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# Effect of Processing Methods and Storage Time on the Content of Bioactive Compounds in Blue Honeysuckle Berry Purees

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**Abstract:** In this study, we aimed to investigate the effect of processing methods and storage time on selected parameters of quality in the purees of blue honeysuckle berries. We investigated the content of bioactive compounds such as anthocyanins, L-ascorbic acid, and total polyphenols (TPs). We tested four processing methods and two varieties of blue honeysuckle berry (*Lonicera caerulea* L.var. *Sineglaska* and *Volshebnica*). The purees were analyzed immediately after production, and after two and four months of storage at 20 °C without exposure to light. According to our results, thermal treatment of the fruits for 5 min resulted in obtaining purees with a higher content of anthocyanins and TPs, but lower content of L-ascorbic acid. However, sieving fruits that were not thermally treated resulted in a higher content of L-ascorbic acid. Furthermore, purees prepared from the fruits of the *Volshebnica* cultivar were richer in bioactive compounds than that of purees prepared from the *Sineglaska* cultivar. In addition, the content of anthocyanins, L-ascorbic acid, and TPs decreased with storage time.

**Keywords:** blue honeysuckle berry; *Lonicera caerulea* L.; polyphenols; anthocyanins; L-ascorbic acid

## 1. Introduction

Growing consumer demand and increasing competition in the market has fueled the need to search for new and nutritionally valuable fruits and vegetables for human consumption. One such not-so-popular fruit is the blue honeysuckle berry (*Lonicera caerulea* L.). It is relatively easy to cultivate and is best-grown at high-altitudes, and in colder climates. It contains high levels of bioactive components [1,2]; therefore, there is a growing interest among researchers to characterize the blue honeysuckle berry for industrial exploitation.

Blue honeysuckle (*L. caerulea* L.) belongs to the family Caprifoliaceae and includes about 200 different varieties. In addition to the name "blue honeysuckle", the literature also refers to them using terms such as "sweet berry honeysuckle," "honeyberry," "haskap berry," or "edible honeysuckle" [3]. In the literature, the first mention of this plant originates from Russia in the 17th century. At present, blue honeysuckle is cultivated across Japan, China, Russia, Central and Eastern Europe—especially Poland—the Czech Republic, Slovenia, Slovakia, North America–Canada, and the USA [3–5].

Blue honeysuckle is a shrub that grows up to 2 m in height. It is a long-lived shrub and can bear fruit up to 30 years. In addition, it is resistant to diseases, pests, and frost. Ripe fruits can reach a length of up to 1.5–2.5 cm, and depending on the variety, they can be either elongated or cylindrical in shape. In the final stage of ripening, the fruits are blue to navy blue in color. The fruits have thin skin with a

characteristic waxy coating. They are very juicy and have sour to sweet taste with a noticeable slight bitterness [3,6–8].

The health-promoting properties of blue honeysuckle berries have been known for centuries in the folk medicine of Russia, Japan, and China [9,10]. Because of its rich content of biologically active ingredients, blue honeysuckle berries can be used to combat many diseases. They significantly contribute to the improved functioning of the visual system, lower blood pressure, reduce the risk of heart attacks and atherosclerosis, have antimicrobial and anti-inflammatory effects, is used to treat anemia, and help to manage diabetes [3,7,11–13].

The pro-health properties of the blue honeysuckle berry are related to its content of bioactive compounds, the most noteworthy among which are anthocyanins [14]. Anthocyanins have been shown to have antioxidant, antimicrobial, anti-diabetic, neuroprotective, anti-cancer, and cardiovascular effects [15]. The other groups of chemical compounds identified in the fruits of blue honeysuckle are phenolic acids, flavonoids, flavan-3-ols, flavons, flavanols, organic acids, and iridoids [1,3,16]. The fruit of blue honeysuckle are a good source of vitamin C [8]. In addition, they also contain macroelements such as calcium, potassium, phosphorus, and magnesium [14].

The fruits of blue honeysuckle are suitable for fresh consumption and for the production of preserves. It has a relatively short shelf life and therefore should be processed as soon as possible [17]. The most popular fruit-processing options for blue honeysuckle are juices, jams, jellies, soft drinks, wines, and liqueurs [7]. Due to the special health qualities of blue honeysuckle berries, it is extremely important to search for various ways to utilize them. However, technology-oriented research to find practical applications for this fruit in the fruit industry is scarce. In addition, it is necessary to use appropriate processing methods so as to minimize the losses during the production process, and to produce products with the highest nutritional value. The production of purees would be an excellent way of using blue honeysuckle berries, which in turn can be used in the preparation of smoothies, ice-creams, yogurt, salad dressings, and marinades. Fruit purees can be prepared with a creamy consistency, and further preserved by thermal or nonthermal methods [18].

Processing of puree can significantly contribute to the loss of bioactive components [19]; therefore, it is best to select a processing method that can cause minimal loss of such bioactive components. To achieve this, the process parameters should be tuned to retain the maximum level of bioactive components from the raw material being tested. In this study, we aimed to determine the effect of processing and storage time on the content of bioactive compounds in purees prepared from blue honeysuckle berries.

## 2. Materials and Methods

### *2.1. Plant Material*

The fruit of the blue honeysuckle (*L. caerulea* L.) cultivars *Sineglaska* and *Volshebnica* were obtained from the plantation of the Institute of Horticulture in Skierniewice. The berries were harvested and immediately frozen in June. Until the preparation of the purees in October, the fruits were stored in a freezer at −29 °C, in vacuum-sealed foil packaging.

