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Haskap Juicing Method Effects on Haskap Juice Quality

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Abstract: This research is the first study on the influence of juicing methods on the 'Aurora' haskap juice quality. Three common juice extraction methods, namely, using a steam juicer, a centrifugal juicer, and a bladder wine press, were applied for haskap juice extraction. Both physicochemical assays and tests of antioxidant activities were employed to evaluate the qualities of the haskap juice. Generally, the centrifugal juicer increased and the steam juicer reduced concentrations of measured juice components relative to the press. The juice from the centrifugal juicer had the highest cloudiness. Sugar concentrations were about 40% lower in steam juice compared to the centrifugal juicer. Pressed juice had a slightly lower soluble solid content than the juice made with the centrifugal juicer and concentrations of glucose and fructose were similar between these methods. The methods altered pH and malic acid content without affecting the concentrations of tartaric and malic acids. Similar effects of juicing methods were seen in secondary compounds associated with health benefits and antioxidant capacities. Anthocyanin concentrations in press and centrifugal juicer extracts were similar, about 1.6 times higher than steam juicer extracts. Total phenolics and antioxidant activities were from two to four times higher in the centrifugal juicer extraction compared to steam juicing. Ascorbic acid and total flavonoid content in the haskap juice was increased by centrifugal juicing and decreased by steam juicing compared to the press. Overall, the juice extracted with the centrifugal juicer had concentrations of juice components that were 1.5–2 times higher than the steam juicer. The pressed juice had similar to slightly lower concentrations of components compared to the centrifugal juicer.

Keywords: haskap; Aurora; juice quality; antioxidants

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1. Introduction

Haskap (*Lonicera caerulea* L.), an emerging commercial berry, has been known for its health benefits among the people of Russia, Japan, and Northeastern China for centuries [1]. Japanese aborigines called it the "elixir of life". Haskap berries are rich in phenolic compounds and ascorbic acid, which can act as antioxidants within biological systems, promoting health [2]. Haskap consumption has also been reported to promote anti-inflammatory, anti-cancer, anti-diabetic, neuroprotective, and cardioprotective health benefits [1,3,4]. Previous studies asserting implications for human health were based on in vitro analysis, as well as preclinical trials in animal models and humans [5,6]. Haskaps' bioactive components include anthocyanins and phenolics, such as cyanidin-3-glucoside, chlorogenic acid, ferulic acid, and rutin [7]. Although it is well known that haskap berries are high in anthocyanins and overall phenolic content compared to some other berries, such as blueberries, the influence of processing procedures on berry basic nutrients remains largely unexamined. Here we assess and compare three common small-scale juicing methods for their effect on berry health attributes, examining the use of a steam juicer, fruit juicer, and fruit press.

There is no optimal juice extraction method for various fruits and vegetables, but methods differ from fruits' and vegetables' juice extraction efficiencies, juice stability, quality, and nutritional values [8]. Household processing techniques usually focus on blending,

juicing, and hand pressing [9]. Previous work has compared the centrifugal juicer, cold press (hydraulic press) juicer, and blenders for the extraction of juice from pineapple, guava, carrot, red dragon fruit, and white dragon fruit, showing superior results from the cold press juicer [10]. Some disadvantages of juicing methods compared to the blending extraction method include a potential reduction in antioxidants and phenolic compounds [11]. Blending, high-speed centrifugal juicing, and low-speed juicing vegetable extraction result in varied metabolites and antioxidant activities [12]. Cooking methods, such as blanching, have also been compared with juicing effects on flavonoids and tannin content in leafy greens [13]. Physico-mechanical properties (dimensional, gravimetrical, frictional, and aerodynamic) are critical to the design of juicing machines [14]. Steam juicer juicing methods had been used in juice extraction and preservation, and are demonstrated to maintain juice microbiological stability, although it may also alter phytochemical compounds and antioxidant capacity [15–17]. The fruit wine press is another widely used extraction method, used for macerated or non-macerated grapes and fruits before, during, or after fermentation [18].

On an industrial scale, advanced techniques are applied for the extraction of juice from fruits and vegetables. Thermal processing, high pressure processing, or combined treatments are often employed to target optimal flavor and storability [19]. Pre-storage processing treatments, such as high pressure and pulsed electric field processing have positive effects on storage for juices [20]. High pressure pasteurization helps maintain juice quality and extend the shelf life of juices [21]. Freeze crystallization has also shown success in maintaining juice qualities while promoting the preservation of juice nutritional properties [22]. UV-C radiation is another method used to maintain juice quality during storage [23]. The different scale of and type of operation (home gardening, U-pick, commercial, etc.) for haskap required different extraction methods and corresponding evaluation.

