive sour taste which may be a negative influencing factor on the consumers' acceptability (Narayanan *et al.*, 2014). The addition of sucrose to the products resulted in an increased sweet to sour ratio. This ratio may present a better-balanced flavor compared to the other products. The two products sweetened with stevia extract were not well received for flavor. The reason may be the unpleasant aftertaste of stevia extract. The best texture was received in the elderberry product with 5.7% sucrose. Sucrose contributes to a more viscous texture which was observed in this product. Effervescence in dairy product induced by fermentation may be unfamiliar to consumers and might be an influencing factor. Best

overall acceptability (6.6) was rated in elderberry kefir sweetened with high sucrose content (5.7%), and the product made with low stevia content (0.4%) received the lowest rating (5.8). Similar to what was observed in previous aronia kefir consumer testing, participants' unfamiliarity with either kefir or elderberry may undermine the overall acceptability of the products. In this test, 40% of the consumers had never consumed kefir and 58% of the participants were unfamiliar or had never heard of elderberry. Elderberry kefir products were better accepted by previous kefir consumers compared to the whole participants group (data not shown).

Elderberry kefir quality parameters (commercial juice)

pH, TA, °Brix and color results of elderberry kefir made with commercial juice are shown in Table 2. The pH values demonstrated that the elderberry kefir products were acidic, which is necessary for the stability of bioactive compounds (Friedman and Jurgens, 2000). TA of products sweetened with 5.7% sucrose was the lowest while stevia-sweetened (0.4%) elderberry kefir was the highest. TA represents the sour taste. The sour taste in elderberry kefir was associated with the lactic acid generated by fermentation and the citric acid from the elderberry juice. There was no significant correlation between TA and pH ($r = 0.49$, $p = 0.25$). The highest °Brix value was observed in 5.7% sucrose-sweetened products as expected. Product sweetened with 4.3% sucrose was second highest. Stevia products both had low °Brix values. Color was analyzed for elderberry kefir products. All elderberry kefir products had similar color intensity based on the Chroma values. Hue angle values of all elderberry products were close to 0 (360) indicating that the products presented as a reddish color. High L^* values indicated that the products had a light color. a^* and b^* values of all products were positive. This indicated that elderberry kefir products present light red color. Since anthocyanins present a red color in an acidic environment, this color was reasonable.

Elderberry kefir phytochemical analyses (commercial juice)

Phytochemical results of elderberry kefir made with commercial juice are shown in Table 3. Kefir products made with commercial elderberry juice exhibited a low amount of ANC (0.95 – 1.13mg C3G/100 g kefir) and a moderate amount of TP (18.76 – 20.13 mg GAE/100g kefir). The ANC and TP contents of commercial elderberry juice were 25.59 mg C3G/100 g kefir and 337.28mg GAE/100 g kefir respectively. These amounts were much

lower than the reported values in the literature (Johnson *et al.*, 2015). The recovery rates of ANC and TP were about 40% and 60% respectively (data not shown). Literature suggests several factors may impact ANC content in elderberry or elderberry products. For instance, plant variety and growing conditions can alter anthocyanin content (Johnson *et al.*, 2015; Veberic *et al.*, 2015). Processing, such as thermal treatments or filtration, could result in anthocyanin loss (Sadilova *et al.*, 2007; Wilkes *et al.*, 2014; Szalóki-Dorkó *et al.*, 2016). The processing parameters and elderberry varieties of commercial juice are unknown, an additional set of kefir made with fresh juice was made to better understand the difference between the commercial and fresh elderberry juice. The same product formulas were used. Phytochemical evaluation of the additional elderberry kefir products is discussed in the next section.

Elderberry kefir evaluation (fresh juice)

The results for the biochemical measures in commercial and fresh elderberry juices are shown in Table 2. °Brix and TA values of the fresh elderberry juice were lower than the commercial juice. Color parameter values are different. Based on the Chroma values, fresh elderberry juice had a more saturated color. Phytochemical properties of the fresh and the commercial elderberry juices were measured to better understand the difference (Table 3). The results demonstrated that the ANC content in fresh elderberry juice was 16 times higher than the commercial juice. Higher TP content was observed in fresh elderberry juice. Gonzalez-Molina *et al.* (2012) reveal that anthocyanin content in elderberry juice decreased more than 50% during 56 days storage at room temperature. Elderberry juice should be utilized fresh to ensure the highest levels of anthocyanins in products like elderberry kefir.