### *2.2. Reagents and Standards*

Formic acid, acetonitrile, phosphoric acid, Folin–Ciocalteu reagent, sodium carbonate, and gallic acid were purchased from Sigma-Aldrich (Steinheim, Germany).

Ascorbic acid Cyanidin-3-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, peonidin-3-*O*-rutinoside, and peonidin-3-*O*-glucoside were purchased from Extrasynthese (Lyon, France).

### 2.3. Blue Honeysuckle Berry Purée Processing

For the production of purees, two varieties of blue honeysuckle—*Sineglaska* and *Volshebnica*—were selected, and four processing methods were used. The following variants of purees were produced from each variety: Puree from fruit that did not undergo a thermal treatment before sieving, and did not contain fruit skins or seeds; puree from fruit that underwent a thermal treatment for 2.5 min before sieving, and did not contain fruit skins or seeds; puree from fruit that underwent a thermal treatment for 5 min before sieving, and did not contain fruit skins or seeds; puree from whole fruit (with skins and seeds), that underwent a high-speed homogenization treatment (HSH).

The analyses were performed immediately after the production of the purees, and after two and four months of storage at 20 °C without access to light.

Table 1 shows the variants of purees produced, which were obtained by sieving or homogenizing the fruit. In the case of purees, in which the sieving process was used, the fruits underwent a thermal treatment at a temperature of 85 °C using a contact method using a thermostatic water bath, and were then sieved using a laboratory fruit grinder machine and a 1.5 mm sieve. For variants in which the homogenization process was used, the IKA T25 Ultra Turrax device was used (10000 rpm/min, 10 s). The obtained purees were heated at 85 °C for deaeration and poured hot into glass containers. Then, pasteurization was performed for 15 min at 85 °C. After pasteurization, the product was immediately cooled and stored at 20 ± 1 °C without exposure to sunlight.

**Table 1.** Variants of purees produced.

| Symbol of the Puree | The Processing Method |
|---|---|
| SP0; VP0 | Non-thermal treatment fruit subjected to a sieving process |
| SP2.5; VP2.5 | Thermal treatmentfruit for 2.5 min at 85 °C, and sieving |
| SP5; VP5 | Thermal treatment fruit for 5 min at 85 °C and sieving |
| SH; VH | Non-thermal treatment fruit subjected to a homogenization |

[S] Blue honeysuckle cultivar *Sineglaska*; [V] Blue honeysuckle cultivar *Volshebnica.*

### 2.4. Physicochemical Parameters

Total soluble solids (TSS) were determined using a Refracto 30PX (Mettler Toledo, Columbus, OH, USA), following the operating instructions. Results are expressed in °Brix.

Total titratable acidity (TTA) was determined using a Hi 221 pH meter (Hanna Instruments, Woonsocket, RI, USA), in accordance with the Polish standard (PN-A-75101/04) [20]. The results were expressed in grams per 100 g sample, and calculated as citric acid.

Active acidity (pH) was determined according to the Hi 221 electric pH meter manual. The results were read with an accuracy of 0.01.

### 2.5. Preparation of Extracts for Testing

The solvent for extraction contained 20% distilled water and 80% methanol, which was acidified with 1 mL HCl per liter of solution. Centrifuge tubes were weighed with an accuracy of 0.01 g, after 2.5 g purees. The extraction mixture was then added to each sample tube up to a volume of 12 mL and placed for 3 min in an ultrasonic cleaner (Sonoswiss SH-3H, Ramsen, Switzerland), to increase the efficiency of extraction. The prepared samples were centrifuged in a laboratory centrifuge (MPW-350R, Warsaw, Poland) (10000 rpm/min, 10 min). The steps were repeated until the samples were completely discolored. The extracts were collected into 100 mL volumetric flasks in which the extraction mixture was added.

### 2.6. HPLC Analysis of Anthocyanins

The anthocyanin content in the prepared juices was determined using the high-pressure liquid chromatography (HPLC-PDA with diode array detector, Shimadzu, Kyoto, Japan) method in accordance

with previous methodology [21,22]. The analysis was conducted in an isocratic flow at a flow rate of 1 mL/min. A water:acetonitrile:formic acid mixture in a volumetric ratio of 810:90:100 was used as the mobile phase. Prior to the analysis, the samples were passed through a 0.45 µm PTFE syringe filter. The results were recorded at a wavelength of 520 nm. The total anthocyanin content was expressed as milligram content per 100 g sample, and calculated as cyanidin 3-*O*-glucoside.

### *2.7. Total Phenolic Analysis*

Total polyphenols (TPs) were determined using the Folin-Ciocalteu reagent method, as described in previous literature [23]. The results were expressed as milligram content per 100 mL sample, and calculated as gallic acid equivalents.

### *2.8. Statistical Analysis*

Statistical analysis was performed using the Statistica version 13.3 program at a significance level of $\alpha = 0.05$. We performed a one-way analysis of variance (ANOVA) test, and the results were divided into homogeneous groups using the Tukey test. Pearson correlation coefficients ($r$) and coefficients of determination ($R^2$) were also calculated.

## 3. Results and Discussion

### *3.1. Physicochemical Parameters*

TSS, often expressed in °Brix, is a term used primarily to determine the weight percentage of solid sugars in pure sucrose solution [24]. According to the guidelines of the AIJN (European Fruit Juice Association) code, TSS is often used, among others, in the analysis of fruit and vegetable product quality [25]. Immediately after production, the TSS of SP0 and VP5 was approximately 12.6 °Brix and 15.6 °Brix, respectively (Table 2).

