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Effect of Ultrasonic, Thermal and Enzymatic Treatment of Mash on Yield and Content of Bioactive Compounds in Strawberry Juice

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Featured Application: An important aspect of food production is achieving high yield in the production process while striving to improve quality. The conducted research indicates the application potential of thermosonication used as a method of mash treatment, both in terms of increasing the efficiency of the pressing process and the content of anthocyanins and other phenolic compounds (an increase of 40% and 20%, respectively, compared to the juice obtained from the mash without treatment). In particular, the increased content of anthocyanins in the juice shows not only health-promoting potential but also affect the formation of the appropriate color of the product.

Abstract: Strawberries are rich in bioactive compounds that may be of health importance. The technological process often significantly reduces the content of such compounds in the product. The study aimed to compare the effect of enzymatic, ultrasonic and thermal mash treatment on the content of ascorbic acid, anthocyanins, phenolic compounds and the antioxidant activity of strawberry juice. In addition, the effect of increased temperature assisting ultrasonic mash treatment and the use of a vacuum for a short period to remove air from the mash during pectinolysis was investigated. A significant increase in the efficiency of juice pressing was obtained for enzymatic treatment (by 40%), thermal and thermosonication (16%). It was found that the applied methods yield different results depending on the tested compounds. In the case of anthocyanin, the most effective method was thermosonication, which contributed to a 40% increase in their content. The enzymatic and thermal methods resulted in a two-fold increase in the content of phenolic compounds. The antioxidant activity of the juice from the treated mash (regardless of the method used) was significantly higher than samples from the untreated mash. A significant correlation (r = 0.77) was noted between antioxidant capacity and non-anthocyanin phenolic compound content in the tested juices.

Keywords: ultrasound; pectinolysis; thermosonication; juice production; strawberry; yield; bioactive compounds

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Citation: Radziejewska-Kubzdela, E. Effect of Ultrasonic, Thermal and Enzymatic Treatment of Mash on Yield and Content of Bioactive Compounds in Strawberry Juice. *Appl. Sci.* **2023**, *13*, 4268. https://doi.org/10.3390/app13074268

Academic Editor: Emanuel Vamanu

Received: 10 February 2023
Revised: 19 March 2023
Accepted: 26 March 2023
Published: 28 March 2023

1. Introduction

Strawberries (*Fragaria* × *ananassa* Duch.) contain a significant amount of vitamin C (37–112 mg/100 g), polyphenols (8–318 mg/100 g), anthocyanins (3.74 to 64.88 mg/100 g) and dietary fiber [1–4]. The high content of bioactive compounds results in their health-promoting properties. The bioactive compounds contained in this raw material show e.g., antioxidant, anti-inflammatory and anti-carcinogenic properties [5–7].

Strawberry is a seasonal fruit. Therefore, the quality of products obtained from it, taking into account its health-promoting properties, is an important issue. The most common directions of its processing are juices, purees, smoothies, nectar and jam. The technological process often contributes to the loss of bioactive compounds. Klopotek et al. [8] reported a 22% loss of vitamin C and a 29% loss of polyphenols as a result of crushing and pressing the strawberry mash. Oszmiański et al. [9] also indicate significant losses of procyanidins after pressing. These compounds are bound to the polysaccharides of the cell walls and remain in the pomace. Therefore, methods should be sought to improve the efficiency of juice and bioactive compounds extraction, especially at the pressing stage.

Enzymatic treatment of mash (crashed fruits) has been used as a common method to increase juice yield. The literature shows that the effectiveness of enzymatic treatment largely depends on the appropriate selection of the enzyme and processing time [10–12]. However, enzymatic maceration is time consuming. Another method is mash heating. This treatment, by denaturing the cell walls in the tissue, facilitates the release of phenolic compounds from the tissue into the juice [13]. On the other hand, thermal treatment may also contribute to the degradation of bioactive compounds [14]. An interesting method is ultrasound treatment. It can be used to extract the desired compounds at low temperature to protect bioactive compounds against degradation [15]. However, some researchers point out that long-term use of ultrasound or the use of high power can also cause the degradation of bioactive compounds [16]. So far, sonication has been used in such applications as extraction, inactivation of enzymes, emulsification, low-temperature pasteurization and homogenization [17]. The impact of ultrasonic mash processing on the efficiency of pressing and the content of bioactive compounds in the juice has not been extensively investigated so far. An attempt to apply this technique to mash treatment was conducted for acerola [12] grape [18,19], noni [20], black, red and white currant [21] and barberry [22]. Ultrasound treatment can be more effective when combined with heating (thermosonication) [23]. Attempts to use this technique have been made mainly to preserve juice [24]. In this study, an attempt was made to apply it to mash processing.

This study aimed to compare the effect of enzymatic, thermal and ultrasonic mash processing on the pressing efficiency and the content of bioactive compounds in strawberry juice. In addition, the effect of thermosonication of the mash and the use of a short vacuum period to remove air from the mash during enzymatic treatment on the above-mentioned quality parameters was also investigated.