The evaluation system of juice quality in publications is typically focused on (1) basic physiochemical qualities, which include juice yield, soluble sugar, pH, acidity, color, cloud value, and viscosity; (2) sensory evaluation, which include human-based evaluation of appearance, texture, aroma, and flavors; (3) health qualities and safety concerns, which might include assays of sugar content, vitamin C, antioxidants, phenolics, flavonoids, and microorganisms; and (4) identification of enzymatic activities and by-products [24–27]. Colloidal stability and enzymatic browning are also part of the evaluation for apple juice and orange juice [28]. Meanwhile, blending juices is an emerging market trend and been evaluated often, including aspects such as the blended juice ratios and their potential storage length [11]. Finally, different brands of juices are typically evaluated to ensure they meet the requirement of customers [29].

Since haskap is a new commercial berry, only few publications are available for haskap juice extraction. A lab-scale wine press extraction method has been evaluated by comparing thawing and osmotic treatment on haskap juice quality at varying times during the pressing process [30]. Juicing techniques have not been widely applied to haskap fruits in both research and commercial industry. Due to the recent interest in haskap production, processing, and marketing, this work evaluates three haskap juicing methods (steam juicer, centrifugal juicer, and wine press), comparing these techniques for multiple characteristics, including basic physiochemical and nutraceutical properties. These lab-scale operations will provide some useful information for expanding haskap processing methods.

2. Materials and Methods

2.1. Chemicals

All chemicals in this experiment were of analytical grade. Methanol, Folin-Cioalteu reagent, catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitrite, sodium carbonate, sodium hydroxide, aluminum trichloride, potassium chloride buffer, sodium acetate buffer, and Polyvinylpolypyrrolidone (PVPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Juice Preparation

Haskap of the 'Aurora' variety was cultivated and harvested at the Western Agricultural Research Center of Montana State University in 2022. Three equal amounts (5 kg) of each replicate of frozen 'Aurora' haskap were used for extracting the juice by three methods (juicer, steam juicer, and wine press) after being defrosted overnight. Steam juice was extracted for 50 min using a Mr. Rodolf Steam Juicer for each amount (Mr. Rodolf, Rock Hill, SC, USA). Juice was extracted from a centrifugal juicer using a Hamilton Beach Juicer Machine with a speed of 13,000 rpm (Hamilton, Reno, NV, USA). A fruit press (SQUEEZE master, Elk Grove Village, IL, USA) with a pressure of 3 Pa was used to press the haskap juice.

2.3. Juice Physiochemical Measurement

The juice was measured for its soluble solids content (SSC [°Brix]) using an Atago 3810 (PAL-1) digital pocket refractometer (ATAGO, Tokyo, Japan), and the pH was measured with an Atago PAL-pH meter (ATAGO, Tokyo, Japan).

Cloud values, a juice clarity parameter, were measured after 5 mL samples were centrifuged (Thermo Fisher, Waltham, MA, USA) at 1000× *g* for 10 min at room temperature in 15 mL centrifuge tubes. Cloud values were determined using a 10 mm pathlength cuvette at 660 nm using a spectrophotometer (Agilent, Santa Clara, CA, USA) with diH_2O as the blank [31].

2.4. Carbohydrate Content Analysis

According to the assay procedure from the Megazyme Sucrose/Fructose/D-Glucose Assay kit (Neogen, Lansing, MI, USA), highly tinted juice samples were incubated with Polyvinylpolypyrrolidone (PVPP, 0.2 g/10 mL) for 10 min, followed by centrifuging at 4000 rpm for 1 min (Thermo Scientific, Waltham, MA, USA). The supernatant from each haskap juice sample was diluted 1000 times and analyzed using the Megazyme Sucrose/Fructose/D-Glucose Assay kit [32].

2.5. Malic Acid, Citric Acid, and Tartaric Acid Assays

For acid tests, PVPP-treated juice supernatant was diluted 10 times followed by malic acid, citric acid, and tartaric acid assays using an L-malic acid assay kit, citric acid assay, and tartaric acid kit from Megazyme in the processed juices (Neogen, Lansing, MI, USA) [33,34].

2.6. Total Anthocyanin Content

The anthocyanin content was assayed using Wrolstade's method [35]. Each sample was mixed with 9.5 mL chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). The absorbance (A) was measured at 520 nm and 700 nm in each mixed solution. Then, the anthocyanin content was calculated with the equation:

$$\text{Total anthocyanin content } (\text{mg}/100\ \text{mL}) = \frac{\text{A} \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times 1}$$

where, $A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$; MW (molecular weight) of malvidin-3-O-glucoside = 493.2 g. DF is dilution factor, 1 is path length (1 cm), ε is an extinction coefficient (28,000 L/mol/cm), and 1000 is conversion for mg/100 mL.