A sensory test was not conducted on the products due to insufficient fresh elderberry. pH, TA, °Brix and color results of elderberry kefir made with fresh juice are shown in Table 2. The elderberry kefir products made with fresh juice exhibited similar pH, TA and °Brix values. No correlation was found between pH and TA ($r = 0.22$, $p = 0.61$). Based on the Chroma value, fresh-elderberry-juice-added kefir product with 0.6% stevia had a more saturated color.

Phytochemical analyses results of elderberry kefir made with fresh juice are shown in Table 3. ANC content of kefir made with fresh elderberry juice was 14 times higher than that in the product made with commercial juice. TP content in fresh-juice elderberry kefir was two times higher compared

Table 2. Quality evaluation of aronia kefir, elderberry kefir made with commercial juice, elderberry kefir made with fresh juice and juices.

Product	pH (n=3)	TSS (*Brix n=3)	%TA (w/w n=2)	Color (n=5)					
				L*	a*	b*	Hue angle	Chro ma	
aronia kefir	sucrose	4.4± 0.01	13.2± 0.10	0.64	53.1± 0.26	16.9± 0.29	-4.9± 0.14	359.7 ±0.01	17.6± 0.24
	stevia	4.4± 0.00	8.9± 0.06	0.67	53.2± 0.04	16.7± 0.11	-5.1± 0.04	359.7 ±0.00	17.5± 0.09
	Monk fruit	4.4± 0.01	9.5± 0.12	0.66	54.0± 0.12	16.9± 0.17	-4.6± 0.09	359.7 ±0.01	17.5± 0.14
	4.3%	4.5±	13.1±	0.73	60.5±	10.9±	1.5±	360.1	11.0±
Elderb- erry kefir (comm- ercial juice)	sucrose	0.01	0.06	0.70	0.11	0.02	0.04	±0.00	0.03
	5.7%	4.6±	14.6±	0.70	60.4±	10.9±	1.6±	360.1	11.1±
	sucrose	0.01	0.06	0.77	0.11	0.03	0.05	±0.00	0.04
	0.4%	4.5±	9.2±	0.77	60.8±	10.8±	1.7±	360.2	10.9±
	stevia	0.01	0.00	0.77	0.48	0.07	0.08	±0.01	0.08
	0.6%	4.5±	9.2±	0.75	61.6±	10.6±	1.6±	360.2	10.7±
Elderb- erry kefir (fresh juice)	stevia	0.01	0.06	0.74	0.27	0.16	0.05	±0.00	0.17
	4.3%	4.5±	13.5±	0.74	60.7±	12.0±	0.1±	360.0	12.0±
	sucrose	0.01	0.01	0.74	0.07	0.09	0.06	±0.01	0.09
	5.7%	4.5±	14.9±	0.74	60.6±	12.1±	-0.3±	360.0	12.1±
	sucrose	0.01	0.06	0.76	0.02	0.03	0.04	±0.00	0.03
	0.4%	4.6±	9.1±	0.76	59.9±	12.2±	0.6±	360.1	12.2±
Juice	stevia	0.01	0.06	0.74	0.04	0.03	0.03	±0.00	0.03
	0.6%	4.6±	9.5±	0.74	59.6±	12.7±	0.3±	360.0	12.7±
	stevia	0.01	0.01	0.74	0.09	0.05	0.07	±0.01	0.05
	aronia	3.4±	17.1±	1.15	0.4±	0.1±	-0.3±	358.7	0.3±
	0.00	0.06	1.90	0.01	0.02	0.03	±0.07	0.02	
	comme- rcial elderbe- rry fresh elderbe- rry	4.0± 0.01	20.7± 0.06	1.90	0.3± 0.00	-0.1± 0.01	-0.8± 0.01	361.4 ±0.01	0.8± 0.01
4.3± 0.01	12.5± 0.00	0.55	2.40± 0.12	2.7± 0.22	3.5± 0.22	360.9 ±0.05	4.4± 0.24		

Data are shown as means ± standard deviation except for %TA.

