The impact of ultrasound-assisted thawing on the bioactive components in juices obtained from blue honeysuckle (Lonicera caerulea L.)

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ABSTRACT

Ultrasound (US) assisted thawing of blue honeysuckle berry was utilized in order to reduce the losses of bioactive components (ascorbic acid, anthocyanins, phenolic acids, iridoids, proanthocyanins) and increase the extraction efficiency during juice processing. It was analysed whether it was more beneficial to apply US (alone or with enzymatic treatment) to the frozen state, until reaching the cryoscopic temperature or thawed state. Both the US and enzymatic treatment significantly increased the extraction efficiency, extract content, acidity and the content of iridoids and chlorogenic acid in juices, especially if the US was applied to 50 °C. It was probably due to a higher extractivity by the greater damage of the tissue and detexturation. Enzymatic treatment due to long heating contributed to a higher degradation of anthocyanins, ascorbic acid and proanthocyanidins, which are more heat-sensitive. The results of the study mainly indicated the possibility of including ultrasound-assisted thawing in the fruit processing before pressing the juices. This may replace costly enzymatic treatment.

1. Introduction

Blue honeysuckle berry (Lonicera caerulea L.), also known as ‘haskap’, ‘sweet berry honeysuckle’ or ‘edible honeysuckle’, is a long-lived shrub originating from Northeast Asia. Its fruits are fleshy, elongated, multi-seeded berries, covered with waxy coating, of a sweet and sour taste with slight bitterness and pleasant aroma [2]. Due to the short harvest time and a relatively short shelf life, fresh fruits should be processed as soon as possible or subjected to freezing [15].

A wide range of bioactive compounds with proven health-promoting properties have been identified in blue honeysuckle berry. Lonicera caerulea is a rich source of vitamin C, polyphenols, among which anthocyanins (especially cyanidin-3-glucoside), flavan-3-ols (proanthocyanidins, catechin), phenolic acids (especially chlorogenic acid), flavonols (quercetin derivatives) and flavones (luteolin derivatives) should be mentioned [38]. Furthermore, iridoids are an important group of compounds identified in blue honeysuckle berry fruits, despite they are not widely present in fruits. Iridoids are a large class of secondary metabolites, belonging to the cyclopentane monoterpene, which act in plants as defensive substances against pests due to e.g. a bitter taste. Similar protective effect is also noticed in the case of tannins, which toxic properties and a bitter taste discourage animals from the consumption of a plant [31]. The basic structure of iridoids consists of a framework composed of a cyclopentane and pyran ring [32]. What is especially important is the fact that depending on the chemical structure of iridoids, they exhibit various biological properties including anti-inflammatory, antispasmodic, hepatoprotective, antiviral or cardiovascular [16]. It is worth noting that making the public aware of the impact of iridoids on health and highlighting their presence in blue honeysuckle berry can help spread and increase consumption of these valuable berries. Until 2018, the sale of the blue honeysuckle berry was prohibited in most European countries. The possibility of market launch of these fruits has been guaranteed for the EU countries under the Regulation of the European Parliament and of the Council (EU) no 2015/2283. The long tradition of safe consumption of the fruits in Japan contributed to the establishment of this regulation [12].

In response to consumer expectations and high market competition, producers are looking for novel raw materials with high healthy and sensory qualities. These raw materials are often used for juice production, which is one of the most popular products of the fruit and vegetable

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industry. Among the whole range of juices, cloudy juices (NFC - “Not From Concentrate”) are gaining popularity because the omission of such operations as clarification, filtration and concentration, as well as possible reconstitution of juices prevents against losses of bioactive components and allows to obtain more nutritionally valuable product \[44\]. Due to the fact that blue honeysuckle berry is a seasonal fruit, thus the freezing and thawing method should also be carried out, which can contribute to worse quality juices. However, nowadays, the researchers are sought novel processing methods to preserve the highest possible amount of bioactive components of the raw material, which is particularly important in the era of prevention and treatment of diseases of modern civilization.

The utilization of ultrasound in order to improve technological methods and obtain a more valuable product is tested widely all over the world. Ultrasound (US) is a sound wave of a frequency above 18 kHz. For enhancement of the unit operations in food processing by the means of alteration of material structure, the high-power ultrasound is used \[6\].

For the alteration of material structure, the high-power ultrasound is used \[6\]. The mechanism of ultrasound-enhanced process is linked to the occurrence of various phenomena, such as: “spunge effect” (compression and decompression of the material causing internal stress), cavitation, and the effects accompanying cavitation (like microjetting or microstreaming) \[7,19\]. Furthermore, when US is applied to the plant material, the bioactive component content may not only be preserved but also increased due to higher extractivity \[29\] owing to cavitation, microjetting and the sonocapillary effect \[41\]. The formation and collapsing of gas bubbles (cavitation), which locally releases high dose of energy (high temperature and pressure) and results in propagation of shock waves, is responses of changes in internal structure of the material. As a result of better mixing of the medium, homogenization and highly efficient mass transfer, the process is also accelerated \[7,21\]. Furthermore, in the case of those bubbles, which collapse near the solid surface, the microjetting takes place causing erosion of the material and microchannels formation \[7,19\]. In turn, the sonocapillary effect is linked to better penetration of solvent into capillaries and crevices and the increased velocity and depth of solvent penetration through pores and canals when the US is generated \[41\].