2.7. Juice Sample Methanol Extraction

The methanol method was used to extract samples with some modifications [36]. Briefly, 5 g of each juice sample was mixed with 25 mL ice-cold 95% methanol (*v*/*v*) and homogenized for 2 min. The homogenates were kept at 4 °C in the dark for 12 h and then centrifuged at 16,100× *g* for 20 min. The supernatants were recovered and stored at −20 °C until analysis for phenolics and antioxidant assays.

2.8. Total Phenolic Assays

The Folin–Ciocalteau (FC) method with a slight modification was used to test the total phenolic content of the juice sample. 0.02 mL of sample extracts were mixed with 0.2 mL of methanol [37]. 0.2 mL of Folin–Ciocaulteau reagent (1:10) was added and mixed. After 3 min, 0.2 mL of 1 N Na_2CO_3 solution was added, and distilled water was added to fill up to a total volume of 2.5 mL. The mixture was kept in darkness at room temperature for 30 min, then the absorbance was measured at 760 nm using a Cary 60 UV-Vis spectrophotometer (Agilent, USA). The results were expressed in mg gallic acid equivalent/100 mL (mg GAE/100 mL) of each sample.

2.9. Antioxidant Capacity Assays

2.9.1. Total Antioxidant Capacity Assessment (DPPH)

The antioxidant activity of juice was evaluated using DPPH radical scavenging assay [38]. For the analysis, 0.02 mL of juice extract was mixed with 0.02 mL of DMSO, then subsequently mixed with 2.96 mL of freshly prepared 0.1 mM DPPH solution. The mixture was reacted in the dark for 30 min at room temperature. Spectrophotometric absorbance at 516 nm was measured afterwards. Three mL of DPPH was taken as the control. The radical scavenging activity was calculated with the equation:

$$\text{RSA (\%)} = (\text{Absorbance of control} - \text{Absorbance of sample})/\text{Absorbance of control} \times 100.$$

2.9.2. FRAP Assay

A ferric reducing antioxidant power (FRAP) assay kit (Sigma-Aldrich, USA) was used to measure antioxidant capacity [39]. Each sample was tested three times and its antioxidant capacity was converted into mM Ferrous Equivalent (nmol/μL).

2.10. Total Flavonoid Content

The total flavonoid content was analyzed using the aluminum chloride colorimetric method [40]. Serial methanol diluted quercetin solution from 5 to 200 μg/mL was prepared as the standard. 0.6 mL of the diluted standard quercetin solution or juice sample extracts was separately mixed with 0.6 mL of 2% aluminum chloride. The mixture was thoroughly mixed and allowed to stand for one hour at room temperature. Absorbance was measured with a UV spectrophotometer (Agilent, USA). The total flavonoid was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of each sample.

2.11. Measurement of Juice Ascorbic Acid Amount

The amount of ascorbic acid amount in the haskap juices was determined using the colorimetric method [41,42]. Firstly, haskap juices were centrifuged at 13,000× *g* for 10 min at room temperature to remove the insoluble materials in the processed juice in advance before the estimated ascorbic acid amount was obtained using an ascorbic acid assay kit (MAK074, Sigma-Aldrich, USA). The 570 nm Absorbance colorimetric results in each sample were recorded through a SPECTROstar Nano Microplate Reader (BMG, New York, NY, USA). Each treatment had three replicates with three technical replicated tests in plates. The concentration of ascorbic acid in samples was evaluated as ng/μL.

2.12. Statistical Analysis

Each treatment was examined using three biological replicates, and each sample was measured via three technical replicates for each assay. Data were assessed for normality and transformed when appropriate. Results were analyzed by one-way analysis of variance (ANOVA) and mean values were separated using Tukey's post hoc test at the significance level of $p < 0.05$.

3. Results and Discussion

3.1. General Physicochemical Properties and Cloud Values

The centrifugal juicer juicing method had the highest SSC (19.17 ± 0.50), followed by the press (16.97 ± 1.55) and steam juicer-processed juice (11.20 ± 1.78) (Table 1). All three methods had significant differences in terms of Brix level. Juice treatments differed in terms of the pH of the final juice. The steam juicer had higher pH than the press and the pH of the centrifugal juicer was the lowest. Ordinarily, optimal wine must have a pH in the range of 3.0–3.5, which increases the wine stability and reduces wine contamination [43]. In this research, the pH was typically around 3.00 for all treatments, indicating that the juice products could be used directly for fruit wine fermentation, which might expand the market of haskap fruits.

Table 1. Basic physicochemical properties of the processed juice.

Treatment	Brix	pH	Cloud Value
Steam juicer	11.20 ± 1.78 ^{a}	3.14 ± 0.04 ^{a}	0.96 ± 0.18 ^{a}
Press	16.97 ± 1.55 ^{b}	3.07 ± 0.04 ^{b}	1.00 ± 0.10 ^{a}
Juicer	19.17 ± 0.50 ^{c}	2.97 ± 0.07 ^{c}	1.51 ± 0.17 ^{b}

Each analysis by each column was represented by the mean ± standard deviation results of triplicate sample analysis from each treatment. Different superscript letters in the same column indicate significant difference at $p < 0.05$ separated via Tukey's HSD.