A significant effect of both the variety and processing method on the value of TSS was found. In the case of purees obtained by the sieving of fruit not subjected to thermal treatment, a higher TSS value was recorded for the VP0 (14.3 °Brix) than that of SP0 (12.6 °Brix). TSS values were significantly affected by applying the thermal treatment process. Thermally treated purees for 2.5 and 5 min showed a higher TSS value than that of purees obtained without thermal treatment (SP0, VP0), and after homogenization (SH, VH). This tendency was the same for purees from both tested varieties. The reason for the increased value of TSS in thermally treated samples might have been the higher rate of water evaporation from these fruit.

An example of another technological process during which the TSS value also increases due to the evaporation of water, is the concentration of fruit juices [26]. In addition, there was no significant effect of storage time on TSS values, as after 4 months of storage the TSS values remained the same as the initial values. Next, the pH and TTA values were evaluated. One of the important parameters from the point of view of microbiological stability is the pH value, whereas TTA indicates the ripeness of the fruit, which provides sensory character to the finished product [27,28].

Table 2 shows the effects of fruit variety and processing methods on pH and TTA values. TTA values ranged from 1.90 to 2.00 for SP0 and VP5, respectively. pH values ranged from 2.60 to 2.91 for SH and VP5, respectively. Puree obtained from the *Volshebnica* variety had higher TTA and pH values compared to puree obtained from the *Sineglaska* variety. There was no significant effect of storage time on changes in TTA and pH values. Wibowo et al. [29] similarly did not obtain any significant effect of storage time on parameters such as TSS, TTA, or pH values of kale purees.

**Table 2.** Impact of storage time, variety and processing methods on selected physicochemical properties and content of L-ascorbic acid in blue honeysuckle berry purees.

| Variety | Symbol | Parameters | Time of Storage | | |
|---|---|---|---|---|---|
| | | | After Production | Two Months | Four Months |
| *Sinoglaska* | SP0 | TSS $^{A}$ | 12.6 ± 0.01 $^{aE}$ | 12.6 ± 0.01 $^{aE}$ | 12.6 ± 0.00 $^{aE}$ |
| | | TTA $^{B}$ | 1.93 ± 0.00 $^{aA}$ | 1.95 ± 0.00 $^{aA}$ | 1.95 ± 0.01 $^{aA}$ |
| | | pH | 2.61 ± 0.10 $^{aB}$ | 2.60 ± 0.01 $^{aB}$ | 2.60 ± 0.02 $^{aB}$ |
| | | L-ascorbic acid | 13.75 ± 0.11 $^{aD}$ | 6.65 ± 0.02 $^{bD}$ | nd |
| | SP2.5 | TSS $^{A}$ | 14.0 ± 0.02 $^{aD}$ | 13.9 ± 0.01 $^{aD}$ | 13.9 ± 0.00 $^{aD}$ |
| | | TTA $^{B}$ | 1.90 ± 0.02 $^{aA}$ | 1.91 ± 0.10 $^{aA}$ | 1.92 ± 0.01 $^{aA}$ |
| | | pH | 2.62 ± 0.07 $^{aB}$ | 2.61 ± 0.02 $^{aB}$ | 2.60 ± 0.02 $^{aB}$ |
| | | L-ascorbic acid | 12.49 ± 0.04 $^{aE}$ | 5.32 ± 0.03 $^{bE}$ | nd |
| | SP5 | TSS $^{A}$ | 14.4 ± 0.01 $^{aC}$ | 14.4 ± 0.00 $^{aC}$ | 14.3 ± 0.00 $^{aC}$ |
| | | TTA $^{B}$ | 1.90 ± 0.06 $^{aA}$ | 1.91 ± 0.05 $^{aA}$ | 1.91 ± 0.04 $^{aA}$ |
| | | pH | 2.63 ± 0.01 $^{aB}$ | 2.62 ± 0.00 $^{aB}$ | 2.62 ± 0.00 $^{aB}$ |
| | | L-ascorbic acid | 10.68 ± 0.04 $^{aF}$ | 4.49 ± 0.03 $^{bF}$ | nd |
| | SH | TSS $^{A}$ | 12.7 ± 0.01 $^{aE}$ | 12.6 ± 0.00 $^{aE}$ | 12.6 ± 0.00 $^{aE}$ |
| | | TTA $^{B}$ | 1.92 ± 0.03 $^{aA}$ | 1.92 ± 0.02 $^{aA}$ | 1.93 ± 0.04 $^{aA}$ |
| | | pH | 2.60 ± 0.00 $^{aB}$ | 2.60 ± 0.02 $^{aB}$ | 2.59 ± 0.00 $^{aB}$ |
| | | L-ascorbic acid | 4.57 ± 0.04 $^{aH}$ | 2.30 ± 0.11 $^{bH}$ | nd |
| *Volshebnica* | VP0 | TSS $^{A}$ | 14.3 ± 0.01 $^{aC}$ | 14.3 ± 0.01 $^{aC}$ | 14.3 ± 0.00 $^{aC}$ |
| | | TTA $^{B}$ | 2.00 ± 0.00 $^{aA}$ | 2.01 ± 0.00 $^{aA}$ | 2.01 ± 0.01 $^{aA}$ |
| | | pH | 2.90 ± 0.05 $^{aA}$ | 2.89 ± 0.01 $^{aA}$ | 2.88 ± 0.02 $^{aA}$ |
| | | L-ascorbic acid | 20.72 ± 0.11 $^{aA}$ | 15.18 ± 0.02 $^{bA}$ | 12.67 ± 0.02 $^{cA}$ |
| | VP2.5 | TSS $^{A}$ | 15.3 ± 0.02 $^{aB}$ | 15.2 ± 0.01 $^{aB}$ | 15.2 ± 0.00 $^{aB}$ |
| | | TTA $^{B}$ | 1.98 ± 0.02 $^{aA}$ | 1.98 ± 0.10 $^{aA}$ | 1.99 ± 0.01 $^{aA}$ |
| | | pH | 2.91 ± 0.20 $^{aA}$ | 2.90 ± 0.02 $^{aA}$ | 2.89 ± 0.00 $^{aA}$ |
| | | L-ascorbic acid | 19.51 ± 0.04 $^{aB}$ | 13.23 ± 0.03 $^{bB}$ | 10.48 ± 0.02 $^{cB}$ |
| | VP5 | TSS $^{A}$ | 15.6 ± 0.01 $^{aA}$ | 15.3 ± 0.00 $^{aA}$ | 15.4 ± 0.01 $^{aA}$ |
| | | TTA $^{B}$ | 1.97 ± 0.02 $^{aA}$ | 1.97 ± 0.01 $^{aA}$ | 1.97 ± 0.01 $^{aA}$ |
| | | pH | 2.91 ± 0.07 $^{aA}$ | 2.89 ± 0.00 $^{aA}$ | 2.89 ± 0.00 $^{aA}$ |
| | | L-ascorbic acid | 17.93 ± 0.04 $^{aC}$ | 11.98 ± 0.03 $^{bC}$ | 7.32 ± 0.02 $^{cC}$ |
| | VH | TSS $^{A}$ | 14.2 ± 0.01 $^{aC}$ | 14.2 ± 0.00 $^{aC}$ | 14.2 ± 0.00 $^{aC}$ |
| | | TTA $^{B}$ | 2.01 ± 0.03 $^{aA}$ | 2.01 ± 0.02 $^{aA}$ | 2.02 ± 0.01 $^{aA}$ |
| | | pH | 2.90 ± 0.05 $^{aA}$ | 2.87 ± 0.02 $^{aA}$ | 2.87 ± 0.00 $^{aA}$ |
| | | L-ascorbic acid | 5.42 ± 0.04 $^{aG}$ | 3.14 ± 0.11 $^{bG}$ | nd |

