EFFECT OF ULTRASOUND AND PASTEURISATION ON BIOACTIVE COMPOUNDS IN SEA BUCKTHORN AND BLACK ELDERBERRY JUICES

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Abstract
Sea buckthorn and black elderberries are valuable ingredients in the food industry. They offer natural colourings, unique flavours, and potential health benefits due to their rich content of bioactive compounds. Pasteurisation (PA) is a widely used method in food processing that extends the shelf life of products and ensures consumer safety. Ultrasound treatment (UT) is a promising technique for enhancing product safety and preserving bioactive compounds in berry juices. This study aimed to investigate the impact of UT and PA on the bioactive compounds and physicochemical properties in black elderberry and sea buckthorn juice. It specifically analyses how PA and UT influence the total polyphenolic compound content (TPC), free radical scavenging activity (DPPH$^•$), and carotenoid content (CC) in sea buckthorn juice, and TPC, DPPH$^•$, and monomeric anthocyanin content (MAC) in black elderberry juice. Additionally, the effects of processing on juice pH and total soluble solids content were examined. The results indicated that UT did not affect the TPC of sea buckthorn juice. In contrast, the TPC of black elderberry juice decreased by 8.3% due to UT, and PA led to an even more significant reduction of 43.8%. Pasteurised sea buckthorn juice had the highest CC. However, the highest levels of MAC were found in untreated and ultrasound-treated black elderberry juice. Furthermore, the DPPH$^•$ results of pasteurised black elderberry juice were lower than those of untreated juice and juice treated with ultrasound. This study highlights UT's potential as an alternative or complementary method to traditional PA for preserving health-promoting compounds in berry juices.
Keywords: *Sambucus nigra*, *Hippophae rhamnoides*, anthocyanin content, carotenoid content, sonication.

Introduction

Fruit juices are popular beverages that provide essential nutrients and bioactive compounds with potential health benefits. Sea buckthorn (*Hippophae rhamnoides* L.) and black elderberry (*Sambucus nigra* L.) juices are particularly rich in bioactive compounds, including phenolics, flavonoids, and vitamin C, which contribute to their high free radical scavenging activity (Vujanoví et al., 2020; Chen et al., 2023). However, the traditional thermal processing method – pasteurisation – used to ensure microbiological safety can negatively impact the bioactive compounds of natural juices (Zhang et al., 2024). Specifically, pasteurisation may reduce the levels of flavonoids and anthocyanins (Vieira et al., 2018). Additionally, it can diminish the content of various volatile compounds in fruit juices, including terpene alcohols, esters, aldehydes, ketones, and sesquiterpenes, which may impact the sensory profile of the juice (Giavoni et al., 2022).

Ultrasound treatment is a promising non-thermal alternative to traditional pasteurisation for fruit juice processing (Margean et al., 2020; Türkol et al., 2024). This technology has shown the potential to enhance the extraction of bioactive compounds, improve juice quality, and maintain nutritional value while ensuring microbiological safety (Kidoń & Narasimhan, 2022; Kobus et al., 2023). Ultrasound treatment offers a significant advantage in enhancing the bioavailability of bioactive compounds. By disrupting cellular structures and minimising particle size, this technique increases the solubility and digestibility of active molecules, leading to more efficient absorption by the human body. Ultrasound treatment can also reduce the molecular weight of polyphenols and polysaccharides, facilitating their uptake and utilisation (Demesa et al., 2024).

This study aimed to investigate the effects of ultrasound treatment and pasteurisation on the bioactive compounds and physicochemical properties of sea buckthorn and black elderberry juices. By comparing these two processing methods, we sought to determine whether ultrasound treatment differed in its impact on the bioactive compounds in the juices compared to traditional pasteurisation. Additionally, we examined whether these effects differed between the two types of juice.

Materials and Methods

LLC Aneva J supplied both fresh and pasteurised sea buckthorn and black elderberry juice. The raw material for both juices originated from Latvia. After extraction, the fresh juice was filled into 500 ml polyethylene terephthalate containers, sealed, frozen, and stored for one month for further analysis.

The pasteurised juice was produced immediately after extraction by heating it to 85 °C for 5 seconds, then filling it into 200 ml glass bottles. Until analysis, the pasteurised juice was stored in a dark, cool (6 ± 2 °C) place for one month.

Before ultrasound treatment and analysis, fresh juice samples were thawed to 18 ± 2 °C for five hours.