A juice cloud is a complex mixture of pectin, proteins, polysaccharides, and lipids [44]. In orange juice, the juice cloud and cloud stability are crucial for orange storage and marketability. Enzymatic treatment and other high techniques have studied the improvement of cloud stability of certain juices [45]. Haskap juice markets are new compared with orange and apple juice, so the desirable level of cloud is undefined. Although freezing is still the main preservative method for haskap fruit [46], otherwise, haskap juice research is currently too limited to provide enough information for processors [47]. According to our results, steam juicing and pressing resulted in less cloudy juice than the centrifugal juicer (Table 1). Although clarifying agents can be used to increase the visual clarity of different kinds of fruits, for haskap juice, sensory evaluation may be required as an assessment method before defining and refining clarity targets and clarifying methods [48].

3.2. Carbohydrate Content in Processed Haskap Juice

Fructose, glucose, sucrose are the main simple carbohydrates (i.e., sugars) in fruits [49]. The amount of D-glucose and D-fructose differed among juice extraction methods (Table 2). Steam juicing resulted in juice with lower fructose and glucose content than the juice made with the press and centrifugal juicer. Regardless of juicing method, the concentrations of glucose and fructose were similar, which was similar to other publications about haskap carbohydrates [50]. No sucrose was detected in each treatment by enzymatic assays. Sucrose was also not detected by HPLC in some prior research; however, a trace quantity of sucrose (less than 0.2 g/100 FW) was detected in some publications [3,51]. Ultimately, the sucrose amount may be trivially low in both haskap fruit and haskap juice based on this and prior research.

Table 2. Evaluation of the processed juice carbohydrates.

Treatment	D-Glucose (g/L)	D-Fructose (g/L)	Sucrose (g/L)
Steam juicer	44.17 ± 7.81 ^{a}	45.63 ± 7.98 ^{a}	ND
Press	62.24 ± 8.48 ^{b}	65.55 ± 9.43 ^{b}	ND
Juicer	69.20 ± 13.14 ^{b}	69.87 ± 14.35 ^{b}	ND

Carbohydrates are measured for fructose, glucose, and sucrose concentration in processed juice. ND indicates none detected. Each assay by each column was represented by the mean ± standard deviation results of triplicate sample analysis from each treatment. Different superscript letters in the same column indicate significant difference at $p < 0.05$ separated via Tukey's HSD.

3.3. Malic Acids, Citric Acid, and Tartaric Acid Amount in Haskap Juices

Our results (Table 3) indicated that citric acid was present at approximately three times the quantity of malic acid in each treatment. The quantity of citric acid did not significantly differ among the three different juicing methods. Malic acid in the steam juicer treated juice was lower than the other two methods, which had a similar malic acid content to each other. Tartaric acids in our juices were approximately similar across the different treatments, and the total quantity of tartaric acid was lower than citric acid and malic acids.

Table 3. Malic acid, citric acid, and tartaric acid amounts in processed juice.

Treatment	Malic Acid (g/L)	Citric Acid (g/L)	Tartaric Acid (g/L)
Steam juicer	1.09 ± 0.37^{a}	3.54 ± 0.38^{a}	0.64 ± 0.22^{a}
Press	1.7 ± 0.42^{b}	3.18 ± 0.28^{a}	0.54 ± 0.17^{a}
Juicer	1.71 ± 0.14^{b}	3.36 ± 0.11^{a}	0.71 ± 0.24^{a}

Enzymatic method was used to detect malic acid, citric acid, and tartaric acid amounts in processed juice. The specific acid amount by each column was represented by the mean ± standard deviation results of triplicate sample analysis from each treatment. Different superscript letters in the same column indicate significant difference at $p < 0.05$ separated via Tukey's HSD.

Our test results in juices based on enzymatic assays showed similar trend of acids in haskap fruits as has been previously reported [52]. It had been reported that the primary organic acids in 'Aurora' were citric acid, followed by malic acids in the HPLC system [53]. Similarly, Senica et al. also indicated that the growing location caused haskap to have different amounts of acids, but haskap acids were generally comprised of citric acid, malic acid, quinic acid, tartaric acid, shikimic, and fumaric acid [54].

3.4. Total Anthocyanin Content in Haskap Juices

Dark fruits derive their color from anthocyanins and photoprotective agents, with the color often correlated with fruit maturity and quality [55].

Prior reports on ultrasound-assisted extraction of anthocyanins from haskap berries identified five anthocyanins in the extract of haskap berries, namely, cyanidin 3,5-diglucoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-glucoside, and peonidin 3-glucoside [56]. The total anthocyanin content in fresh haskap berries was from about 4 to 7 mg cyd-3-glu/g FW by using pH differential methods [57].