$^{a,b,c}$, mean values marked with the same letters do not differ significantly, differences in lines; $^{A,B,C}$, mean values in columns (presented single parameters) denoted with the same letter are not significantly different, Tukey's test, $\alpha$ = 0.05; nd = not detected; TSS = total soluble solids; TTA Total titratable acidity.

### 3.2. L-Ascorbic Acid Content

Vitamin C is an important antioxidant that shows several beneficial effects in the human body [30,31]. Research has shown that the content of vitamin C is higher in blue honeysuckle berry than that of citruses, which are widely recognized as one of the best sources [14]. Unfortunately, it is a highly labile and heat-sensitive compound [32,33]. L-ascorbic acid and its oxidation product—dehydroascorbic acid—are commonly known as "Vitamin C" [34]. In this study, we assessed the content of L-ascorbic acid after the production of puree, and during storage. According to our results, the content of L-ascorbic acid was 4.57 and 20.72 mg/100 g for SH and VP0, respectively. The content of L-ascorbic acid significantly depended on the variety of the raw material. The puree of the *Volshebnica* variety showed higher levels of vitamin C. In addition, the thermal treatment before sieving had a significant effect on the content of vitamin C. All samples in which the thermal treatment was used during

production (SP2.5, SP5, VP2.5, and VP5), and immediately after production, showed lower values of L-ascorbic acid than that of the respective non-thermally treated purees (SP0 and VP0). In addition, the content of vitamin C was significantly affected by storage time. After two and four months of storage, a loss in the content of L-ascorbic acid were found in all the tested variants. The greatest amount of loss was found in purees obtained from the *Sineglaska* variety, with after four months of storage, there was no presence of L-ascorbic acid. However, in the case of the *Volshebnica* variety, only the puree obtained by the process of homogenization (VH) after four months of storage, showed no content of L-ascorbic acid. In other variants of the *Sineglaska* variety, 41% (VH) to 61% (VP0) of the initial L-ascorbic acid content remained after storage. The use of homogenization alone may cause so much aeration that it results in the degradation of vitamin C, which is susceptible to oxidation [35]. In our study, the most beneficial method in terms of preserving the greatest amount of L-ascorbic acid, was to use a process of sieving fruit that had not undergone thermal treatment. In the context of preserving natural vitamin C, it is therefore more beneficial to obtain purees without using thermal treatment.

### *3.3. Anthocyanin and Total Phenolic Content*

Blue honeysuckle berries contain anthocyanins, belonging to a group of compounds called flavonoids, which protect our body against the harmful effects of free radicals [3,14]. These compounds are responsible for the red, blue to navy blue, and black colors of various plant parts such as flowers, fruits, and stems. In plant cells, they are synthesized in the cytoplasm and stored in vacuoles [36]. Table 3 shows the values of anthocyanins in the tested purees.