2. Materials and Methods

2.1. Strawberry

Frozen strawberries were purchased at a local market. The berries were stored at −18 °C until processing.

2.2. Technological Process

The berries were thawed for 12 h at 4 °C. Then they were crushed in a Thermomix laboratory mill (Wuppertal, Vorwerk, Germany). Each batch was obtained from 1000 g of the fruit. Mash treatment was carried out in six variants:

- The first batch was left without mash treatment as a sample control.
- The second batch was heated up to a temperature of 80 °C for 15 min and then held for 5 min at this temperature. Processing parameters were selected based on the literature data [13]. The parameters used are also in the range used in the industrial production of fruit juices.
- The third batch was subjected to ultrasonication. Mash was directly poured into an ultrasonic bath. The sonication was carried out in an SW3H ultrasonic cleaner (Sonoswiss AG, Ramsen, Switzerland) at a frequency of 37 kHz. The process was carried out for 10 min at the effective power of the equipment (80 W). The conditions of ultrasonic mash treatment were selected from the range described as optimal for the extraction of active compounds from the plant matrix [25] and the author of earlier research.
- The next batch was subjected to thermosonication under the same conditions as the one treated ultrasonically at 50 °C. The selected temperature corresponds to the temperature used for enzymatic treatment and results from the review of the literature data [26].

- The fifth batch was subjected to enzymatic treatment. The mash was heated up to 50 °C. Pectinase (Rohapect 10L AB, Enzymes, Darmstadt, Germany) was added to the mash in the amount of 0.23 mL/1000 g. Processing was carried out at 50 °C for 60 min. Enzymatic maceration conditions were used according to the manufacturer's recommendations.
- For the sixth batch, enzymatic treatment was carried out with additional deaeration for 10 min. The mash, heated to 50 °C with the addition of an enzyme, was placed in a VC1621S vacuum chamber (VacuumChambers.eu, Białystok, Poland) connected to a vacuum pump and manometer. An absolute pressure of 80 mbar was used. The samples were held for 10 min in vacuum conditions. Further maceration was carried out at atmospheric pressure. The maceration was carried out for 60 min. Vacuum conditions were applied to reduce oxidative changes.

Then, the treated mashes were pressed in a laboratory press (Para-press, Arauner Kitzingen, Kitzingein, Germany) using these parameters: pressure—0.28 MPa for 10 min. Each experiment was performed in duplicate. The juices were stored at −50 °C for further analysis.

2.3. Yield of Strawberry Juices

The juice yield was calculated from Equation (1):

$$Y = \frac{mass\ of\ the\ juice\ [\mathrm{g}]}{mass\ of\ the\ mash\ [\mathrm{g}]} \times 100\% \tag{1}$$

2.4. Ascorbic Acid Content

Ascorbic acid was extracted from the juice with metaphosphate acid. The extraction was performed according to the procedure described by Howard et al. [27].

Analysis was performed using an Agilent Technologies LC 1200 Rapid Resolution system (Waldbronn, Germany). Methanol (phase A) and 0.005 mol/L KH_2PO_4 solution (phase B) were used as mobile phases. Gradient elution was used from 5% to 22% phase A for 6 min. The separation was carried out on a Poroshell 120, SB-C18 column (4.6 × 150 mm, 2.7 µm) (Agilent Technologies, Wilmington, USA). The flow was 0.7 mL/min. Detection was carried out on a UV-Vis detector (DAD 1260, Waldbronn, Germany). The content of ascorbic acid was determined at a wavelength of 245 nm.

2.5. Content of Phenolic Compounds

2.5.1. Anthocyanin Content

Extraction and determination of anthocyanins were carried out according to the procedure described by Oszmiański and Sapis [28]. The chromatographic system used for separation was the same as in the case of other bioactive compounds. The assay was carried out at a wavelength of 520 nm. Cyanidin-3-O-glucoside was used as a standard.

2.5.2. Content of Non-Anthocyanin Phenols

Phenolic compounds were extracted from the juice according to the procedure described by Vallejo et al. [29].

Chromatographic separation was carried out on the same equipment used for the determination of ascorbic acid. The mobile phase was 60 g/L acetic acid in 0.002 mol/L sodium acetate (solvent A) and acetonitrile (solvent B). A flow of 1 mL/min was used. The separation was carried out with an increasing share of phase B, with 0–15% for 15 min, 15–30% for 25 min, 30–50% for 5 min and 50–100% for 5 min. Quantification of phenols was carried out at the wavelengths of 280 nm, 320 nm and 360 nm. Catechin (Sigma-Aldrich, St. Louis, MO, USA), chlorogenic acid and quercetin (Sigma-Aldrich, Buchs, Switzerland) were used as standards [30].

2.6. Antioxidant Activity by ABTS$^{\cdot+}$ Free Radical Scavenging Assay

Antioxidant activity was determined according to the procedure described by Re et al. [31]. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard. Results were expressed as µmol Trolox/1 g f. w. (fresh weight).

