beverages

MDPI

Article

Ultrasonic Effects on the Quality of Mulberry Juice

Chi L. Nguyen † and Ha V. H. Nguyen *,†

Department of Food Technology, Biotechnology School, International University, Vietnam National University, Ho Chi Minh City 700000, Vietnam; linhchi.nguyen2401@gmail.com

* Correspondence: nvhha@hcmiu.edu.vn; Tel.: +84-28-3724-4270 (ext. 3824)

† These authors contributed equally to this work.

Received: 17 June 2018; Accepted: 27 July 2018; Published: 3 August 2018

Abstract: This study was conducted to investigate the effects of ultrasonic treatments on the extraction yield and the quality of mulberry juice. The mulberry mash was treated with ultrasound at different incubation times from 30 to 120 min and different temperatures from 30 to 75 °C. The determination of the juice yield, total phenolic content, total anthocyanin content, antioxidant capacity, L-ascorbic acid content, total soluble solids, and the titratable acidity of the juice were carried out. Overall, applying ultrasound at 45 °C for 60 min resulted in the highest juice yield and antioxidant contents for the mulberry juice. The ultrasonic treatment increased the extraction yield (29.6%), the total soluble solid (8.7%), the titratable acidity (39.3%), the L-ascorbic acid content (94.3%), total phenolic content (174.1%), total anthocyanin content (156.9%) and the antioxidant capacity (40.7%) of the mulberry juice as compared to pressing only. A strong positive correlation between the total phenolic content and the antioxidant capacity indicated that phenolic compounds were the main antioxidants in the beverage.

Keywords: mulberry juice; ultrasound treatment; polyphenols; anthocyanins; antioxidant capacity

1. Introduction

Mulberry, the edible fruit of the Moraceae family, is widely grown in many regions around the world such as Southern Europe, Northern Africa, East Asia, and the Americas. There are three most common mulberry species including white mulberry (*Morus alba*), black mulberry (*Morus nigra*), and red mulberry (*Morus rubra*). This fruit is extremely rich in phenolic compounds and also has a high antioxidant activity. Among those, anthocyanins are the most important antioxidants [1]. Furthermore, these anthocyanins, which have been found in mulberry, have many positive health effects, such as fighting against aging, cancer, and bacterial infections [2–5]. The pigment components of this group of fruits may improve human health or lower the risk of disease [6]. Besides, mulberries also contain several nutritive compounds such as vitamins, fatty acids, amino acids, minerals, rutin, quercetin, and polysaccharides [7]. Although this fruit contains a rich source of bioactive compounds, it is easily and quickly deteriorated after harvesting. Hence, it is required that the fruit is processed into food products.

There are some conventional techniques for juice extraction including pressing and enzymatic maceration. However, these methods are often time and energy consuming and their extraction efficiency is usually low. The sonication (ultrasound) treatment is an emerging technology that can be cheap, simple, reliable, environmentally friendly, and effective in achieving microbial decontamination [8]. Moreover, the ultrasound method has established its ability to reduce the maceration time while increasing the yield of extraction when compared to the enzymatic treatment [9]. Sonication can be applied during fruit juice processing in order to disrupt the pulp particles and to affect the particle size distribution. A smaller particle size results in a lower setting of velocity, leading to a

reduction of sedimentation and improved storage stability. Furthermore, this disintegration of particles can lead to an increased release of flavor components, color pigments, and cell constituents, such as sugar or volatile aroma compounds, into the juice. The result is an improvement in color intensity, sweetness, and aroma impression [10].

Ultrasonic waves in the ultrasound treatment are generated by mechanical vibrations of frequencies that are higher than 18 kHz. When these waves propagate into liquid media, alternating compression and expansion cycles are produced. During the expansion cycle, high intensity ultrasonic waves make small bubbles grow in liquid. When they attain a volume at which they can no longer absorb enough energy, they implode violently. This phenomenon is known as cavitation. During implosion, very high temperatures (approximately 5000 K) and pressures (estimated at 50,000 kPa) are reached inside these bubbles [11]. There are also several mechanisms that act when ultrasound is applied in fluids, for example, thermal effects that are produced by bubble implosion, mechanical stresses that are produced by micro-streaming, implosion shock waves, and free radical production [12]. The advantages of using ultrasound come from the consequences of the various effects on the medium through which it is transmitted [13].