The highest content of total anthocyanins immediately after the production was obtained for VP5 (649.80 mg/100 g) and the lowest for SP0 (401.89 mg/100 g). Interestingly, among the purees obtained from thermally treated fruits, anthocyanin content was found to be higher in samples thermally treated for 5 min (SP5 and VP5) than for 2.5 min (VP2.5 and VP5). Longer thermal treatment time could have contributed to the better extraction of anthocyanin from vacuoles. Heat treatment is widely used in the food industry to inactivate the enzymes of raw materials. This includes during the enzymatic processing of fruit before juice pressing, which in turn allows for the better extraction of anthocyanins responsible for the color of berry juices [37]. However, many scientific studies point to the negative effect of thermal treatment on the stability of anthocyanins, which can easily degrade at higher temperatures [38–40]. Nevertheless, Casati et al. [38] obtained interesting results when they studied the effect of heat treatment times on elderberries at 90 °C, 80 °C, and 70 °C, on the kinetics of anthocyanin degradation. It turned out that the higher the temperature, the greater the degree of anthocyanin degradation, but the duration of thermal treatment is also very important. Based on the calculated Q10 parameter, it was found that the treatment of puree at 90 °C for 1 min showed similar anthocyanin degradation as treatment for 5 min at 80 °C. In addition, Casati et al. [38] concluded that the use of thermal treatment at higher temperatures (i.e., 90 °C), but for a shorter time, may allow for a greater degree of inactivation of the raw material's own enzymes, i.e., PPO (polyphenol oxidase) and PDO (peroxidase), with a higher retention of color from the bioactive components.

Storage time is another important factor that determines the content of anthocyanin in the tested purees. Loss of anthocyanin content was found in all the tested purees after two and four months of storage. After four months of storage, the *Sineglaska* variety showed the smallest loss in SP2.5 samples, where 70% of the initial total anthocyanin content remained. The largest loss was recorded for SP0 and SH samples, where 64% and 63%, respectively, of the original total anthocyanin content remained. As for the purees made from the *Volshebnica* variety, the smallest loss was recorded for VP2.5 puree (67% of the initial anthocyanin content remained), whereas the largest loss was recorded for VP0 puree (59% of the initial anthocyanin content remained). Interestingly, SP2.5 and VP2.5 contained lower total anthocyanin contents than that of purees SP5 and VP5, whereas after four months of storage, SP2.5 and VP2.5 had higher total anthocyanin content than that of purees SP5 and VP5. This indicates that the process of thermal treatment for a longer time contributes to the better extraction of anthocyanins,

but during storage they may degrade faster. Probably at a higher initial concentration, the rate of degradation is higher.