Freezing is an essential part of the processing of seasonal raw materials that are harvested within a very short period of time, which gives more production flexibility. It was proven that ultrasound during freezing contributed to shorter process, better bioactive compounds’ retention and more fresh-like quality (especially texture is greatly preserved) than freezing alone mainly due to lower and evenly distributed crystals created as a consequence of higher freezing rate and lower supercooling \[6\]. Many publications show the beneficial effect of using ultrasound (sometimes together with mild temperature) during juice processing on microbiological safety, increased stability during storage (due to e.g. enzyme inactivation) and greater stability or increased level of bioactive compounds \[1,21\]. However, relatively few studies investigated the effect of ultrasound on fruits before juice extraction. To the best of our knowledge, there is no published literature on the effect of high-power ultrasound during blue honeysuckle thawing. Furthermore, also this is the first paper focused on production of cloudy blue honeysuckle berry juices.

As large amount of bioactive compounds are lost during thawing, this study attempts to the use of ultrasound while thawing the blue honeysuckle berry fruits. The following hypothesis was investigated: Ultrasound-assisted thawing increases the extraction efficiency and bioactive components content in cloudy juices from blue honeysuckle berries. It was analysed whether it is more beneficial to apply ultrasound (alone or with enzymatic treatment) only at the initial stage of thawing (to the frozen state), until reaching the cryoscopic temperature, or until reaching the thawed state.

2. Material and methods

2.1. Material

Blue honeysuckle berry fruits (\textit{Lonicera caerulea} L.) cv. ‘Wojtek’ (~10 kg) came from a blue honeysuckle berry plantation in Kujawsko-Pomorskie Province (Nutracevit, Poland). The fruits after harvest were frozen and stored at ~23 °C until the juice was extracted. Freezing was a necessary step in order to obtain homogeneous raw material for all experiments. This is due to the fact that blue honeysuckle berry has a short time of yielding and harvesting and its prolongation would result in different degrees of post-harvest maturity or even obtaining overripe material. All the fruits were frozen at the same time and the material was used in the experiments within 2 weeks.

2.2. Ultrasonic-assisted thawing (US)

Before juice pressing, the raw material was thawed and further heated to 50 °C, which was the optimum temperature for the action of the pectinolytic enzyme used for enzymatic treatment of the mash. At this stage, usually utilized air thawing, which causes high losses of bioactive components, was replaced by ultrasound-assisted thawing. For this purpose, the MKD-3 ultrasound bath (MKD ULTRASONIC, Stary Konik, Poland) was used, giving frequency and outlet power of 21 kHz and 300 W, respectively. The bath was equipped with a heating mantle, so that water at a constant temperature washed the beakers with the material, which caused them to heat up to the set temperature of 50 °C. Frozen fruits weighing 1 kg were placed in beakers (4 beakers, 250 g each) in the ultrasonic bath when the water temperature reached 58 °C, which ensured that the fruit reached 50 °C. The temperature of fruits was controlled by thermocouple (±0.1 °C) placed in the center of the beaker. The blue honeysuckle berry fruits were gently mixed manually.

In order to select the optimal sonication conditions for preserving bioactive compounds, different ultrasound application times were used during thawing and further heating:

1) until the material was still in the frozen state (-10 °C),
2) until the phase transition (around the cryoscopic temperature and the freezing point of pure water ~ -3 ± 0 °C),
3) until the material was in the thawed state, but below room temperature (10 °C),
4) until the material reached 50 °C.

On the basis of introductory studies (data not shown), during which the temperature changes of fruits during thawing were analyzed, the ultrasonic operation times were selected, which were: 5 min (~10 °C), 15 min (~3 ± 0 °C) and 20 min (10 °C), respectively. After the aforementioned time, the ultrasound was turned off and the fruits were left in the bath for further heating to reach 50 °C. For the variant in the case of which the ultrasound operated constantly until the temperature reached 50 °C, the time length was around 30 min. In the case of the control variants (with and without enzymatic treatment), the berries were heated in the ultrasonic bath until 50 °C was reached, but without US (time of 35 min). The juice variants that were obtained in 2 replicates are summarized in Table 1.