2.7. Statistical Analysis

The analyses were conducted in triplicate. Analysis of variance to indicate significant main effects and post-hoc analysis performed with Tukey's test to determine significant differences between the means. Correlations were analyzed using Pearson's coefficient. Statistica 13.1 (TIBCO Software Inc., Palo Alto, CA, USA) was used.

3. Results

3.1. Yield of Strawberry Juices

The impact of mash treatment on the yield of strawberry fruit juice is shown in Figure 1. The yield of strawberry juice depending on the cultivar of raw material can range from 48% to 90% [32]. The samples obtained from untreated mash showed an average pressing efficiency. Thermal treatment of the mash and thermosonication caused an increase in yield by 16%, while enzymatic treatment caused an increase of approximately 40%. No additional effect of using vacuum conditions during enzymatic treatment and ultrasonic mash treatment was found.

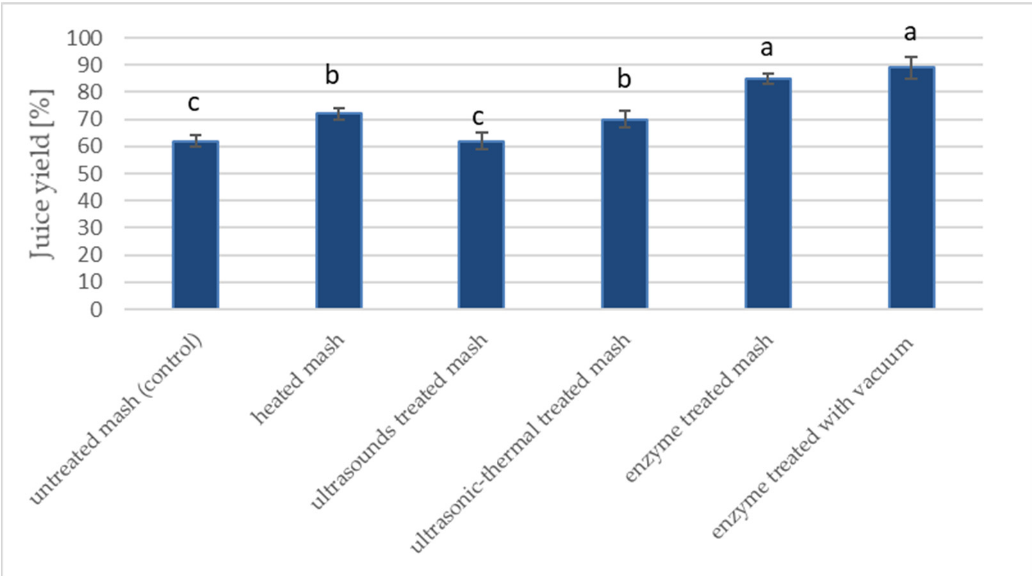

Figure 1. Effect of mash treatment on juice yield (values are means ± standard deviation; the different letters (a–c) indicate a significant difference at $p < 0.05$ for analysis of variance (ANOVA) and post-hoc analysis performed with Tukey's test).

An increase in yield (by approximately 30%) resulting from the enzymatic treatment of the mash was obtained by Dadan et al. [33] for blue honeysuckle berry and Marsol-Vall et al. [10] for lingonberry. In the case of mash thermal treatment for 1 h at 50 °C, they noted a decrease in the yield of lingonberry juice. An increase was found after 3 h of treatment. In this study, the strawberry mash was heat treated for a shorter time (5 min) but at a higher temperature (80 °C), which could have contributed to the improved yield. In the case of ultrasonic mash treatment, no effect of this method on the pressing efficiency was also noted by Bora et al. [34] for banana and by Radziejewska-Kubzdela et al. [22] for barberry fruit. Lieu and Le [18], subjecting grapefruit mash to ultrasonic treatment, found that the yield of juice is additionally affected by temperature and ultrasonic exposure time. The highest juice yield was noted at the temperature of 70 °C and the time of 13 min. Higher temperatures make it possible to reduce mash viscosity and thus facilitate the formation of violently collapsing cavitation bubbles. Simultaneously formed microjets increase higher cellular degradation [17]. In this study, the rise of temperature during ultrasonic treatment resulted in an increase in juice yield, but it was comparable to the process in which only thermal treatment was used. However, it is worth noting that a lower temperature was used during thermosonication.