**Table 3.** Impact of storage time, variety, and processing method on anthocyanin content [mg/100 g].

| Variety | Symbol | Compounds | Time of Storage | | |
|---|---|---|---|---|---|
| | | | After Production | Two Months | Four Months |
| *Sinoglaska* | SP0 | Cyanidin 3,5-*O*-diglucoside | 20.31 ± 0.47 $^{aF}$ | 12.83 ± 1.09 $^{bE}$ | 8.63 ± 0.15 $^{cE}$ |
| | | Cyanidin 3-*O*-glucoside | 350.49 ± 1.44 $^{aG}$ | 283.61 ± 0.38 $^{bH}$ | 228.28 ± 1.62 $^{cH}$ |
| | | Cyanidin 3-*O*-rutinoside | 10.36 ± 1.62 $^{aE}$ | 8.53 ± 0.72 $^{bG}$ | 7.21 ± 0.03 $^{cG}$ |
| | | Pelargonidin 3-*O*-glucoside | 3.53 ± 0.13 $^{aB}$ | 2.85 ± 0.06 $^{bC}$ | 2.68 ± 0.05 $^{cC}$ |
| | | Peonidin 3-*O*-glucoside | 15.69 ± 0.19 $^{aF}$ | 11.84 ± 0.03 $^{bG}$ | 9.73 ± 0.07 $^{cG}$ |
| | | Peonidin 3-*O*-rutinoside | 1.51 ± 0.08 $^{aB}$ | 1.03 ± 0.02 $^{cDE}$ | 1.06 ± 0.10 $^{bBC}$ |
| | | Total anthocyanins | 401.89 ± 3,93 $^{aH}$ | 320.69 ± 2,30 $^{bG}$ | 257.59 ± 2,02 $^{cF}$ |
| | SP2.5 | Cyanidin 3,5-*O*-diglucoside | 25.22 ± 0.09 $^{aC}$ | 12.73 ± 0.08 $^{bE}$ | 10.58 ± 0.10 $^{cC}$ |
| | | Cyanidin 3-*O*-glucoside | 384.11 ± 0.39 $^{aE}$ | 316.13 ± 0.40 $^{bF}$ | 275.44 ± 0.18 $^{cE}$ |
| | | Cyanidin 3-*O*-rutinoside | 10.18 ± 0.06 $^{aE}$ | 9.18 ± 0.02 $^{bF}$ | 9.06 ± 0.02 $^{cE}$ |
| | | Pelargonidin 3-*O*-glucoside | 3.56 ± 0.16 $^{aB}$ | 3.15 ± 0.01 $^{bB}$ | 3.03 ± 0.06 $^{cB}$ |
| | | Peonidin 3-*O*-glucoside | 16.76 ± 0.15 $^{aE}$ | 13.25 ± 0.01 $^{bF}$ | 10.14 ± 0.02 $^{cF}$ |
| | | Peonidin 3-*O*-rutinoside | 1.44 ± 0.07 $^{aB}$ | 1.08 ± 0.00 $^{cCD}$ | 1.15 ± 0.00 $^{bB}$ |
| | | Total anthocyanins | 441.27 ± 0,92 $^{aF}$ | 355.40 ± 0.52 $^{bE}$ | 310.27 ± 0.38 $^{cD}$ |
| | SP5 | Cyanidin 3,5-*O*-diglucoside | 24.05 ± 0.05 $^{aD}$ | 15.52 ± 0.10 $^{bB}$ | 10.67 ± 0.19 $^{cC}$ |
| | | Cyanidin 3-*O*-glucoside | 443.49 ± 0.18 $^{aC}$ | 376.14 ± 0.13 $^{bC}$ | 303.16 ± 0.96 $^{cD}$ |
| | | Cyanidin 3-*O*-rutinoside | 12.88 ± 0.01 $^{aD}$ | 10.84 ± 0.01 $^{bE}$ | 9.14 ± 0.06 $^{cE}$ |
| | | Pelargonidin 3-*O*-glucoside | 4.22 ± 0.04 $^{aA}$ | 3.75 ± 0.04 $^{bA}$ | 3.54 ± 0.03 $^{cA}$ |
| | | Peonidin 3-*O*-glucoside | 19.68 ± 0.06 $^{aD}$ | 15.90 ± 0.06 $^{bC}$ | 12.84 ± 0.04 $^{cD}$ |
| | | Peonidin 3-*O*-rutinoside | 1.88 ± 0.00 $^{aA}$ | 1.53 ± 0.01 $^{bA}$ | 1.35 ± 0.02 $^{cA}$ |
| | | Total anthocyanins | 506.20 ± 0.34 $^{aD}$ | 423.68 ± 0.35 $^{bC}$ | 340.70 ± 1.30 $^{cC}$ |
| | SH | Cyanidin 3,5-*O*-diglucoside | 25.13 ± 0.10 $^{aC}$ | 15.54 ± 0.08 $^{bB}$ | 6.41 ± 0.07 $^{cF}$ |
| | | Cyanidin 3-*O*-glucoside | 372.38 ± 0.58 $^{aF}$ | 338.91 ± 2.09 $^{bE}$ | 242.40 ± 0.51 $^{cF}$ |
| | | Cyanidin 3-*O*-rutinoside | 9.97 ± 0.07 $^{aEF}$ | 9.13 ± 0.08 $^{bF}$ | 8.09 ± 0.15 $^{cF}$ |
| | | Pelargonidin 3-*O*-glucoside | 3.28 ± 0.11 $^{aC}$ | 3.10 ± 0.01 $^{bB}$ | 2.68 ± 0.10 $^{cC}$ |
| | | Peonidin 3-*O*-glucoside | 16.51 ± 0.09 $^{aE}$ | 13.91 ± 0.82 $^{bE}$ | 11.37 ± 0.16 $^{cE}$ |
| | | Peonidin 3-*O*-rutinoside | 1.45 ± 0.00 $^{aB}$ | 1.27 ± 0.00 $^{bB}$ | 1.10 ± 0.00 $^{cB}$ |
| | | Total anthocyanins | 428.72 ± 0.95 $^{aG}$ | 381.86 ± 3.08 $^{bD}$ | 272.05 ± 0.99 $^{cE}$ |
| *Volshebnica* | VP0 | Cyanidin 3,5-*O*-diglucoside | 19.43 ± 0.01 $^{aG}$ | 10.19 ± 0.88 $^{bF}$ | 10.09 ± 0.04 $^{bD}$ |
| | | Cyanidin 3-*O*-glucoside | 391.10 ± 0.17 $^{aD}$ | 290.52 ± 0.02 $^{bG}$ | 232.25 ± 1.21 $^{cG}$ |
| | | Cyanidin 3-*O*-rutinoside | 21.62 ± 0.00 $^{aC}$ | 15.41 ± 0.01 $^{bD}$ | 10.94 ± 0.01 $^{cD}$ |
| | | Pelargonidin 3-*O*-glucoside | 2.37 ± 0.12 $^{aD}$ | 1.95 ± 0.04 $^{bE}$ | 1.62 ± 0.01 $^{cG}$ |
| | | Peonidin 3-*O*-glucoside | 21.65 ± 0.02 $^{aC}$ | 14.71 ± 0.00 $^{bD}$ | 14.13 ± 0.65 $^{bC}$ |
| | | Peonidin 3-*O*-rutinoside | 1.22 ± 0.01 $^{aC}$ | 0.95 ± 0.08 $^{bE}$ | 0.82 ± 0.00 $^{cD}$ |
| | | Total anthocyanins | 457.39 ± 0.33 $^{aE}$ | 333.73 ± 1.03 $^{bF}$ | 269.85 ± 1.92 $^{cE}$ |
| | VP2.5 | Cyanidin 3,5-*O*-diglucoside | 21.57 ± 0.10 $^{aE}$ | 14.76 ± 0.04 $^{bC}$ | 11.16 ± 0.06 $^{cB}$ |
| | | Cyanidin 3-*O*-glucoside | 466.82 ± 0.18 $^{aC}$ | 371.42 ± 0.16 $^{bD}$ | 306.57 ± 0.80 $^{cC}$ |
| | | Cyanidin 3-*O*-rutinoside | 24.14 ± 0.02 $^{aB}$ | 17.99 ± 0.01 $^{bB}$ | 17.70 ± 0.05 $^{cA}$ |
| | | Pelargonidin 3-*O*-glucoside | 3.14 ± 0.06 $^{aC}$ | 2.89 ± 0.01 $^{bC}$ | 2.50 ± 0.06 $^{cD}$ |
| | | Peonidin 3-*O*-glucoside | 24.88 ± 0.02 $^{aB}$ | 16.98 ± 0.00 $^{bB}$ | 15.09 ± 0.09 $^{cB}$ |
| | | Peonidin 3-*O*-rutinoside | 1.20 ± 0.00 $^{aC}$ | 1.18 ± 0.02 $^{aBC}$ | 0.98 ± 0.05 $^{bC}$ |
| | | Total anthocyanins | 541.75 ± 0.38 $^{aC}$ | 424.83 ± 0.22 $^{bC}$ | 364.39 ± 1.11 $^{cB}$ |
| | VP5 | Cyanidin 3,5-*O*-diglucoside | 34.54 ± 0.08 $^{aA}$ | 25.51 ± 0.51 $^{bA}$ | 20.55 ± 1.44 $^{cA}$ |
| | | Cyanidin 3-*O*-glucoside | 556.75 ± 0.40 $^{aA}$ | 431.74 ± 2.98 $^{bA}$ | 360.96 ± 1.62 $^{cA}$ |
| | | Cyanidin 3-*O*-rutinoside | 26.69 ± 0.02 $^{aA}$ | 20.32 ± 0.35 $^{bA}$ | 17.44 ± 1.09 $^{cB}$ |
| | | Pelargonidin 3-*O*-glucoside | 2.52 ± 0.01 $^{aE}$ | 2.36 ± 0.06 $^{bD}$ | 2.32 ± 0.08 $^{bE}$ |
| | | Peonidin 3-*O*-glucoside | 28.24 ± 0.01 $^{aA}$ | 19.21 ± 0.22 $^{bA}$ | 17.31 ± 0.72 $^{cA}$ |
| | | Peonidin 3-*O*-rutinoside | 1.26 ± 0.00 $^{aC}$ | 0.98 ± 0.32 $^{bDE}$ | 0.79 ± 0.03 $^{cE}$ |
| | | Total anthocyanins | 649.8 ± 0.52 $^{aA}$ | 500.28 ± 4.44 $^{bA}$ | 419.41 ± 5.28 $^{cA}$ |
| | VH | Cyanidin 3,5-*O*-diglucoside | 26.75 ± 0.47 $^{aB}$ | 13.88 ± 0.02 $^{bD}$ | 11.21 ± 0.15 $^{cB}$ |
| | | Cyanidin 3-*O*-glucoside | 488.55 ± 2.60 $^{aB}$ | 379.18 ± 0.61 $^{bB}$ | 321.88 ± 1.62 $^{cB}$ |
| | | Cyanidin 3-*O*-rutinoside | 21.76 ± 0.31 $^{aC}$ | 17.43 ± 0.01 $^{bC}$ | 15.75 ± 0.15 $^{cC}$ |
| | | Pelargonidin 3-*O*-glucoside | 2.28 ± 0.13 $^{aD}$ | 2.30 ± 0.04 $^{aD}$ | 2.02 ± 0.03 $^{bF}$ |
| | | Peonidin 3-*O*-glucoside | 24.48 ± 0.21 $^{aB}$ | 18.92 ± 0.04 $^{bA}$ | 15.08 ± 0.09 $^{cB}$ |
| | | Peonidin 3-*O*-rutinoside | 1.56 ± 0.05 $^{aB}$ | 1.23 ± 0.00 $^{bB}$ | 0.95 ± 0.05 $^{cC}$ |
| | | Total anthocyanins | 565.38 ± 3.77 $^{aB}$ | 432.94 ± 0.72 $^{bB}$ | 366.89 ± 2.09 $^{cB}$ |