2.3. Enzymatic treatment

After thawing and heating up to 50 °C, one part of berries was subjected to enzymatic treatment. For this purpose, the enzyme BremPect (Brenntag, Kędzierzyn-Koźle, Poland) was used at the amount of 0.5 mL/kg, selected based on the preliminary study (data not published) giving the best results in terms of content of bioactive components. The dose was within the range suggested by the enzyme’s manufacturer. The mash was obtained without blending but the berries after the step of thawing and heating released the cellular content due to the very delicate structure. After the addition of the enzyme, the mash was kept in a water bath at 50 °C for 60 min. In order to analysed the influence of
The technological proceedings

| The symbol of the juice type | The technological proceedings                                                                                     |
|-----------------------------|---------------------------------------------------------------------------------------------------------------|
| Control                     | juice from fruits not subjected to ultrasonic treatment, heated to 50 °C (for 35 min)                        |
| −10_US                      | juice from fruits treated with ultrasonic until −10 °C (for 5 min) followed by heating without US to 50 °C    |
| 0_US                        | juice from fruits treated with ultrasound until cryoscopic temperature −3 ± 0 °C (for 15 min) followed by heating without US to 50 °C |
| 10_US                      | juice from fruits treated with ultrasound until 10 °C (for 20 min) followed by heating without US to 50 °C     |
| 50_US                      | juice from fruits treated with ultrasound until reaching 50 °C (for 30 min)                                    |
| Control_E                  | juice from fruits not subjected to ultrasonic treatment, heated to 50 °C (for 35 min) followed by enzymatic treatment |
| −10_US_E                   | juice from fruits treated with ultrasonic until −10 °C (for 5 min) followed by heating without US to 50 °C and finally subjected to enzymatic treatment |
| 0_US_E                     | juice from fruits treated with ultrasound until cryoscopic temperature −3 ± 0 °C (for 15 min) followed by heating without US to 50 °C and finally subjected to enzymatic treatment |
| 10_US_E                    | juice of fruits treated with ultrasound until 10 °C (for 20 min) followed by heating without US to 50 °C and finally subjected to enzymatic treatment |
| 50_US_E                    | juice from fruits treated with ultrasound until reaching 50 °C and finally subjected to enzymatic treatment (for 30 min) |

Table 1: Obtained types of juices from blue honeysuckle berry fruits.

Enzymatic treatment, one part of juices was prepared without enzyme addition (Table 1). For these variants the juices were obtained immediately after reaching 50 °C.

2.4. Juice production technology

The juices after the enzymatic treatment or after only thawing and heating were pressed by centrifuging the pulp in the MPW-35OR laboratory centrifuge (Warsaw, Poland) at 5000 rpm for 10 min, which is one of the methods used to obtain juice [3]. All the juices were afterwards heated to 85 °C and rapidly bottled in 40-ml glass containers and pasteurized at this temperature, followed by cooling to 20 °C. For each of the variant of juices, three separate containers were prepared and used for further analytical determinations.

Extraction efficiency (%) was determined as the weight of juice obtained after pressing divided by weight of fruits subjected to pressing.

2.5. Analytical methods

2.5.1. Determination of refractometric extract and titratable acidity

The extract was determined with a Refracto 30PX refractometer (Mettler Toledo, Columbus, Ohio, USA).

The acidity was determined by potentiometric titration with NaOH solution (0.1 M) until a pH of 8.1 was reached. The acidity was expressed as grams of citric acid in 100 mL of juice.

2.5.2. Determination of l-ascorbic acid (AA) content by HPLC

The l-ascorbic acid (AA) content was determined using H_3PO_4 solution (0.1 %) as an eluent [33]. For this purpose, the HPLC system with Onyx Monolithic C18 100 × 4.6 mm (Phenomenex, Torrance, California, USA) column and UV–vis detector were used. The juices from blue honeysuckle berry fruits were filtered through 0.45 μm PTFE syringe filters. The conditions during analysis were as follow: the flow rate: 1 mL/min, the column’s temperature of 25 °C, the wavelength of λ = 254 nm. The AA content was expressed in mg/100 mL. All chemicals were of HPLC purity. The concentration of compounds was determined from the standard of l-ascorbic acid (ChromaDex). Determination was carried out in three repetitions.

2.5.3. Determination of anthocyanin content by HPLC

The content of individual anthocyanins in the obtained juices was determined by gradient HPLC using a DAD detector based on the method proposed by Mieszczakowicz-Frac et al. [28] with own modifications. The flow rate of the mobile phase consisting of 5 % HCOOH (reagent A) and 100 % ACN (reagent B), was 1 mL/min. The gradient programme was as follows: 0–5 min 3 % B, 5–20 min 9 % B, 20–30 min 9–12 % B, 30–35 min 12 % B, 35–38 min 12–40 % B, 38–40 min 40 % B, 40–41 min 3 % B, 41–56 min 3 % B. The gradient varied linearly. The separation was carried out on a Luna 5 μm C18(2) 250x4.6 mm column (Phenomenex, Torrance, California, USA) precolumn at 520 nm and an oven temperature of 25 °C. Anthocyanin content was expressed in mg/100 mL of juice. The concentration of compounds was determined from a cyanidin-3-glucoside standard (Sigma-Aldrich). Analyses were repeated thrice for each material. The total anthocyanins content (TA) was calculated as a sum of individual anthocyanins.