3.2. Ascorbic Acid Content

The content of ascorbic acid in the obtained juices ranged from 29 to 36 mg/100 g and was at the level described in the literature (17–53 mg/100 g) [2,16]. Degradation of ascorbic acid may take place under the influence of ascorbinase or peroxidase. Other factors affecting oxidation are light, pH value, oxygen and the presence of metal ions. Furthermore, ascorbic acid is thermolabile [35]. Subjecting the mash to thermal, enzymatic and enzymatic treatment assisted by vacuum had no impact on the content of ascorbic acid in the juice in comparison to the sample obtained from the untreated mash (Figure 2). As indicated in the references, the effect of thermal or enzymatic treatment on the level of ascorbic acid content in the juice is ambiguous. In the case of barberry fruits, a beneficial effect of heat treatment of the mash on the content of ascorbic acid in the juice was noted. This may be due to the inactivation of oxidative enzymes. No such effect has been observed for enzymatic treatment [22]. In turn, Bender et al. [36] examining the impact of enzymatic treatment for blueberry, black currant and raspberry mash on ascorbic acid content in juice found an increase in the case of blueberry and raspberry and no effect in black currant. A similar effect for black currant was noted by Mieszczakowska-Frąc et al. [11]. Clegg and Morton [35] indicate that the profile of phenolic compounds can have a protective function in relation to vitamin C oxidation. They point out that quercetin had the greatest protective effect, followed by dihydroquercetin, kaempferol, quercitrin, chlorogenic acid and p-coumaric acid. The results presented in Table 1 indicate that some of these compounds are present in the profile. They may have a stabilizing effect on the content of ascorbic acid in thermal or enzymatic treatment. The protective effect is generally attributed to the metal chelating properties. During mash treatment, the oxidation of ascorbic acid may also be related to an enzymatic reaction. Ascorbate oxidase is the major enzyme responsible for this process. This enzyme contributes to the oxidation of ascorbic acid in the presence of oxygen. Enzyme activity can occur both during pectinolysis or preheating the mash to 80 °C (15 min) during thermal treatment. Deaeration of the mash in the first 10 min during enzymatic treatment may also be insufficient to inhibit oxidative changes. Thus, as a result of pectinolysis and heat treatment, the content of ascorbic acid in the juice did not increase, but a stabilizing effect was only achieved compared to the control sample.

The ultrasonic or thermosonic treatment of the mash caused a 20% decrease in ascorbic acid content in the juice in comparison to samples from the untreated mash (Figure 2). In the case of sonication, most studies are related to juice preservation. Similarly to enzymatic or thermal treatment, the influence of ultrasound on ascorbic acid stability is ambiguous. In the case of orange, watermelon and mango, there was a decrease from 5 to 32%, while for guava, grapefruit and apple there was an increase from 8% to 34% [37–43]. Portenlänger and Heusinger [42] found that ascorbic acid degradation can be caused by sonolysis. They suggest that ascorbic acid can react with free radicals generated during cavitation. Sivasankar et al. [43] indicate that the partial deaeration of the mash (caused by ultrasound) may be one of the factors that cause the increased intensity of free radical generation. Additionally, non-inactivated enzymes in the sonicated mash may have an impact on ascorbic acid degradation in the tested samples. Hence, losses of ascorbic acid in juices obtained from mash after sonication or thermosonication may result from two overlapping effects: oxidation and sonolysis. In the case of thermosonication (despite the process being carried out at elevated temperatures), ascorbic acid content in the tested juices was not found to be higher (Figure 2). Abid et al. [44] noted that significant inhibition of oxidative enzymes can only be achieved at 60 °C. Thus, when the process was run at 50 °C, the decrease in ascorbic acid content may also have been caused by enzymatic oxidation. The improvement in the effectiveness of the ultrasonic treatment of the mash in terms of increasing the content of ascorbic acid seems to be related to the reduction of oxidation processes, which could be achieved by better deaeration of the tissue. Research conducted by Aguailar et al. [45] also shows that the total deaeration of the sample results in a decrease in the intensity of sonolysis.