Ultrasound treatment

The samples were processed using an ultrasonic homogeniser, the Sonopuls HD4200 (Bandelin Electronics GmbH & Co., Germany), equipped with a TS 104 probe with a diameter of 4.5 mm. 100 mL of each juice sample was poured into a beaker and sonicated in a cold water bath. The temperature of the samples at the end of the processing did not exceed 23 ± 2 °C, and the sonication was performed at a 15% amplitude level (which corresponded to 36 μm amplitude) for 10 min.

Total soluble solids content (TSS)

TSS was analysed using a digital refractometer DR301-95 (A.KRÜSS Optronic GmbH, Germany). The analyses were performed according to the ISO 2173:2003 standard. The results are expressed in degrees Brix (°Bx), a measure of soluble solids. Measurements were performed in triplicate for each sample.

pH

The pH of each sample was determined using a Milwaukee MW102-FOOD digital pH meter (Milwaukee Electronics Kft., Hungary). The analyses were performed according to the ISO 5542:2010 standard, and the measurements of each sample were performed in triplicate.

Extract preparation for analysing total phenolic compound content and free radical scavenging activity (DPPH•)

40 mL of 80% (v/v) ethanol-water solution is added to 1 g of sample, then filtered through filter paper into a 50 mL volumetric flask. After filtering, the flask is filled to the calibration mark with the same ethanol-water mixture (80:20) (Tomsone, 2015).

Total phenolic compound content (TPC)

The quantification of TPC utilised a modified version of the Folin-Ciocalteu method, adapting protocols from Singleton et al. (1999) and Ozola & Kampuse (2017). The procedure combined 0.5 mL of the prepared extract with 2.5 mL of ten-fold diluted Folin-Ciocalteu reagent. After a 5 minute interval, 2 mL of 7.5% sodium carbonate solution was introduced and mixed. The mixture was then allowed to react for 30 minutes. A Jenway 6300 spectrophotometer (Baroworld Scientific, Great Britain) was employed to measure absorbance at 765 nm (Singleton et al., 1999; Avena et al., 2024).

Results were expressed as grams of Gallic acid equivalent per 100 grams of fresh weight (g GAE 100 g^{-1}). The analysis was conducted in triplicate for each juice sample to ensure reliability.

Free radical scavenging activity (DPPH•)

The free radical scavenging activity was assessed using the DPPH• (2.2-diphenyl-1-picrylhydrazyl) assay, following a protocol of Yu et al. (2003) (Tomsone, 2015). The absorbance of the test solution was measured using a UV-1900i UV-VIS spectrophotometer (Shimadzu Corporation, Japan) at 517 nm.

The results of the radical scavenging activity were quantified in fresh weight and expressed as micromoles of Trolox equivalent per gram of sample (µmol TE g^{-1}). To ensure reliability, the analysis was performed in triplicate for each juice sample.

Carotenoid content (CC)

The quantification of CC was performed using spectrophotometry, utilising a UV-1900i UV-VIS spectrophotometer (Shimadzu Corporation, Japan). The absorbance was measured at 440 nm, adhering to the methodology described by Kampuse et al. (2012). The calculation of CC followed the procedure outlined by Kampuse et al. (2015). The results were expressed in milligrams per 100 grams of fresh weight (mg 100 g^{-1}).

Total monomeric anthocyanin content (MAC)

The MAC content was determined using spectrophotometry based on the pH differential method outlined by Lee et al. (2005). Absorbance readings were taken at 520 nm and 700 nm using a UV-1900i UV-VIS spectrophotometer (Shimadzu Corporation, Japan).

Sample preparation was performed using the methodology described by Krasnova et al. (2023) with slight modifications. 2 g of juice was diluted with 30 mL of a solvent mixture (85% EtOH and 15% 1.5 N HCl). The mixture was then vortexed and allowed to extract for 30 minutes. Afterwards, the mixture was centrifuged and filtered. The MAC content was expressed by cyanidin-3-o-glucoside equivalents (CGE) in mg per CGE 100 g (mg CGE 100 g^{-1}) of fresh weight. The analysis was conducted in triplicate.

Statistical analysis

The average value and standard deviation were calculated for all sample measurements collected. A one-way analysis of variance (ANOVA) was conducted to evaluate the data, with a significance level set at $p < 0.05$. Tukey's Honest Significant Difference (HSD) test was applied to analyse the results further. Data processing and analysis were performed using LibreOffice Calc version 7.1.6.2, a software package for Linux-based operating systems.