2.5.4. Determination of iridoids content by HPLC

The content of iridoid compounds was determined in three repetitions according to the method of Duan et al. [11], with own modifications. The mobile phase of the flow rate of 1 mL/min was: H_3PO_4 (0.1 %) - reagent A and methanol (100 %) - reagent B. The results were recorded at a wavelength of 210 nm. Luna 5 μm C18(2), 250x4.6 mm column (Phenomenex, Torrance, California, USA) and PDA detector were used for chromatographic separation. The gradient programme varied linearly: 0–4 min 0–25 % B, 4–10 min 25–35 % B, 10–22 min 35–40 % B, 22–27 min 40–0 % B, 27–42 min 0 % B. The amounts of iridoids were calculated based on standards of loganic acid (ChromaDex), loganin (Sigma-Aldrich), secolloganin (ChromaDex) and sweroside (ChromaDex) and the total iridoids (TI) was calculated (mg/100 mL) as a sum of identified iridoids.

2.5.5. Determination of phenolic acids contents by HPLC

The HPLC with DAD detector and Luna 5 μm C18(2), 250x4.6 mm column with precolumn (Phenomenex, Torrance, California, USA) were used to analyze the content of phenolic acids in juices from blue honeysuckle berry fruits. The mobile phase of flow rate of 1 mL/min consisted of 5 % HCOOH (Eluent A) and 100 % ACN (Eluent B), both of the HPLC purity. The following linear gradient was set: 0 min 0 % B, 5 min 10 % B, 10 min 25 % B, 20 min 35 % B, 25 min 50 % B, 30 min 70 % B, 35 min 75 % B, 40 min 100 % B. The peaks were analyzed at 254 nm. The results were expressed in mg/100 mL. The sum of individual phenolic acids was presented as total phenolic acids (TPA) [5].

2.5.6. Determination of proanthocyanidins content by HPLC

Proanthocyanidins content was determined according to the method proposed by Kennedy & Jones [18]. Prior to the determination, 0.5 mL of juice was freeze-dried in 2 mL tubes (freeze-drying parameters:
vacuum 0.954 mbar, shelf temperature 20.8 °C, time 26 h). Then, 0.8 mL of a solution of fluoroglucinol (75 g/L) with ascorbic acid (15 g/L) in anhydrous methanol and 0.4 mL of HCl in anhydrous methanol (0.3 mol) were added to the eppendorf tubes with lyophilized juice. After thorough mixing in a thermoshaker for 30 min at 50 °C, the reaction was stopped by placing the samples in ice-cold water and adding 0.6 mL of acetate buffer (0.2 mol/L) immediately to the reaction solution. Directly after the mixing, the samples were centrifuged for 5 min at 4 °C and 14000 rpm. (MPW-350R laboratory centrifuge, Warsaw, Poland).

The determination was carried out in three repetitions by HPLC using the gradient technique and a fluorescence detector by excitation of the particles with light of 278 nm and emission of 360 nm. The mobile phase flow rate was 1 mL/min, the phase consisted of 5 % HCOOH (reagent A) and 100 % ACN (reagent B). The gradient programme was as follows: 0.01–30 min 10–25 % B, 30–33 min 25–50 % B, 33–38 min 50 % B, 38–40 min 50–10 % B, 40–55 min 10 % B. Separation was carried out with a Luna 5 µm C18(2) 250x4.6 mm column (Phenomenex, Torrance, California, USA) with a precolumn. Results are expressed in mg/100 mL. The standards of procyanidin B2 and catechin (Sigma-Aldrich) were used to calculate the amounts of total proanthocyanidins (TA) in juices.

2.5.7. Statistical analysis
Statistical analysis of the results was performed with Statistica v.13.3 (StatSoft, Poland). The one-way analysis of variance (ANOVA) was carried out with a Tukey test to determine the homogeneous groups. The significance level was set at 0.05.