Since hue values were close to 0/360 on hue angle scale, hue angle values were transformed by adding 360 for comparison (McLellan *et al.* 1995).

to the products made with commercial juice. Smaller IC₅₀ values in the products made with fresh elderberry juice were observed. This indicates that kefir made with fresh elderberry juice exhibits stronger antioxidant capacity than the commercial-elderberry-juice kefir. The higher ANC and TP contents in fresh-elderberry-juice kefir due to the larger amount of ANC and TP in the fresh juice. One serving (8oz) of the elderberry kefir products made with fresh juice contains more than 44 mg ANC and 100mg TP. Elderberry kefir products made with fresh juice could contribute to the enhancement of consumers' dietary intake for both ANC and TP. Increased consumption of ANC and TP may contribute to a decrease in the chronic inflammation and in the risk of type 2 diabetes (Wedick *et al.* 2012; Dall'Asta *et al.*, 2015). Therefore, the freshness of juice and a shorter shelf-life are important to maximize the delivery of bioactive compounds.

The recovery rate of ANC in products made with fresh elderberry juice was around 45%. For TP, the recovery rate exceeded 100%. Similarly, increased total phenolics were observed in myrtle berry homogenate after fermentation with *Lactobacillus plantarum* C2 and the main increase was in phenolic acids (Curiel *et al.*, 2015). This study suggested that the increase was due to the esterase activity in the

lactic bacteria. Esterase cleaves the ester bond which liberates the phenolic acids from their glycosides. During fermentation, the constituents of elderberry juice are metabolized by the living kefir culture. This enhancement may lead to a functional food with an increased amount of bioavailable phenolic compounds.

Conclusion

Kefir products made with aronia or elderberry were evaluated for their sensory attributes, quality parameters and phytochemical properties. Both sensory tests indicated that consumers preferred sucrose over non-nutritive sweeteners. Phytochemical results revealed that the freshness of juice was critical for maximum bioactive compounds in the products. The fermentation process contributes to the liberation of phenolic compounds in part by esterase activity and may be important to enhance the bioavailability of bioactive compounds. Further research is needed to better understand the impact of fermentation on bioavailability and liberating the phenolic compounds to potentially increase their absorption. Developing value-added functional food products could be a good way to utilize aronia or elderberries. Kefir products with berries may increase dietary

Table 3. Phytochemical evaluation results of aronia kefir, elderberry kefir made with commercial juice, elderberry kefir made with fresh juice and juices.

Product		Anthocyanin Content (mg C3G/100ml sample)	Total Phenolic Compounds (mg GAE/100ml sample)	DPPH IC ₅₀ (mg sample/ml)
Aronia kefir	Sucrose	17.22 ± 0.17	43.04 ± 1.05	28.84 ± 0.26
	Stevia	16.57 ± 0.44	40.32 ± 1.02	27.59 ± 0.26
	Monk fruit	16.94 ± 0.38	40.37 ± 0.51	27.84 ± 0.26
Elderberry kefir (commercial juice)	4.3% sucrose	1.02 ± 0.22	19.61 ± 0.25	59.19 ± 0.37
	5.7% sucrose	0.95 ± 0.01	18.76 ± 0.19	65.52 ± 0.13
	0.4% stevia	1.13 ± 0.02	19.86 ± 0.32	60.36 ± 0.56
	0.6% stevia	1.06 ± 0.02	20.13 ± 0.58	61.65 ± 0.76
	4.3% sucrose	18.67 ± 0.08	42.31 ± 0.96	20.86 ± 0.43
Elderberry kefir (fresh juice)	5.7% sucrose	20.10 ± 0.14	43.66 ± 1.90	20.36 ± 0.86
	0.4% stevia	17.05 ± 0.06	39.00 ± 0.46	24.11 ± 0.67
	0.6% stevia	18.90 ± 0.07	43.50 ± 0.82	20.68 ± 0.55
	aronia	275.64 ± 3.18	604.49 ± 14.90	1.54 ± 0.04
Juice	commercial elderberry	25.59 ± 1.58	337.28 ± 4.75	4.39 ± 0.03
	fresh elderberry	416.92 ± 3.18	369.47.59 ± 1.38	2.14 ± 0.01

Data are shown as means ± standard deviation.

intake of anthocyanins and total phenolics, which may contribute to the prevention of type 2 diabetes and other inflammatory chronic diseases.