Results and Discussion

The primary flavour characteristics of berry juice are determined by pH and total soluble solids (TSS) content. pH indicates the juice's acidity, while TSS represents the sugar content and other dissolved solids. This study found no significant differences ($p > 0.05$) in TSS and pH values among the different treatments of sea buckthorn and black elderberry juice samples. Specifically, there were no significant differences ($p > 0.05$) in TSS and pH values between untreated, pasteurised, and those treated with ultrasound. The TSS for sea buckthorn juice samples ranged from 8.5 to 8.7 (°Bx), while the TSS for black elderberry juice samples ranged from 9.6 to 9.9 (°Bx). Additionally, all sea buckthorn juice samples had a pH of 2.6, whereas the pH of the black elderberry juice samples ranged from 3.7 to 3.8.

Other authors also report that the effect of ultrasonic treatment and pasteurisation on juice pH and TSS is minuscule or insignificant (Salem et al., 2002; Renard & Maingonnat, 2012; Shen et al., 2021; Zhang et al., 2024).

Total phenolic compound content (TPC)

Table 1 presents the TPC of sea buckthorn juice. No significant differences ($p>0.05$) in TPC were found between the fresh and ultrasound-treated juice. However, PA led to a 5% reduction in TPC from the sea buckthorn samples.

In contrast, the fresh sample of black elderberry juice exhibited the highest TPC (Table 2). The TPC of the ultrasonically treated juice decreased by 8%, whereas the pasteurised juice showed a reduction of 44%. There were significant differences ($p < 0.05$) in TPC among all elderberry juice samples.

Interestingly, while ultrasound treatment did not significantly ($p > 0.05$) alter the TPC of sea buckthorn juice, it did lead to a significant ($p < 0.05$) decrease in the TPC of black elderberry juice. However, for both types of juice, TPC decreased after pasteurisation. Wu et al. (2021) reported that TPC increased in blueberry juice after both pasteurisation and ultrasound treatment. Meanwhile, Xu et al. (2023) found that TPC in strawberry juice decreased with both pasteurisation and ultrasound. Similarly, Bhutkar et al. (2023) noted that neither pasteurisation nor ultrasound treatment significantly changed the TPC of kiwi juice. This indicates that the stability of TPC after processing varies depending on the specific product being processed.

The large difference in the effect of PA between the two analysed juices could be because the TPC profile of black elderberries is mainly composed of cyanidin-3-glucoside and cyanidin-3-sambubioside, which are thermolabile (Jiménez et al., 2017). In contrast, many phenolic compounds in sea buckthorn exist as glycosides (for example, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside), which are more heat-stable than aglycones. These glycosides resist degradation better under heat treatment (Liu et al., 2022). The observed differences in the effect of PA and UT between the two juices studied could be due to the different phenolic profiles of the berries.

Table 1

Bioactive compounds in sea buckthorn juice depending on the processing method

Sample	*TPC, g GAE 100 g^{-1}*	*DPPH$^{\cdot}$, μmol TE g^{-1}*	*CC, mg 100 g^{-1}*
Sea buckthorn juice fresh	0.582 ± 0.017^{a}	7.99 ± 0.13^{a}	129.6 ± 5.6^{b}
Sea buckthorn juice ultrasonically treated	0.593 ± 0.016^{a}	8.05 ± 0.30^{a}	126.4 ± 3.3^{b}
Sea buckthorn juice pasteurised	0.550 ± 0.007^{b}	7.74 ± 0.34^{a}	139.8 ± 1.1^{a}

TPC - Total phenolic compound content; GAE - Gallic acid equivalent; TE - Trolox equivalent; CC - carotenoid content; Results are expressed as mean value ± standard deviation (n = 3); Significant differences in the column are marked with different letters ($p < 0.05$, Tukey HSD test).

Table 2

Bioactive compounds in black elderberry juice depending on the processing method

Sample	*TPC, g GAE 100 g^{-1}*	*DPPH$^{\cdot}$, μmol TE g^{-1}*	*MAC, mg CGE 100 g^{-1}*
Black elderberry juice fresh	1.467 ± 0.005^{a}	14.13 ± 0.16^{a}	95.12 ± 1.85^{a}
Black elderberry juice ultrasonically treated	1.345 ± 0.002^{b}	14.75 ± 0.56^{a}	100.45 ± 7.95^{a}
Black elderberry juice pasteurised	0.824 ± 0.01^{c}	13.89 ± 0.46^{b}	56.12 ± 1.02^{b}

TPC - Total phenolic compound content; GAE - Gallic acid equivalent; TE - Trolox equivalent; MAC - monomeric anthocyanin content; CGE - Cyanidin 3-glucoside equivalent; Results are expressed as mean value ± standard deviation (n = 3); Significant differences in the column are marked with different letters ($p < 0.05$, Tukey HSD test).