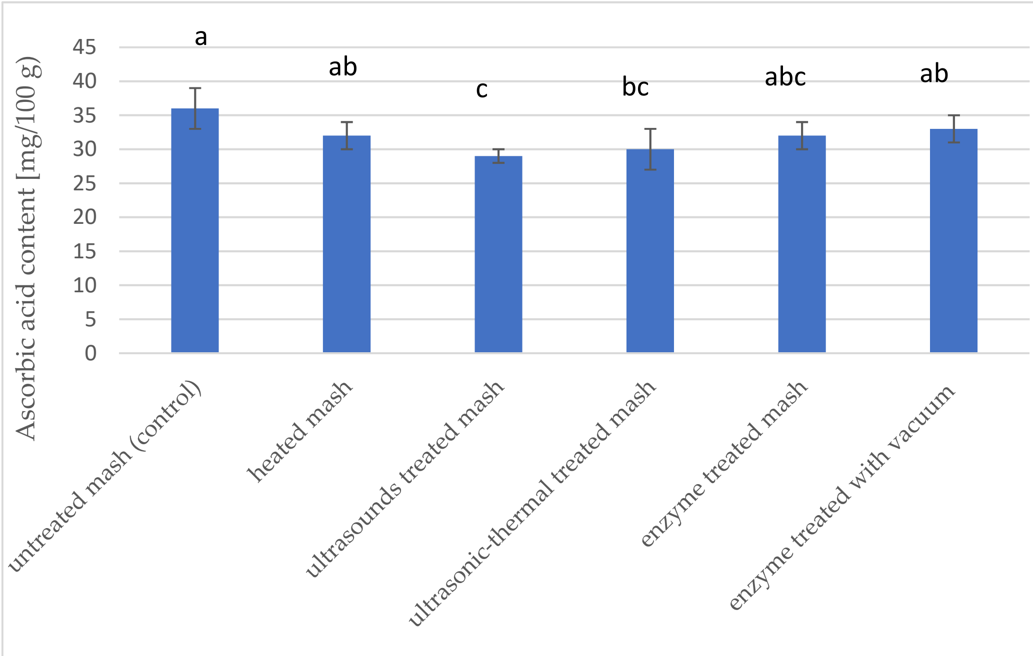

Figure 2. Effect of mash treatment on ascorbic acid content (values are means ± standard deviation; the different letters (a–c) indicate a significant difference at $p < 0.05$ for analysis of variance (ANOVA) and post-hoc analysis performed with Tukey's test).

Table 1. Effect mash treatment on the content of non-anthocyanin phenolic compounds (mg/100 g f. w.).

Samples	Procyanidins	Catechin	Quercetin	Kaempferol	Ellagic Acid Derivates	p-Coumaric Acid	Total
Untreated mash (control)	7.5 ± 0.5 d	1.1 ± 0.3 b	0.9 ± 0.2 c	0.16 ± 0.03 c	0.4 ± 0.3 c	0.90 ± 0.03 e	11 ± 2 c
Heated mash	15.4 ± 0.8 a	2.0 ± 0.3 b	1.0 ± 0.2 bc	0.20 ± 0.04 c	1.7 ± 0.3 ab	1.51 ± 0.08 d	22 ± 1 a
Ultrasounds treated mash	8.2 ± 0.8 d	1.3 ± 0.2 b	1.1 ± 0.1 bc	0.45 ± 0.02 ab	0.81 ± 0.05 bc	1.00 ± 0.05 e	13 ± 1 c
Thermosonicated mash	10.7 ± 0.3 c	1.4 ± 0.2 b	1.2 ± 0.1 b	0.55 ± 0.07 a	1.0 ± 0.1 bc	1.64 ± 0.03 c	17 ± 1 b
Enzyme-treated mash	12.1 ± 0.4 bc	3.5 ± 0.6 a	1.2 ± 0.1 b	0.36 ± 0.02 b	1.9 ± 0.3 ab	2.37 ± 0.04 a	21.0 ± 0.3 a
Enzyme treated with vacuum mash	12.9 ± 0.7 b	4.1 ± 0.6 a	1.7 ± 0.1 a	0.41 ± 0.02 b	2.7 ± 0.3 a	1.89 ± 0.03 b	24 ± 1 a

Values are means ± standard deviation; the different letters (a–e) within a given parameter indicate a significant difference at $p < 0.05$ for analysis of variance (ANOVA) and post-hoc analysis performed with Tukey's test.

3.3. Content of Phenolic Compounds

3.3.1. Content of Non-Anthocyanin Phenols

The content of phenolic compounds in the tested juices was at the level determined in juices obtained from 14 strawberry cultivars by Teleszko et al. [46] (approximately 14–48 mg/100 mL) (Table 1).

The highest content of phenolic compounds was found in strawberry juice from mash after enzymatic and thermal treatment. The use of vacuum conditions during pectinolysis had no significant effect on the level of these compounds. The influence of enzymatic and thermal treatment on the content of phenolic compounds may be different. Szajdek et al. [13], comparing the thermal treatment of bilberry mash with enzymatic treatment using different enzymes, found a higher content of phenols in samples obtained from the mash enzymatic treatment. However, the effect depended on enzyme activity. On the other hand, Ramadan and Moersel [47] did not note the impact of enzymatic treatment on phenol compound content in goldenberry juice. The different effect of enzymes can indicate a relationship between the structure of the tissue and the efficiency of phenols recovery into the juice. Therefore, the content of structure-forming compounds, such as cellulose or hemicelluloses, and the share of individual fractions of pectin compounds (protopectins, soluble pectins, pectic acid, their methylation degree or the type of polysaccharides present in the polygalacturonic acid chain), distribution of phenolic compounds in the tissue and their bonding with the structure of the raw material seem to significantly impact the efficiency of the process.