Acknowledgments

The authors are very grateful to the following contributors to this study: Dr. John Brewer from Wyldewood Cellars for his generous gift of elderberry juice and Dr. Mark Brand from the University of Connecticut for growing the aronia berries, and the authors would like to acknowledge Dr. Mary Ellen Camire, the director of Sensory Evaluation Center of University of Maine.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

- Altay, F., Karbancioglu-Guler, F., Daskaya-Dikmen, C. and Heperkan, D. 2013. A review on traditional Turkish fermented non-alcoholic beverages: microbiota, fermentation process and quality characteristics. *International Journal of Food Microbiology* 167(1): 44-56.
- Arslan, S. 2015. A review: chemical, microbiological and nutritional characteristics of kefir. *Cyta-Journal of Food* 13(3): 340-345.
- Bourrie, B. C., Willing, B. P. and Cotter, P. D. 2016. The microbiota and health promoting characteristics of the

fermented beverage kefir. *Frontiers Microbiology* 7: 647.

- Cardello, H., Da Silva, M. and Damasio, M. H. 1999. Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. *Plant Foods for Human Nutrition* 54(2): 119-130.
- Chang, S. K., Alasalvar, C. and Shahidi, F. 2016. Review of dried fruits: Phytochemicals, antioxidant efficacies, and health benefits. *Journal of Functional Foods* 21: 113-132.
- Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., Han, J. T. and Lee, C. Y. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture* 85(10): 1715-1724.
- Curiel, J. A., Pinto, D., Marzani, B., Filannino, P., Farris, G. A., Gobetti, M. and Rizzello, C. G. 2015. Lactic acid fermentation as a tool to enhance the antioxidant properties of *Myrtus communis* berries. *Microbial Cell Factories* 14(1): 67
- Dall'Asta, M., Bayle, M., Neasta, J., Scazzina, F., Bruni, R., Cros, G., Rio, D.D. and Oiry, C. 2015. Protection of pancreatic β -cell function by dietary polyphenols. *Phytochemistry Reviews* 14(6): 933-959.
- de Oliveira Leite, A. M., Miguel, M. A. L., Peixoto, R. S., Rosado, A. S., Silva, J. T. and Paschoalin, V. M. F. 2013. Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. *Brazilian Journal of Microbiology* 44(2): 341-349.
- De Vrese, M., Keller, B. and Barth, C. A. 1992. Enhancement of intestinal hydrolysis of lactose by microbial beta galactosidase EC. 3.2.1.23 of kefir. *British Journal of Nutrition* 67(1): 67-75.
- Denev, P. N., Kratchanov, C. G., Ciz, M., Lojek, A.