3. Results and discussion

3.1. Extraction efficiency of juice and primary physico-chemical parameters
The efficiency of extraction of juice from blue honeysuckle berry fruits after US-assisted thawing with and without enzymatic treatment is presented in Fig. 1. The extraction efficiency was in the range of 38.25–42.40 % in the case of juices obtained without enzymatic treatment and 57.02–62.69 % in the case of juices subjected to enzymatic treatment. Thus, as predicted, the application of enzymatic treatment on the mash resulted in a significant increase in the extraction efficiency. For all juices obtained by enzymatic treatment, the efficiency was 32–33 % higher than for analogous variants of juices from mash without enzyme addition. The results of our study are in line with many other scientific reports which indicated that juice pressing yields increased after application of an enzymatic treatment of the mash [34]. Moreover, the present results indicated also the effect of ultrasonic treatment on juice extraction efficiency. As the duration of the ultrasound treatment increased, the extraction efficiency increased (significantly in most cases), irrespectively if the enzymatic treatment was applied. For instance, the efficiency in the case of 50_US juice was 10 % higher than for Control juice and for 50_US E juice the yield was 9 % higher than that for Control_E juice. Also, Lieu and Le [25] observed increasing extraction yield with increasing both sonication time and enzyme concentration when grape mash was treated simultaneously by US and enzyme before juice obtaining. In comparison to enzymatic treatment, the extraction yield increased maximally by 2 % for combined enzymatic and sonication treatments and by 7.3 % when US followed by enzymatic treatment was applied. However, when compared with untreated sample, the extraction yield was higher maximally by 11.4 % for combined treatment. In turn, Radziejska-Kubzdela et al. [36] observed that sonication and enzymatic treatment applied alone did not increase the juice yield from barberry when mash was sonicated but for shorter time and power (10 min, 140 W) and by pectinase for 60 min, respectively. Interestingly, in our study, irrespectively of the application of enzymatic treatment, there were no differences between extraction efficiency of samples thawed with US assistance up to 0 and up to 10 °C. In the case of fruits not subjected to enzymatic treatment, also for 50_US the efficiency was statistically the same. It seems that the highest increase of efficiency was when the generation of ultrasound waves during thawing up to phase transition temperature of material was set (~0 °C). The use of both ultrasound and enzymatic treatment were the most efficient in obtaining the juice from blue honeysuckle berry fruits. In comparison to the sample without enzymatic treatment and without US application (Control), the extraction efficiency was around 64 % higher when US up to 50 °C and enzyme were used (50_US_E). The use of ultrasound, especially when combined with further enzymatic treatment resulted in higher extraction efficiency presumably due to better tissue breakdown of the raw material. If the greater destruction of cells’ membranes and cells’ walls occurred owing to both enzymatic treatment and sonication, the higher was the release of cellular content and in a consequence, the higher was the extraction efficiency. Furthermore, the US contributed to higher mass transfer due to better mixing of the medium. These effects resulted from the cavitation and microjetting – collapsing bubbles, especially those, which collapse occurs close to the blue honeysuckle berry fruits microjets the tissue causing its greater

Fig. 1. Extraction efficiency of the juices from blue honeysuckle berry untreated or treated with enzymes after US-assisted thawing for various time until: −10 °C, the phase transition (~−3 °C), 10 °C, and until the material reached 50 °C. a-f - The values with the same letter did not differ significantly.
damage and greater release of intercellular content. The destruction of material and detexturation of plant structures are linked to the breakage of filaments and intercellular junctions which occurs when microjetting bubble “attack” the pores and weakened cell walls. Moreover, probably during the US-assisted thawing, the cellular content released from the fruits better penetrates the capillaries and crevices in fruits and the deeper penetration into pores and canals of material occurred, analogous to US-assisted extraction, during which the sonocapillary effect increases the extraction yield [41]. The results of primary physicochemical parameters, such as the refractometric extract and total acidity were in accordance with this theory (Table 2). It was shown that the application of enzymatic treatment of the mash caused a significant increase in the values of extract and total acidity. The highest values of these parameters were obtained in the case of the 50_US_E juice, whose total acidity was 2.34 g citric acid/100 mL and extract value was equal to 16.80 °Brix. The use of a longer duration of ultrasound treatment resulted mainly in a higher extraction of organic acids but the combined application of ultrasound followed by enzymatic treatment was the most efficient in the improvement of both extract and the total acidity. Similarly, the highest acidity was noted for combined US + enzyme treatment of grape mash in the work of Lieu and Le [25].

### 3.2. L-ascorbic acid (AA) content

L-ascorbic acid is essential for maintaining the normal functioning of the human immune system. It acts as a natural antioxidant, preventing oxidative stress in the body. Ascorbic acid and dehydroascorbic acid play an important role in the non-enzymatic browning of products. The oxidoreductive system ascorbic acid ↔ dehydroascorbic acid can participate in regulating the oxidoreductive potential in the cell and take part in electron transport [10].

All variants of obtained blue honeysuckle berry juices were analysed in terms of the L-ascorbic acid content (Table 3). The AA content was in the range of 34.73–47.97 mg/100 mL. Enzymatic treatment affected the degradation of vitamin C, regardless of the variant of sonication. Compared to the non-enzymatically treated material, losses ranged from 17 to 23 %. This was mainly due to the fact that l-ascorbic acid is a very labile component and sensitive to high temperature and oxidation [39]. Probably, the long heating time of the mash during enzymatic treatment and the availability of oxygen contributed to the oxidation and loss of this component in juices obtained from berries subjected to enzymatic treatment. Other studies have also demonstrated a negative effect of mash enzymatic treatment on l-ascorbic acid content in juices [24]. Immediately after production, the highest AA content was found in juices obtained from fruit treated with US up to 0 and 10 °C without enzymatic treatment (0_US and 10_US), due to a higher extractivity and/ or a protective effect of shortening of thawing time (~5 min between non-US and US-assisted process to 50 °C) by ultrasound. A higher determined content of different bioactive compounds as a result of US-enhanced process was reported in the scientific literature previously [29]. The cavitation with shock waves generation, microjetting of the material by the bubbles, mixation of the released juice and sonocapillary effects are the main reasons responsible for enhancement of bioactive components extraction as a result of ultrasound application [41], as we explained this in the 3.1. section. When ultrasound was applied continuously up to reaching 50 °C, (50_US and 50_US_E), it promoted a significant degradation of AA, amounting to 8 % in the case of treatment without enzyme and 11 % in the case of material not subjected to ultrasound, compared to material sonicated up to reaching 10 °C. The prolongation of the ultrasound treatment resulted in increased generation of free radicals, which could lead to the degradation of l-ascorbic acid and thus limited the beneficial effect of the increased extractability of this compound observed after a shorter sonication time [30]. It should be therefore emphasised, that only in the case of juices obtained without enzymatic treatment, the AA contents were significantly lower after thawing with the longest US application than in juices before obtaining which the fruits were thawed without US. Therefore, from the point of view of obtaining the highest l-ascorbic acid content, the most beneficial application of ultrasound was until the pulp temperature reached phase transition state or the temperature of +10 °C without enzymatic treatment. Other studies also conform that too long ultrasonic treatment has a negative effect on the stability of l-ascorbic acid. Wang et al. [43] reported lower content of this compound in kiwi juices subjected to prolonged ultrasound treatment. In another study by Ordóñez-Santos et al. [30], the ascorbic acid content of sonicated gooseberry juices was significantly lower compared to untreated juices.