In the tested samples from the mash after thermosonication, the content of phenolic compounds was about 20% lower in comparison to the juices after enzymatic and thermal treatment. In turn, in juices obtained from mash without treatment or after sonication, their content was lower by approximately 40% (Table 1). An approximately 20% lower content of phenolic compounds in the juice obtained from the mash after ultrasonic treatment at 50 °C compared to the enzymatic treatment for acerola mash (Pectinex Ultra SP-L preparation) was also noted by Dang et al. [12]. Dzah et al. [25] indicate that the effectiveness of ultrasound largely depends on parameters, such as temperature, frequency and power. In the conducted studies, a beneficial effect of the use of temperature (50 °C) on the content of phenolic compounds was noted. Some researchers indicate that the most optimal extraction of phenolic compounds is with ultrasound at temperatures up to 60 °C [48]. They suggest that phenolic compounds can be hydrolyzed and oxidized at higher temperatures when they are extracted for a longer time [26]. The profile of phenolic compounds present in the raw material also seems to be an important issue. For instance, Lieu and Le [18] found that the most optimal temperature for the ultrasonic treatment of grapes is 74 °C. These findings may indicate an important role of the phenolic profile but also of the tissue structure of the raw material in the optimization of process parameters. The aforementioned authors noted a more favorable effect of ultrasonic mash treatment compared to enzymatic treatment. Thus, it seems that further research on the use of thermosonication for fruit mash processing in a wider range of temperatures should be conducted. In the case of the use of ultrasounds alone for mash treatment, as in the case of ascorbic acid, degradation of phenolic compounds by sonolysis or oxidation may play a significant role. Especially as non-anthocyanin phenols are often directly oxidized in enzymatic reactions. In this case, an increase in the content of the tested compound in the juice may also be associated with more effective deaeration of the mash.

Procyanidins, catechin, quercetin, kaempferol, ellagic acid derivatives and p-coumaric acid were identified in the profile of non-anthocyanin phenols. Procyanidins accounted for 55–63% of the content of these compounds. The dominant share of these compounds was also noted by Aaby et al. [2] who examined different strawberry genotypes. In the tested samples, the highest yield of procyanidins was found for juices from mash after thermal treatment followed by enzymatic treatment. Salazar-Orbea et al. [49] found an approximately 15% increase in procyanidins in strawberry puree under the influence of heat treatment used in the technological process. In turn, the effect of enzymatic treatment on the improvement of extractability of procyanidins into juice is described by Laaksonen et al. [50] for blackcurrant and by Marsol-Vall et al. [10] for lingonberry. In the case of p-coumaric acid, pectinolysis was the most effective. For catechins, the highest content in the juice was obtained after pectinolysis and vacuum-assisted pectinolysis. The latter method was also most effective for quercetin and ellagic acid derivatives. The highest content of kaempferol was as a result of thermosonication. The content of individual phenolic compounds in the juice can be determined both by improving the extractability of the tested compounds from the tissue and by the degrading effect. The literature data show that compounds, such as procyanidins, catechins, quercetin and ellagic acid derivatives, occurring largely in achene are strongly associated with the tissue matrix. Hence, the higher efficiency of their extraction as a result of the use of mash pectinolysis may result [51]. Only in the case of kaempferol, which is mainly located in the epidermis, a higher efficiency of sonication was noted [52]. In addition, some of these compounds, especially catechins, are good substrates in the process of enzymatic oxidation, which may cause their degradation during ultrasonic treatment of the mash.

3.3.2. Anthocyanin Content

The highest content of anthocyanins was found in the juices obtained from mash after thermosonication. It was about 20% lower in juices pressed from mash after ultrasonic treatment and subjected to vacuum-assisted pectinolysis. In the remaining samples (from mash without treatment, with heat treatment, or pectinolysis), the content of anthocyanins was about 40% lower (Table 2).

Table 2. Effect of mash treatment on anthocyanins content (mg/100 g f. w.).

Samples	Cyanidin-3-Glucoside	Pelargonidin-3-Glucoside	Pelargonidin-3-Rutinoside	Pelargnidin Derivative	Total Anthocyanins
Untreated mash (control)	0.67 ± 0.03 c	18.6 ± 0.8 b	0.6 ± 0.2 d	0.10 ± 0.02 c	20 ± 1 d
Heated mash	0.87 ± 0.05 bc	18.8 ± 0.4 b	1.04 ± 0.03 de	0.08 ± 0.03 c	20.8 ± 0.4 d
Ultrasounds treated mash	1.37 ± 0.08 ab	26 ± 2 a	1.28 ± 0.04 cd	0.34 ± 0.04 ab	29 ± 2 b
Thermosonicated mash	1.73 ± 0.14 a	29 ± 2 a	1.53 ± 0.09 c	0.50 ± 0.14 a	33 ± 1 a
Enzyme-treated mash	1.20 ± 0.40 bc	20 ± 1 b	2.1 ± 0.2 b	0.22 ± 0.05 bc	24 ± 2 cd
Enzyme treated with vacuum mash	1.26 ± 0.15 b	21.6 ± 0.8 b	2.7 ± 0.1 ab	0.23 ± 0.02 bc	26 ± 1 bc

Values are mean ± standard deviation; the different letters (a–e) within a given parameter indicate a significant difference at $p < 0.05$ for analysis of variance (ANOVA) and post-hoc analysis performed with Tukey's test.