- and Kratchanova, M. G. 2012. Bioavailability and antioxidant activity of black chokeberry (*aronia melanocarpa*) polyphenols: in vitro and in vivo evidences and possible mechanisms of action: a review. *Comprehensive Reviews in Food Science and Food Safety* 11(5): 471-489.
- Duymus, H. G., Goger, F. and Baser, K. H. 2014. *In vitro* antioxidant properties and anthocyanin compositions of elderberry extracts. *Food Chemistry* 155: 112-119.
- Fernandes, I., Faria, A., de Freitas, V., Calhau, C. and Mateus, N. 2015. Multiple-approach studies to assess anthocyanin bioavailability. *Phytochemistry Reviews* 14(6): 899-919.
- Friedman, M. and Jurgens, H. S. 2000. Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry* 48(6): 2101-2110.
- Gonzalez-Molina, E., Girones-Vilaplana, A., Mena, P., Moreno, D. A. and Garcia-Viguera, C. 2012. New beverages of lemon juice with elderberry and grape concentrates as a source of bioactive compounds. *Journal of Food Science* 77(6): C727-C733.
- Harris, C. S., Cuerrier, A., Lamont, E., Haddad, P. S., Arnason, J. T., Bennett, S. A. and Johns, T. 2014. Investigating wild berries as a dietary approach to reducing the formation of advanced glycation endproducts: chemical correlates of in vitro antiglycation activity. *Plant Foods for Human Nutrition* 69(1): 71-77.
- Hunaefi, D., Gruda, N., Riedel, H., Akumo, D. N., Saw, N. M. M. T. and Smetanska, I. 2013. Improvement of antioxidant activities in red cabbage sprouts by lactic acid bacterial fermentation. *Food Biotechnology* 27(4): 279-302.
- Jennings, A., Welch, A. A., Spector, T., Macgregor, A. and Cassidy, A. 2014. Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. *The Journal of Nutrition* 144(2): 202-208.
- Johansen, S. B., Næs, T., Øyaas, J. and Hersleth, M. 2010. Acceptance of calorie-reduced yoghurt: Effects of sensory characteristics and product information. *Food Quality and Preference* 21(1): 13-21.
- Johnson, M. C., Thomas, A. L. and Greenlief, C. M. 2015. Impact of frozen storage on the anthocyanin and polyphenol contents of American elderberry fruit juice. *Journal of Agricultural and Food Chemistry* 63(23): 5653-5659.
- Kokotkiewicz, A., Jaremicz, Z. and Luczkiewicz, M. 2010. Aronia Plants: A review of traditional use, biological activities, and perspectives for modern medicine. *Journal of Medicinal Food* 13(2): 255-269.
- KoŁAkowski, P. and Ozimkiewicz, M. 2012. Restoration of kefir grains subjected to different treatments. *International Journal of Dairy Technology* 65(1): 140-145.
- Lee, J., Durst, R. W. and Wrolstad, R. E. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of Aoac International* 88(5): 1269-1278.
- Lee, M.-Y., Ahn, K.-S., Kwon, O.-K., Kim, M.-J., Kim, M.-K., Lee, I.-Y., Oh, S.-R. and Lee, H.-K. 2007. Anti-inflammatory and anti-allergic effects of kefir in a mouse asthma model. *Immunobiology* 212(8): 647-654.
- Li, X. E., Lopetcharat, K. and Drake, M. A. 2015. Parents' and children's acceptance of skim chocolate milks sweetened by monk fruit and stevia leaf extracts. *Journal of Food Science* 80(5): S1083-1092.
- Maria Salinas-Hernandez, R., Gonzalez-Aguilar, G. A. and Ernesto Tiznado-Hernandez, M. 2015. Utilization of physicochemical variables developed from changes in sensory attributes and consumer acceptability to predict the shelf life of fresh-cut mango fruit. *Journal of Food Science and Technology-Mysore* 52(1): 63-77.
- Masisi, K., Beta, T. and Moghadasian, M. H. 2016. Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. *Food Chemistry* 196: 90-97.
- McLellan, M. R., Lind, L. R. and Kime, R. W. 1995. Hue angle determinations and statistical-analysis for multiquadrant hunter L,a,b data. *Journal of Food Quality* 18(3): 235-240.
- Mena, P., Garcia-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D. and Marti, N. 2011. Phytochemical characterisation for industrial use of pomegranate (*Punica granatum* L.) cultivars grown in Spain. *Journal of the Science of Food and Agriculture* 91(10): 1893-1906.
- Narayanan, P., Chinnasamy, B., Jin, L. and Clark, S. 2014. Use of just-about-right scales and penalty analysis to determine appropriate concentrations of stevia sweeteners for vanilla yogurt. *Journal of Dairy Science* 97(6): 3262-3272.
- Orjuela-Palacio, J. M., Zamora, M. C. and Lanari, M. C. 2014. Consumers' acceptance of a high-polyphenol yerba mate/black currant beverage: Effect of repeated tasting. *Food Research International* 57: 26-33.
- Porto Cardoso, J. M. and Andre Bolini, H. M. 2008. Descriptive profile of peach nectar sweetened with sucrose and different sweeteners. *Journal of Sensory Studies* 23(6): 804-816.
- Riso, P., Klimis-Zacas, D., Del Bo, C., Martini, D., Campolo, J., Vendrame, S., Moller, P., Loft, S. and Porrini, M. 2013. Effect of a wild blueberry (*Vaccinium angustifolium*) drink intervention on markers of oxidative stress, inflammation and endothelial function in humans with cardiovascular risk factors. *European Journal of Nutrition* 52(3): 949-961.
- Rodrigues, K. L., Caputo, L. R. G., Carvalho, J. C. T., Evangelista, J. and Schneedorf, J. M. 2005. Antimicrobial and healing activity of kefir and kefir extract. *International Journal of Antimicrobial Agents* 25(5): 404-408.
- Sadilova, E., Carle, R. and Stintzing, F. C. 2007. Thermal degradation of anthocyanins and its impact on color and *in vitro* antioxidant capacity. *Molecular Nutrition and Food Research* 51(12): 1461-1471.
- Sarkar, S. 2007. Potential of kefir as a dietetic beverage - a