Carotenoid content (CC)

CC in the analysed sea buckthorn juice samples are presented in Table 1. Pasteurised sea buckthorn juice had a significantly ($p < 0.05$) higher CC than both fresh and ultrasonically treated juice. However, no significant ($p > 0.05$) difference in CC was observed between fresh juice and juice treated with ultrasound.

Staicu et al. (2024), in the research on β-carotene extraction from sea buckthorn berries, reported that the content of β-carotene increased significantly when the temperature was raised from 50 to 70 °C.

An increase in carotenoid levels is often observed during heat treatment, which is usually associated with an improved ability to obtain these compounds. Moderate heat exposure to plant tissues increases the release of carotenoids from their natural matrix. Consequently, these beneficial pigments become more accessible for quantification during analysis (Renard & Maingonnat, 2012).

Carotenoid compounds are typically associated with pulp content when producing fruit juices. Juices that retain a significant amount of pulp, such as sea buckthorn juice, can contain notably high levels of these beneficial pigments (Renard & Maingonnat, 2012).

Total monomeric anthocyanin content (MAC)

The highest MAC levels were observed in fresh and ultrasonically treated black elderberry juice samples, with no significant differences ($p > 0.05$) between the two samples. However, pasteurised black elderberry juice showed a 41% decrease in MAC, resulting in a value of 56.12 ± 1.02 mg CGE 100 g^{-1}.

The observed significant effect of pasteurisation could be due to the fact that anthocyanins are sensitive to high temperatures, which can lead to degradation during processes like pasteurisation. As the temperature increases, the speed at which anthocyanins break down also accelerates. This degradation follows first-order reaction kinetics. As described by other researchers in the case of black elderberry juice, both the temperature during pasteurisation and the processing time significantly influence the extent of anthocyanin degradation (Casati et al., 2015). The elevation in temperature triggers a breakdown of the bonds between sugar molecules and anthocyanins, resulting in the degradation of vibrant anthocyanin pigments. As a result, the original colour degrades into brownish compounds known as chalcones. This transformation not only changes the visual appearance of the juice but also affects its overall quality (Teneva et al., 2022).

Free radical scavenging activity (DPPH˙)

The results of this study indicate that the juice treatment did not affect the DPPH˙ levels in sea buckthorn juice, which ranged from 7.74 to 8.05 µmol TE g^{-1}. In contrast, significant differences were observed among some samples of black elderberry juice. Following pasteurisation, the DPPH˙ value decreased to 13.89 µmol TE g^{-1}, which is significantly lower ($p < 0.05$) than the values of untreated and ultrasonically treated black elderberry juice, which were 14.13 and 14.75 µmol TE g^{-1}, respectively.

However, Wu et al. (2021) reported in their study that DPPH˙ in blueberry juice increases in both pasteurised and ultrasonically treated juice. In addition, increasing the ultrasonic treatment intensity increases the juice's DPPH˙. Meanwhile, Khalil et al. (2023) reported in a study on carrot-orange juice blend that increasing the pasteurisation time decreases the DPPH˙ in the juice, while increasing the ultrasonic treatment time increases the DPPH˙ in the juice. These findings suggest that pasteurisation parameters and ultrasound treatment influence the DPPH˙.

Conclusions

1. Pasteurisation generally had the most detrimental effect on bioactive compounds in sea buckthorn and black elderberry juices.
2. Ultrasonic treatment showed potential as a gentler alternative to pasteurisation, particularly for preserving anthocyanins and phenolic compounds.
3. Sea buckthorn juice appeared more resistant to processing-induced changes in DPPH˙ levels.
4. Black elderberry juice showed more pronounced changes in bioactive compounds across different processing methods.
5. Further research may be needed to optimise ultrasonic treatment parameters for maximum retention of bioactive compounds.

Acknowledgements

The research was carried out under contract number 5.1.1.2.i.0/1/22/A/CFLA/003, established between 'Latvian Food Competence Centre' Ltd. and the Central Finance and Contracting Agency. It was conducted by 'Aneva J' Ltd. (Research project No. 28) with financial support from the European funds - Recovery and Resilience Facility.

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