Here, we used the pH differential method to calculate the monomeric anthocyanin content. The results indicate that the juicer and press juicing methods yielded an approximately equal amount of anthocyanin (about 35 mg/100 mL). However, the steam juicer produced juice with 32.5% lower anthocyanin concentrations than the other methods (Figure 1). In our research, although steam juicing could pasteurize food with a higher temperature and extract haskap juice at the same time in principle, it extracted less anthocyanins compared to the other two methods. Furthermore, due to the increases in processing temperature, anthocyanin profiles might also be changed [47].

3.5. Total Phenolics Contents and Antioxidant Activities Affected by Juicing Methods

The total phenolics content of the juices was analyzed spectrophotometrically with the Folin–Ciocalteu method [58]. Therefore, the assays were performed to detect the reductive capacity of the total phenolics in haskap juices. The results (Figure 2) indicated that the juicer produced juices contained about two times amount of total phenolics compared with steam juicer juices (150 GAE mg/mL). The total phenolics in the press juices was approximately 200–250 GAE mg/mL. In our research, we used methanol extraction methods, which were reported to have a relatively high extraction yield compared to acetone extraction methods [59].

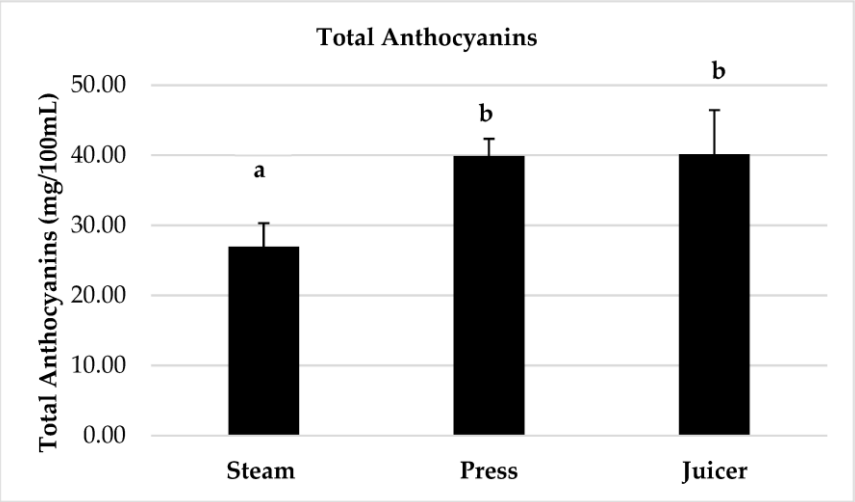

Figure 1. Total anthocyanin content in different juices. Juicing methods include the steam juicer (steam in the figure), wine press (press in figure), and centrifugal juicer (juicer in figure). Each value of each treatment is an average of three replicate measurements ± standard deviation. Different letters above each bar indicate statistically significant difference ($p < 0.05$) separated via Tukey's HSD.

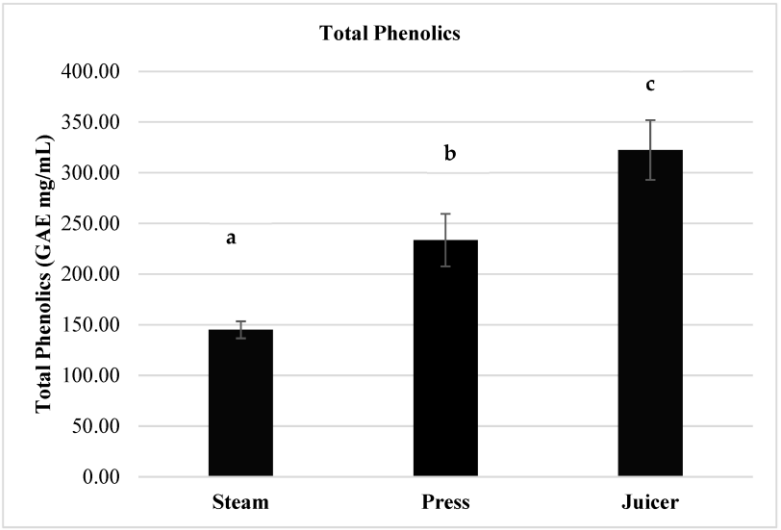

Figure 2. Total phenolics content was affected by haskap juicing methods, including steam juicer, press and the centrifugal juicer. Each method contains three replicates, and each sample has three technical replicates. Total phenolic assay is expressed as gallic acid equivalent (GAE mg/mL). Each value of each treatment is an average of three replicate measurements ± standard deviation. Different letters above each bar indicate statistically significant difference ($p < 0.05$) separated via Tukey's HSD.