The high content of anthocyanins in the samples obtained from the mash after thermosonication, ultrasonic treatment and vacuum-assisted pectinolysis may result from better extraction of anthocyanin into the juice. Ultrasonic treatment can induce cavitation effects and accelerate plant cell disruption, which can facilitate the extraction of anthocyanins from tissue [47]. On the other hand, higher anthocyanin content may be related to the lower oxygen content in the mash. Cao et al. [53] compared the content of anthocyanins in cloudy and clear juices, where higher content was noted for clear juices and was associated with a lower oxygen level and thus with a lower effect of enzymatic oxidation. Anthocyanins are less susceptible to oxidation than ascorbic acid because they do not participate directly in this reaction but are degraded by o-quinones formed as a result of oxidation of non-anthocyanine phenolic compounds. Patras et al. [54] indicate that anthocyanins can be degraded by o-quinones as a result of coupled oxidation. Thus, partial deaeration of the mash by thermosonication, sonication or vacuum-assisted pectinolysis may be sufficient to limit changes related to the oxidation of anthocyanins. However, this effect may be dependent on the degree of deaeration in the particular processes used for mash treatment. Lower anthocyanin content in juices obtained from mash subjected only to ultrasonic treatment may be the result of a greater share of enzymatic oxidation or sonolysis in their degradation in comparison to thermosonication. The sonochemical reaction is related to cavitation phenomena that contribute to the formation of free radicals. The generated free radicals subsequently can oxidize the bioactive compounds. Some researchers indicate that the deaeration of the mash may intensify cavitation [43]. The lower efficiency of sonication in relation to thermosonication may be the result of more effective inhibition of the activity of the enzymes responsible for oxidation reactions and more effective evacuation of oxygen from the mash as a result of using a higher temperature.

In the samples from the mash after thermal or enzymatic treatment, the content of anthocyanins did not differ significantly from juice obtained from the untreated mash. Both thermal and enzymatic treatments are reported to increase the extraction of anthocyanins from the mash into the juice [55]. In the tested samples, the lack of the increase in anthocyanin content may be related to the predominance of degradation processes over extraction improvement. In the case of thermal treatment, especially in oxygenated mash, processes causing thermal degradation may intensify. This effect is indicated by Kim et al. [56] examining the content of anthocyanins in strawberry puree heated under aerobic and anaerobic conditions. Markakis and Jurd [57] indicate that the degradation of anthocyanins during heating may result from the ring opening of the colorless pseudobase, which is in equilibrium with the flavylium salt and the formation of a colorless chalcone. An increase in temperature shifts the equilibrium towards the chalcone. Another pathway (in thermal degradation) is the formation of an unstable aglycone through the hydrolysis of the glycosidic bond. In the case of enzymatically treated mash, the content of anthocyanins depends on the type of enzyme and treatment time [10,11]. For example, Marsol-Vall et al. [10] found an approximately 180% increase in anthocyanin content compared to untreated lingonberry juice. However, some of the enzyme-aided juices in their study showed a lower content of anthocyanins than was in juices obtained without enzymatic treatment of the mash, which was only heated at 45 °C. They also noted the beneficial effect of extending the pectinolysis time from 1 h to 3 h. In the enzymatic method, the degrading effect may also be significant. The decrease in anthocyanin content may be related to the activity of such oxidative enzymes, such as polyphenoloxidase and peroxidase. This assumption may be confirmed by the higher content of anthocyanins in juices obtained from mash, where the use of pectinolytic enzymes was supported by vacuum conditions.

Pelargonidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-3-glucoside, pelargonidin-3-rutinoside and pelargonidin derivative were identified in the profile. Pelargonidine-3-glucoside was found to account for the largest share (80% to 90%) in the total content of anthocyanins. Dzhanfezova et al. [58], examining the anthocyanin profile in 14 strawberry cultivars, also found a dominant share of pelargonidin-3-glucoside, which accounted for 50–90% of the total content of these compounds. The influence of the applied processing methods on the content of individual anthocyanins in the juice was varied. The highest content of pelargonidin-3-glucoside was recorded for samples obtained from mash subjected to ultrasonic treatment and thermosonication. Thermosonication was the most effective for cyanidin-3-glucoside and pelargonidin derivatives. In turn, for pelargonidin-3-rutoside, the beneficial effect was obtained as a result of vacuum-assisted pectinolysis. The different effectiveness of the anthocyanin treatment methods may, on the one hand, be related to their distribution in tissues and, on the other hand, result from their different susceptibility to degradation. Some differences in the distribution of anthocyanins in the tissue are indicated by Wang et al. [59]. They show that pelargonidin-3-glucoside occurs both in around the skin and cortical tissues and in the pith tissue. Pelargonidin-3-rutinoside is located in the skin and outer cortical tissues. In turn, cyanidin-3-glucoside is found mainly in the skin.