| Total acidity | Refractometric extract [°Brix] |
|--------------|-------------------------------|
| [g citric acid/100 mL] |                              |
| **Juices from fruits without enzymatic treatment** |                         |
| Control     | 1.98 ± 0.01 f                 |
| 0_US        | 2.06 ± 0.01 e                 |
| 10_US       | 2.11 ± 0.02 d                 |
| 50_US       | 2.13 ± 0.01 c ed              |
| **Juices from fruits subjected to enzymatic treatment** |                         |
| Control_E   | 2.16 ± 0.02 c                |
| 10_US_E     | 2.23 ± 0.02 b                 |
| 0_US_E      | 2.25 ± 0.01 b                 |
| 10_US_E     | 2.24 ± 0.02 b                 |
| 50_US_E     | 2.34 ± 0.01 a a              |

### 3.3. Anthocyanins’ content

Blue honeysuckle berry is an exceptionally abundant raw material rich in anthocyanins. Their content may vary from 400 to 1500 mg/100 g and is comparable to the content in chokeberry and elderberry, which are considered a particularly rich source of these compounds [17]. Anthocyanins are pigments commonly found in the plant world, imparting colours ranging from pink through blue to dark purple. Many scientific publications have also confirmed antioxidant, anticancer, inflammatory [4], cardio-protective and anti-diabetic properties of blue honeysuckle berry fruit [38].

The dominant anthocyanin in blue honeysuckle berry juices was cyanidin-3-glucoside, with cyanidin-3-rutinoside, cyanidin-3,5-diglucoside, peonidin-3-glucoside and pelargonidin-3-glucoside also present in smaller amounts (Table 3). Analysing the results, it could be stated that as the duration of US treatment during fruits thawing increased, the determined content of total anthocyanins (TA) in the juices increased. The juice of 50_US contained the highest amount of TA, which was 442.22 mg/100 mL. This shows that the extractability of the anthocyanins increased with sonication time, regardless of whether enzymatic treatment was carried out after thawing. Only for the shortest time of ultrasound application during thawing, when sonication lasted only until the material was still frozen (-10_US and –10_US_E), the TA content was statistically at the same level as in the case of no ultrasound application (Control and Control_E, respectively). This indicates the relevance of generating ultrasound at least until the cryostatic temperature with recommendation to continue the application during the entire thawing stage until the temperature reaches 50 °C. The higher anthocyanin content in the 50_US juice can be explained by the fact that the continuous ultrasonic treatment reduced the thawing time and increased the extraction of anthocyanins from the fruit peel. Due to the detexturation and destruction of the material by sonication, the bioactive components may be released to juice to a greater extent.
Furthermore, the juice released from the blue honeysuckle berries during thawing deeper penetrates the pores and capillaries [41] and thus the bioactive components contents may increase, as well. Another explanation is that the sonication may deactivate the enzymes responsible for degradation of anthocyanins. Sun et al. [42] confirmed that longer sonication > 15 min and higher temperatures (45–60 °C) contributed to inhibition of β-D-glucosidase. Glucosidases are the enzymes that are most responsible for degradation of anthocyanins because they break the covalent bond between aglycone and glycosyl residue, which leads to the formation of unstable anthocyanidins [13]. Other studies also indicated the beneficial effect of using ultrasound in reducing both freezing and thawing times as well as increasing the TA content in the obtained product [35]. As confirmed by studies, more anthocyanins are present in the peel than in the flesh of the blue honeysuckle berry [2], thus the utilization of ultrasound-assisted thawing contributes to obtaining a higher content of anthocyanins in juices from blue honeysuckle berry. The thawing method is a very important factor affecting the stability of anthocyanins. Khattab et al. [22] showed that thawing the berries at room temperature resulted in higher losses of anthocyanins, compared to shorter microwave thawing.