It has been reported that the antioxidant capacities in plant materials were largely correlated with total phenolics content [60], and our results also generally supported this. The antioxidant activities were examined via two different assays in haskap juices (Figure 3), DDPH and FRAP. The DPPH assay is considered an accurate and economic method to evaluate the radical scavenging activity of antioxidants in a compound, an extract, or other biological sources [61]. The FRAP assay is also used to determine the antioxidant activities in different tissues from varieties of plants [62]. FRAP showed the similar trend of antioxidants differences in the three treatments, although the differences were not significant. The DPPH assay indicated that antioxidant activities were the highest in centrifugal juicer-processed juices, followed by the juices from the press and steam juicer.

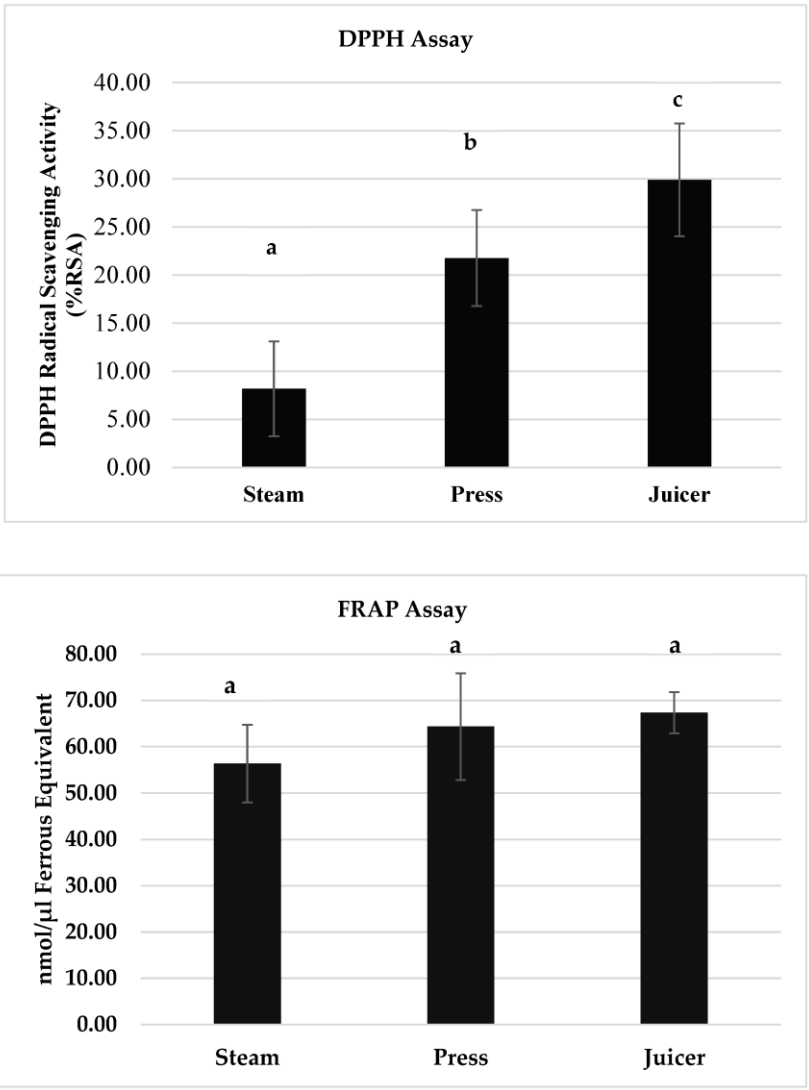

Figure 3. Antioxidant activity assays in haskap juices. The two different antioxidant activity assays are the DPPH assay and FRAP assay. Treatments (x-axis) for processing haskaps are steamer, press, and centrifugal juicer. Total phenolic assay is expressed as gallic acid equivalent (GAE mg/mL); DPPH assay was measured as radical scavenging activity (RSA%); FRAP assay results were converted into nmol/µL ferrous equivalent. Each value of each treatment is an average of three replicate measurements ± standard deviation. Different letters above each bar indicate statistically significant difference ($p < 0.05$) separated via Tukey's HSD.

Although our tests were all determined by colorimetry, the principles of detecting antioxidants and antioxidant activity were different. The [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) assay was based on an antioxidant reaction with free organic radicals. The total phenolic assay was performed using Folin–Ciocalteu solution to detect the reductive capacity of antioxidants. The FRAP assay was performed to measure the antioxidant potential through the reduction of Fe (III) to Fe (II) by antioxidants [22].

For total phenolics assays, in alkaline conditions (Na_2CO_3 in this research), the dissociation of phenolic proton resulted in the creation of a phenolate ion, which could reduce the Folin–Ciocalteu reagent. Therefore, the electron from the phenolic antioxidants was the assay target. In some reports, the total phenolic assay results can be overstated when compared to data from HPLC techniques [63]. The DPPH assay also screens many bioactive substances, such as polyphenols and flavonoids. Limitations of DDPH include potential reaction with weak antioxidants and the influence from the reaction with solvents [64].