3.4. Antioxidant Activity

The antioxidant activity of the juices from the mash after treatment (regardless of the method used) was significantly higher than that of the samples from the mash without treatment. Juices from mash subjected to vacuum-assisted enzymatic treatment had the highest antioxidant capacity (Figure 3). Szajdek et al. [13] noted a significant effect of both thermal and enzymatic treatment of the mash on the increased antioxidant activity of bilberry juice. A significant correlation (r = 0.77) was found between the value of antioxidant capacity and the content of phenolic compounds (sum of phenolic acids, flavanols and flavan-3-ols) (Table 3). Oszmiański and Wojdyło [60] also noted a significant correlation between phenols, especially procyanidines and ellagic acid, and the antioxidant activity determined by the ABTS and FRAP methods in strawberry juices and purees. Significant correlations between phenols (especially to ellagitannins) and antioxidant activity determined by the ABTS method are also confirmed by studies by Nowicka et al. [1] conducted on 90 cultivars of strawberries in two growing seasons.

Antioxidant activity only indicates a certain pro-health potential of the tested product. However, the assessment of the benefits that consumption of strawberry juice may bring requires further research related to the availability of the tested compounds. For example, according to the research of Azzini et al. [61] on the strawberry fruit, a significant increase in plasma concentration after consumption was noted for vitamin C, coumaric acid, pelargonidin-3-glucoside and cyanidin-3-glucoside. However, the presence of quercetin and anthocyanins was not found.

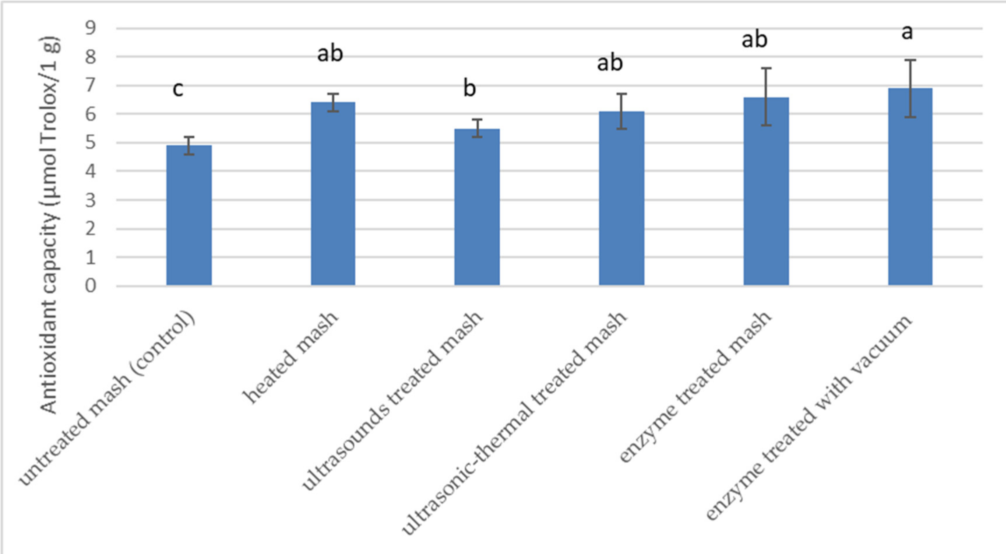

Figure 3. Effect of mash treatment on antioxidant activity (data are means ± standard deviation; the different letters (a–c) indicate a significant difference at $p < 0.05$ for analysis of variance (ANOVA) and post-hoc analysis performed with Tukey's test).

Table 3. Pearson's correlation coefficients.

	Correlation Coefficients (r)			
	Ascorbic Acid Content	**Anthocyanin Content**	**Content of Non-Anthocyanin Phenolic Compounds**	**Total Content of Phenolic Compounds**
Antioxidant activity	−0.16	0.01	0.77 *	0.49 *

* Significant at $p < 0.05$.

4. Conclusions

The influence of the applied mash processing methods on the efficiency and content of bioactive compounds is varied. The conducted research shows that the use of mash treatment, regardless of the method used (thermal, ultrasonic or enzymatic), results in a significant increase in the antioxidant activity of strawberry juice compared to juice from the untreated mash. Among the tested methods, it seems that thermosonication may be a good alternative to the thermal and enzymatic method. It results in a significant increase in the yield and anthocyanins and other phenolic compounds' content (40% and 20%, respectively, compared to a sample obtained from an untreated mash) in strawberry juice. However, in the conducted studies, a 20% decrease in the content of ascorbic acid and phenolic compounds in the juice obtained from the mash after thermosonication was noted in comparison to the sample from the mash without treatment. Therefore, the effective application of this method requires further research related mainly to the optimization of the parameters of the thermosonication process.

Funding: This study was supported by the grant for the project 'Bioactive food' POIG 01.01.02-00-061/09.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the author on request.

Acknowledgments: The author would like to thank the colleagues in the Department of Food Technology of Plant Origin.

Conflicts of Interest: The author declares no conflict of interest.

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