$^{a,b,c}$, mean values marked with the same letters do not differ significantly, differences in lines; $^{A,B,C}$, mean values in columns (presented single compounds) denoted with the same letter are not significantly different, Tukey's test, $\alpha$= 0.05.

It is noteworthy that the stability of anthocyanins depends not only on the type and chemical structure, but also on their concentration [41]. It is also noteworthy that SP0 and VP0 purees, obtained from fruit not subjected to thermal treatment, showed lower anthocyanin content than SH and VH, respectively. This might be due to the homogenization of the whole fruit, which also contained the skin removed during sieving, and here it was intensively crumbled thereby liberating dyes enclosed in vacuoles. Scientific studies indicate that anthocyanins are compounds that accumulate primarily in the skin cells of the blue honeysuckle berry [3].

In this study, blue honeysuckle berries showed the presence of six types of anthocyanins. On average, the greatest content of anthocyanin was of cyanidin 3-*O*-glucoside, which accounted for 87% of all anthocyanins. Similar to some other studies, in this study, cyanidin 3-*O*-glucoside was the dominant anthocyanin in blue honeysuckle berries; its content ranged from 79% to 92% of the total anthocyanin content [14]. Other anthocyanins identified in this study include cyanidin 3,5-*O*-diglucoside; cyanidin 3-*O*-rutinoside; pelargonidin 3-*O*-glucoside; peonidin 3-*O*-glucoside; and peonidin 3-*O*-rutinoside. The anthocyanins found in lesser quantities were pelargonidin 3-*O*-glucoside and peonidin 3-*O*-rutinoside. This was consistent with the results reported by Chaovanalikit et al. [42], who showed that these two types of anthocyanins constitute the smallest share in the total anthocyanin fraction of blue honeysuckle berries.