The major limitation of the FRAP assay is that antioxidants must be water soluble. It has been reported that FRAP assay results can be less associated with other antioxidant activity assays [65]. In our research, both the total phenolic assay and DPPH assay indicated there were significant differences ($p < 0.05$) among haskap juices from different processing treatments. Although FRAP also indicated the highest antioxidants from juicer-processed juices, followed by press, and then steamed haskap juices, juicing methods were similar in this assay, which might be due to lower effect size or the amount of water-soluble antioxidants (Figure 3). In conclusion, steam extraction might dilute or break down more antioxidants compared to press and juicers, whereas the centrifugal juicer-processed haskap juice had more antioxidants compared to press-processed haskap juices in the same volume.

3.6. Ascorbic Acid Content in Haskap Juices

Vitamin C (ascorbic acid) is well known for its antioxidant properties [66]. Haskap has high levels of ascorbic acid and anthocyanin content in comparison to other berries, such as blueberries and raspberries [67]. Here we also estimated the ascorbic acid content in processed juices (Figure 4). The results showed patterns similar to total antioxidant capacities. The centrifugal juicer juices contained the most the ascorbic acid (about 250 ng/μL), followed by the press with 225 ng/μL ascorbic acid content and the steam with the lowest amount of ascorbic acid (about 150 ng/μL). Since the stabilities of ascorbic acid depends on air exposure and temperature, it is worth optimizing the processing methods in the future [68].

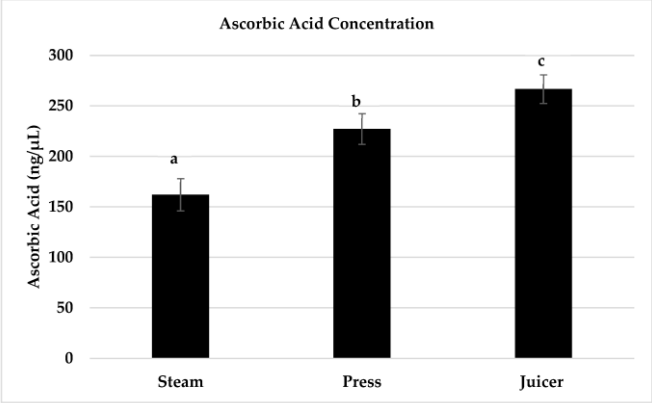

Figure 4. Ascorbic acid content in steam juicer-, press- and centrifugal juicer-processed samples. Ascorbic acid concentration was evaluated as ng/μL in each sample. Each sample had three replicates in the assay. Different letters above each bar indicate statistically significant difference ($p < 0.05$) separated via Tukey's HSD.

3.7. Total Flavonoid Contents in Processed Haskap Juices

Flavonoids, as a class of polyphenolic secondary metabolites in plants, play very important roles in biological activities in plants [69]. Flavonoids are responsible for the color and aromas of flowers and fruits and abiotic and biotic stress resistance. For human and animal health, flavonoids are applied to neurosciences, diabetes and obesity treatment, cancer treatment, and cardiovascular disease treatment [70]. In our research, the centrifugal juicer has demonstrated advantages for the extraction of flavonoids (Figure 5), which were about 40 mg QE/100 g higher than the processed juices obtained from the steam juicer method, and more than 10 mg QE/100 g flavonoids compared to the press method. Corresponding to the total phenolic contents in each treatment (Figure 2), the total flavonoid contents in three treatments showed similarly ranking trends by method.

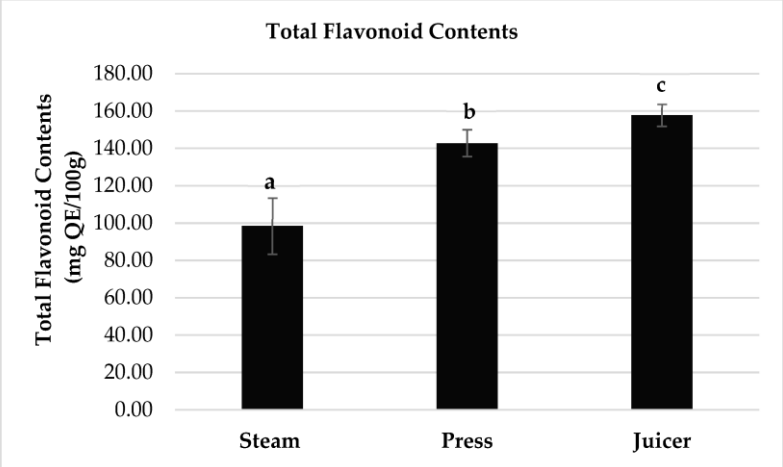

Figure 5. Total flavonoid contents in processed haskap juices. The flavonoid is measured in haskap juice methanol extracts. The values are estimated as mg quercetin equivalent (QE)/100 g. Each value of each treatment is an average of three replicate measurements ± standard deviation. Different letters above each bar indicate statistically significant difference ($p < 0.05$) separated via Tukey's HSD.