- review. *British Food Journal* 109(4-5): 280-290.
- Satir, G. and Guzel-Seydim, Z. B. 2015. Influence of Kefir fermentation on the bioactive substances of different breed goat milks. *LWT - Food Science and Technology* 63(2): 852-858.
- Ścibisz, I., Ziarno, M., Mitek, M. and Zaręba, D. 2012. Effect of probiotic cultures on the stability of anthocyanins in blueberry yoghurts. *LWT - Food Science and Technology* 49(2): 208-212.
- Seymour, E. M., Warber, S. M., Kirakosyan, A., Noon, K. R., Gillespie, B., Uhley, V. E., Wunder, J., Urcuyo, D. E., Kaufman, P. B. and Bolling, S. F. 2014. Anthocyanin pharmacokinetics and dose-dependent plasma antioxidant pharmacodynamics following whole tart cherry intake in healthy humans. *Journal of Functional Foods* 11: 509-516.
- Sidor, A. and Gramza-Michałowska, A. 2015. Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – a review. *Journal of Functional Foods* 18: 941-958.
- Szaloki-Dorko, L., Csizmadia, G., Abranko, L. and Steger-Mate, M. 2015. Examination of anthocyanin content of some elderberry cultivars grown in Hungary. In Thomas, A.L. (Ed.). I International Symposium on Elderberry 1061: 79-86.
- Szalóki-Dorkó, L., Stéger-Máté, M. and Abrankó, L. 2016. Effects of fruit juice concentrate production on individual anthocyanin species in elderberry. *International Journal of Food Science and Technology* 51(3): 641-648.
- Temiz, H. and Kezer, G. 2015. Effects of fat replacers on physicochemical, microbial and sensorial properties of kefir made using mixture of cow and goat's milk. *Journal of Food Processing and Preservation* 39(6): 1421-1430.
- Veberic, R., Slatnar, A., Bizjak, J., Stampar, F. and Mikulic-Petkovsek, M. 2015. Anthocyanin composition of different wild and cultivated berry species. *LWT - Food Science and Technology* 60(1): 509-517.
- Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* 46(10): 4113-4117.
- Wedick, N. M., Pan, A., Cassidy, A., Rimm, E. B., Sampson, L., Rosner, B., Willett, W., Hu, F. B., Sun, Q. and van Dam, R. M. 2012. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *American Journal of Clinical Nutrition* 95(4): 925-933.
- Wilkes, K., Howard, L. R., Brownmiller, C. and Prior, R. L. 2014. Changes in chokeberry (*aronia melanocarpa* L.) polyphenols during juice processing and storage. *Journal of Agricultural and Food Chemistry* 62(18): 4018-4025.
- Wu, T., Tang, Q., Yu, Z., Gao, Z., Hu, H., Chen, W., Zheng, X. and Yu, T. 2014. Inhibitory effects of sweet cherry anthocyanins on the obesity development in C57BL/6 mice. *International Journal of Food Sciences and Nutrition* 65(3): 351-359.
- Wu, X. L., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E. and Prior, R. L. 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry* 54(11): 4069-4075.
- Youdim, K. A., Martin, A. and Joseph, J. A. 2000. Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. *Free Radical Biology and Medicine* 29(1): 51-60.
- Young, K. N., Man, R. J. and Yong, L. H. 2015. Effect of lactic acid fermentation on cognitive activities of aronia merlanocarpa elliot. *Research Journal of Biotechnology* 10(9): 80-89.
- Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P. and Li, H. B. 2015. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules* 20(12): 21138-21156.