The content of TPs was also estimated. In the case of plant materials, TP content varies, which may depend on many factors such as variety, growing season, agrotechnical operations, date of harvest, and geographical location of the crop [3,10]. Immediately after production, the puree of the *Sineglaska* variety showed TP content in the range of 1160.5–1398.3 mg/100 g for SP0 and SP5, respectively (Table 4). However, purees from the *Volshebnica* variety showed TP content in the range of 1321.97–1635.79 mg/100 g for VP0 and VP5, respectively (Table 3). In addition, a very strong correlation was demonstrated between TP and total anthocyanin content for purees immediately after production. In the case of purees obtained from the *Sineglaska* variety, the correlation coefficient was 0.994531 ($R^2$ = 0.9891), whereas in the case of purees from the *Volshebnica* variety, it was 0.998801 ($R^2$ = 0.9976).

**Table 4.** Impact of storage time, variety, and processing method on total polyphenol (TP) content in the purees of *Sineglaska* and *Volshebnica* varieties [mg gallic acid equivalent/100 g].

| Variety | Symbol | Time of Storage | | |
|---|---|---|---|---|
| | | After Production | 2 Months | 4 Months |
| ***Sinoglaska*** | SP0 | 1160.5 ± 1.24 $^{aH}$ | 986.6 ± 2.21 $^{bH}$ | 920.6 ± 1.32 $^{cH}$ |
| | SP2.5 | 1207.7 ± 0.84 $^{aF}$ | 1141.4 ± 2.54 $^{bF}$ | 1003.3 ± 1.54 $^{cF}$ |
| | SP5 | 1398.3 ± 1.52 $^{aD}$ | 1189.5 ± 1.42 $^{bE}$ | 1058.6 ± 0.98 $^{cD}$ |
| | SH | 1192.8 ± 2.42 $^{aG}$ | 1099.7 ± 3.53 $^{bG}$ | 954.5 ± 0.42 $^{cG}$ |
| ***Volshebnica*** | VP0 | 1321.9 ± 1.21 $^{aE}$ | 1272.9 ± 0.08 $^{bC}$ | 1027.7 ± 1.43 $^{cE}$ |
| | VP2.5 | 1446.8 ± 0.02 $^{aC}$ | 1216.3 ± 0.07 $^{bD}$ | 1170.1 ± 0.03 $^{cB}$ |
| | VP5 | 1635.7 ± 1.31 $^{aA}$ | 1467.9 ± 1.40 $^{bA}$ | 1206.5 ± 1.10 $^{cA}$ |
| | VH | 1498.3 ± 1.21 $^{aB}$ | 1311.1 ± 0.09 $^{bB}$ | 1162.3 ± 0.08 $^{cC}$ |

$^{a,b,c}$, mean values marked with the same letters do not differ significantly, differences in lines; $^{A,B,C}$, mean values in columns denoted with the same letter are not significantly different, Tukey's test, $\alpha$ = 0.05.

Storage time also affected the TP content. After four months of storage, losses in TP content were recorded for the tested samples. However, it is noteworthy that the storage losses of TP are much smaller than that of anthocyanins. After four months of storage, the *Sineglaska* variety samples (SP2.5) showed the lowest loss in TP content (they contained 83% of the initial TP content), whereas the highest loss was recorded for SP5 samples (they contained 76% of the initial TP content).

Among the purees obtained from the *Volshebnica* variety, the lowest loss was found in the VP2.5 puree (containing 81% of the initial TP content), and the highest loss was found in the VP5 puree (containing 74% of the initial TP content). Our results are consistent with the results obtained by Oancea and Călin [43], who showed higher retention of TPs than that of the total anthocyanin content, in fruit jams during five months of storage at 18 °C.

## 4. Conclusions

Along with the growing health awareness of consumers, the demand for new plant materials that could provide fruit and vegetable products enriched with high contents of nutrients, is increasing. Blue honeysuckle berries deserve special attention due to their high content of bioactive compounds, and therapeutic properties. Therefore, in order to fully utilize these fruit, it is important to look for new ways of processing them. An interesting way of processing blue honeysuckle berries is the production of purees, because they can be a valuable component during the production of many other products, especially popular convenience foods. For example, this raw material can be potentially used in the production of baby food, where the high content of bioactive ingredients is particularly important. According to our results, puree from blue honeysuckle berries is a rich source of anthocyanins, polyphenols, and vitamin C. However, the processing method, storage time, and fruit variety had a great effect on the content of the tested compounds. Puree obtained from the fruit of the *Volshebnica* variety was richer in biologically active compounds than that obtained from the fruit of the *Sineglaska* variety. On the one hand, the thermal treatment of the fruits at 5 min increased the content of anthocyanin, but on the other hand, it decreased the content of vitamin C. In addition, the use of high-speed homogenization resulted in a higher content of total anthocyanins compared to purees obtained by sieving fruits not subjected to thermal treatment. However, high-speed homogenization resulted in a greater loss of vitamin C. It should be remembered, that the loss of vitamin C caused by the application of technological processes can be compensated by its addition to the final product, which is not feasible in the case of anthocyanins. Therefore, it is considered more expedient to pay attention to limiting the loss of anthocyanin content in the final product. In addition, there was no significant effect of storage on the physicochemical parameters of the purees, i.e., TSS, pH, and TTA. The results of this study might be useful in the better exploitation of this fruit in future.

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