Based on our various test results, the steam juicer showed lower flavonoid contents compared with the other two methods, and the haskap products from the centrifugal juicer contained more components and nutritional compounds. The mechanisms of the juicing methods might explain some of our results. The centrifugal juicer uses spinning blades and a high-speed centrifuge to extract every nutrient from the fruits. Therefore, the juice usually contains lots of pulp. This might explain why the haskap juices obtained from the centrifugal juicer contained the most components. The steam juicer processes juice through the steaming process, which could reduce microorganisms and most of the enzymes in the fruits. Due to the high temperature, many of the health benefits associated with fruit juice can be destroyed [71]. With the bladder wine pressmethod, water pressure is used to press the fruits, more gently than the centrifugal juicer, and accordingly it contained a moderate amount of haskap components compared with the other two methods in our research.

Although it had been reported that haskap has a high juice yield, its nutrition was easily destroyed by thermal processing [72]. To improve its juice quality and customer consumption, haskap juicing methods need to be studied and developed further. Juicing processes from other fruits may be examined and applied to haskap research. For example, fully automatic fresh apple juicers have been developed to make the juicing process more convenient [73]. Likewise, banana juicing machines functions on several processes, such as kneading, compressing, and filtration [74]. Analytically, Fourier transform infrared (FT-IR) spectroscopy has been applied for the evaluation of juice qualities [75]. Ohmic heating methodology may also be applied to heat rapidly and uniformly, and its treatment could have a positive effect on juice qualities [76]. Cold plasma treatment is another technique that has been shown to effectively reduce mycotoxins and positively affect the physical stability and brightness of jujube juice [77]. The high-pressure carbon dioxide (HPCD) technique as a non-thermal technology could be applied to several inactive enzymes, ultimately elongating the juice's storage life [28]. Pasteurization systems, such as helical heat exchanger and ohmic heating, could not only deactivate bacteria and endogenous enzymes, but it also could influence favorable sensory and nutritional characteristics [78]. Advanced technologies may be helpful in future haskap juice research, such as ultra-high-performance liquid chromatography or ion-trap time-of-flight mass spectrometry [12]. Supercritical fluid extraction of iridoids and fatty acids have previously been applied for haskap [17]. The dynamic streamlined extraction process will benefit the broad applicability of haskap. Further, membrane technology may be applied for clarifying juices if deemed appropriate [79,80].

Mixed fruit juice might be a potential new market for haskap because haskap berries contain high amounts of anthocyanins, phenolics, flavonoids, and vitamins. Meanwhile, haskap is also a candidate for fermentation among specialty fruits, and blending or pure juice fermentation may be used to create marketable fermented products. Comparative evaluation of juice preservation and the fermentation products from different treatments is also a great topic worthy of deeper studies [81,82]. Beyond techniques and chemistries, consumer acceptability also warrants further research to expand the broader markets for haskap and other specialty fruits.

4. Conclusions

This study showed that haskap lab-scale juicing methods influence most juice qualities, from basic physicochemical characteristics to nutritional values. In general, the metrics differed among all three methods with centrifugal juicer juice have the highest values of multiple compounds, the wine press with intermediate values, and the lowest values in the steam juices.

In terms of sugar and acid content, the differences among the juices obtained from the three methods varied by assay. The centrifugal juicer produced haskap juice with a sugar content 1.7 times higher than juice produced by steam juicing across SCC and specific sugar types. Juice made with centrifugal juicer had higher SCC than the pressed juice, but glucose and fructose levels were similar between these two methods. Similarly, the effects of juicing methods on juice acids and acidity varied among metrics. All methods differed in pH (centrifugal < press < steam). Steam juicing reduced levels of malic acid but levels of the most abundant acid (citric) were similar among juice methods.

In terms of nutraceutical benefits, centrifugal juicers seemed to extract and/ or retain more beneficial compounds. The DPPH assay of antioxidant capacity provided some of the most stark effects of juice methods with the juice for the centrifugal juicer have four times the antioxidant capacity compared to steam juicing, and 50% greater antioxidant capacity than press juicing. In other measures of "health-promoting" compounds (i.e., total phenolics, ascorbic acid, flavonoids, and anthocyanins), juice made with the centrifugal juicer also had better performances compared to steam-juiced juice.

This study will provide general information for haskap fruit applications. The results suggest that the centrifugal juicer might be the most effective method to extract juice from haskap berries without consideration for the cloud value and sensory evaluations. Further aroma and taste evaluations of haskap juice are necessary to provide more information for processors.

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