Table 3

|                | AA     | TA     | cy-3,5-diglu | cy-3-glu | cy-3-rut | pealr-3-glu | peonid-3-glu |
|----------------|--------|--------|--------------|----------|----------|-------------|--------------|
| Juices from fruits without enzymatic treatment |        |        |              |          |          |             |              |
| Control        | 44.82 ±| 407.33 ±| 17.11 ±      | 361.11 ± | 23.11 ±  | 1.23 ±     | 4.77 ±       |
| −10_US         | 45.71 ±| 407.81 ±| 17.72 ±      | 361.62 ± | 22.43 ±  | 1.24 ±     | 4.80 ±       |
| 0_US           | 47.97 ±| 420.23 ±| 18.36 ±      | 373.55 ± | 22.05 ±  | 1.30 ±     | 4.98 ±       |
| 10_US          | 47.25 ±| 425.44 ±| 19.11 ±      | 379.40 ± | 22.21 ±  | 1.28 ±     | 4.96 ±       |
| 50_US          | 41.87 ±| 442.25 ±| 19.78 ±      | 391.15 ± | 24.61 ±  | 1.33 ±     | 5.38 ±       |
| Juices from fruits after enzymatic treatment |        |        |              |          |          |             |              |
| Control_E      | 35.70 ±| 384.57 ±| 16.25 ±      | 339.41 ± | 23.45 ±  | 1.22 ±     | 4.24 ±       |
| −10_US_E       | 45.08 ±| 378.70 ±| 15.93 ±      | 333.02 ± | 23.61 ±  | 1.20 ±     | 4.29 ±       |
| 0_US_E         | 37.50 ±| 416.21 ±| 17.53 ±      | 368.89 ± | 23.47 ±  | 1.31 ±     | 5.00 ±       |
| 10_US_E        | 37.76 ±| 414.14 ±| 17.65 ±      | 366.45 ± | 24.26 ±  | 1.30 ±     | 5.12 ±       |
| 50_US_E        | 34.73 ±| 428.02 ±| 18.74 ±      | 377.87 ± | 25.08 ±  | 1.30 ±     | 5.04 ±       |

Table 3

The contents of l-ascorbic acid (AA), total anthocyanins (TA) and individual anthocyanins (mg/100 mL) in juices from blue honeysuckle berry untreated or treated with enzymes after US-assisted thawing for various time until: −10 °C, the phase transition (−3 ± 0 °C), 10 °C, and until the material reached 50 °C. a-f - The values with the same letter in each column did not differ significantly.

3.4. Iridoids content

Blue honeysuckle berries are distinguished from many popular fruits by the content of biologically active compounds from the iridoid group. Iridoids are a large group of secondary metabolites belonging to the monoterpenes. Iridoids are compounds which may have a defensive function in plants against predators. Some of them are bitter substances (like secoiridoids) which may affect the taste of raw materials containing these compounds [23]. Data on the content of iridoids in blue honeysuckle berry is poor. In a study of Kucharska et al. [23], it was reported that loganic acid was the dominant iridoid. According to other available report, in one year (2011) secologanin was dominant, while in the other year (2012) it was loganin [91]. This discrepancy may be related to the environmental factors of cultivation, which can have a very strong influence on the synthesis and accumulation of these
secondary metabolites in fruits [45]. Due to the particular health-promoting properties of iridoids, it is advisable to search for methods to obtain the highest possible content of these compounds in juices, despite their potential impact on the bitter taste sensation.

The content of individual iridoids and the total iridoids (TI) are shown in Table 4. In the present study, the iridoid found in the highest amount in all the studied juices was loganic acid, which accounted for about 65 % of all iridoids. The next most abundant iridoid was loganic acid. Two iridoids present in smaller amounts in the fruits were also identified - secologanin and sweroside. These compounds could potentially affect sensory properties as they are secoiridoids, a group of iridoids with an unusually bitter taste [32].

Both in the case of individual iridoids and in relation to the TI content, a statistically significant tendency to obtain juices richer in iridoids was observed with increasing duration of ultrasound application during fruit thawing, in particular when after the thawing the enzymatic treatment was utilized. The only exception was loganic acid, whose content for no enzymatic treatment variants was statistically independent of ultrasound application. In relation to Control juice, the TI content was higher by 6 and 15 % for 50_US and 50_US_E, respectively. Among different iridoids the increased content due to the longest US application coupled with enzymatic treatment was observed especially in the case of sweroside. Its content was by 45 % higher in 50_US_E juice than in Control sample.

Generally, the ultrasound contributed to their greater extraction in particular when sonication was applied continuously until the material reached cryoscopic temperature (loganic acid) or positive temperature (10 or 50 °C) and the mash was treated by the enzyme. Therefore, it can be concluded that the ultrasound enhanced the iridoids contents more effectively when the material was in thawed state followed by enzymatic treatment due to the greater availability of the compounds and possible greater damage to cells and release of cells contents. Ultrasound may have contributed to increased extractability of iridoids also as a result of better penetration of the cellular content into capillaries, pores and channels [41].

### 3.5. Phenolic acids content

The determination revealed also the presence of phenolic acids in the blue honeysuckle berry juices, which belong to the polyphenols group. Three types of hydroxycinnamic acids were identified: chlorogenic acid, caffeic acid and p-coumaric acid (Table 5). According to the available literature, chlorogenic acid is the phenolic acid most abundant in blue honeysuckle berry [38], which is consistent with present study. However, studies have also proven that the content of this acid is highly dependent on climatic and agrotechnical conditions of cultivation [17]. Chlorogenic acid has also been shown to have many medicinal and therapeutic properties, such as blood pressure-lowering, obesity-preventing, anticancer and antioxidant properties [8].

Statistically the highest total phenolic acids (TPA) content was determined in 50_US_E juice (Table 5). A shorter ultrasonic treatment time (until 10 °C and lower) did not contribute to a significant increase of TPA in the obtained juices. Similar tendency was noted also regarding the chlorogenic acid content. Interestingly, also in the Radziejewska-Kubzda et al. study [36], no effect of enzymatic treatment or ultrasound treatment on chlorogenic acid content was found for 10 min treatment. Hence, this indicates that higher contents of acids were obtained in juices obtained by the longest ultrasonic treatment followed by enzymatic treatment probably due to greater destruction of the tissue and thus better release of them also from the cell walls.

On the other hand, in our study, caffeic acid and p-coumaric acid were present at very low levels. Generally, no effect of ultrasonic treatment as well as enzymatic treatment of fruits on the content of p-coumaric acid was found in juices from blue honeysuckle berry. In turn, the tendency in the case of caffeic acid was not clear. On the one hand, the lower content was noted in the juices not subjected to enzymatic treatment from the temperature of 0 °C and higher. On the other hand, statistically the same content was noted in both Control_E, 50_US_E, Control and –10_US samples. This probably was due to the fact that phenolic acids are compounds incorporated into the cell wall and a significant proportion of them occur in plants in the form of esters [27].

#### 3.6. Proanthocyanidins content (TP)

Proanthocyanidins are compounds belonging to the flavan-3-ols group. Flavan-3-ols are divided into monomers, which are catechin and epicatechin, and polymers, which are proanthocyanidins - predominant in blue honeysuckle berry. Proanthocyanidins are components which can affect the sensory characteristics of raw materials, imparting bitterness or astringency. It is also important to emphasise their activity against modern civilisation diseases - they have been reported to decrease LDL cholesterol, support treat urinary tract diseases, and have antioxidant and anticancer properties [37].

In the present study, the highest total proanthocyanidins (TP) content was determined in the juice of Control (64.98 mg/100 mL) and –10_US (64.86 mg/100 mL) (Table 5). Significantly lower content was noted in the case of all others variants of juices, obtained both with and without enzymatic treatment. Thus, the degradation of proanthocyanidins took place predominantly at positive temperatures. As demonstrated by Khanal et al. [20] temperature is a factor affecting the stability of these compounds. Generally, enzymatic treatment resulted in a statistically lower content of TP in juices obtained without US (Control) and with US but applied until reaching –10 or 0 °C, probably due to further heating of mash during enzymatic treatment. When ultrasound assisted thawing until reaching higher temperatures, the differences were not noted.

Analysing the results in the context of proanthocyanidins content it can be concluded that in the case of proanthocyanidins it was more beneficial to omit longer ultrasonic and enzymatic treatment of the mash, thanks to which higher contents of these compounds were
obtained in the juices.

4. Conclusions

The study revealed the possibility to obtain high-quality juices from blue honeysuckle berry, rich in ascorbic acid, anthocyanins, iridoids, phenolics acids and proanthocyanidins. In this study, a combination of enzymes or untreated after US-assisted thawing for various time until: –10 °C, the phase transition (−3 ± 0 °C), 10 °C, and until the material reached 50 °C a-d. The values with the same letter in each column did not differ significantly.

### Table 5

| Juices from fruits without enzymatic treatment | Control | 50-US | 10-US | 0-US | –10-US | Treatment |
|----------------------------------------------|---------|-------|-------|------|-------|-----------|
| TPA                                          | 58.73 ± 0.57 | 56.67 ± 0.57 | 56.57 ± 0.57 | 56.56 ± 0.57 | 56.72 ± 0.57 | 56.72 ± 0.57 |
| Chlorogenic acid                              | 0.02a | 0.02a | 0.02a | 0.02a | 0.02a | 0.02a |
| Caffeic acid                                 | 0.02a | 0.02a | 0.02a | 0.02a | 0.02a | 0.02a |
| p-coumaric acid                              | 0.01ab | 0.01ab | 0.01ab | 0.01ab | 0.01ab | 0.01ab |
| TP                                           | 0.02ab | 0.02ab | 0.02ab | 0.02ab | 0.02ab | 0.02ab |

### Data availability

Data will be made available on request.

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Magdalena Danad: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing – original draft, Editing, Review & editing, Visualization, Supervision. Anna Grobelna: Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization. Stanislaw Kalisz: Methodology, Validation, Resources, Writing – review & editing. Dorota Witrowa-Rajchert: Conceptualization, Validation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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