Ultrasound has already been evaluated as an alternative to heat treatments to process fruit juices without comprising their health benefits and nutritional quality [14,15]. The application of the low frequency high power ultrasound ($\leq$ 0.1 MHz, 10–1000 W·cm^{-2}) in the food industry has been widely investigated over the last decade [16]. In recent years, there have been several researches on the application of the ultrasonic treatment of various bioactive compounds, for instance, the ultrasound treatment of phenolic compounds from strawberry [17], coconut shell powder [18], citrus peel [19,20], and olive fruit [11]. The ultrasound treatment of fruit juice is reported to have a minimal effect on the ascorbic acid content during processing and results in improved stability during storage when compared to thermal treatment [21]. Moreover, it is considered to be a potential technology for the processing of red juices because of its minimal effect on anthocyanins [22]. Thus, the ultrasonic treatment of fruit mash has recently been used in the extraction of antioxidant-rich juice from strawberries [17] and grapes [23]. However, the application of ultrasonic treatment, especially ultrasonic duration, to mulberry fruit mash for juice extraction has rarely been reported. Therefore, the objective of this study was to investigate the effects of different ultrasonic temperatures and times on the extraction yield and the quality attributes of mulberry juice.

2. Materials and Methods

2.1. Sample Preperation

The fully ripe, fresh mulberry fruits (*Morus rubra*), which were free from insects and damage, were purchased from Lam Dong Province, Vietnam and were transported to the laboratory within 8 h. The fruits were washed under tap water, were drained, and were packed in plastic bags before being stored at −20 °C for further juice processing.

2.2. Experimental Design

The procedure was carried out following Zou et al. [24] with minor modifications. The selected mulberries were separated into two groups:

- Group one: The mulberry mash was introduced into the ultrasound at different times of 30, 60, 90, and 120 min. The ultrasonic temperature was fixed at 60 °C.
- Group two: The mash was treated with the ultrasound at 30, 45, 60, and 75 °C. The treatment time was fixed at 60 min.

In detail, each juice sample was collected from 100 g of selected fresh mulberry fruit. Before applying the ultrasound treatment, the fruits were blended for 3 min and were placed into 250 mL beakers. The beakers containing the mulberry mash were covered with aluminum-foil papers to prevent the

oxidative change from the light and were immersed in an ultrasonic bath (Daihan WUC-A10H, Seoul, Korea) containing 3 L of water as the coupling fluid. The operating frequency and the power of the bath were fixed at 40 kHz and 265 W. In both of the groups, after sonication, the samples were immediately cooled by being immersed in a water bath at room temperature for 10 min. The raw juice was produced by hand squeezing. The pressed mulberry juice was prepared by using a home juicer (Panasonic MJ-68MWRA, Panasonic, Malaysia) and was used as a control sample. All of the samples were filtered through four layers of cheesecloth to remove pomace and to obtain the clear juice. The obtained juice samples were then pasteurized at 90 °C for 10 s and were refrigerated at 4 °C until further analyses could commence.

2.3. Analytical Methods

2.3.1. Determination of the Juice Yield

Juice yield (%) was calculated according to the following equation of Lee et al. [25]

$$\%\ \text{Yield} = \frac{m_2 \times C}{m_1 \times (100 - w)} \times 100\% \quad (1)$$

where:

- m_1: the weight of the mulberry fruit mash, g
- m_2: the weight of the mulberry juice, g
- C: the concentration of the soluble solid compounds in the obtained juice, % (w/w)
- w: the moisture of the initial mulberry mash, %

2.3.2. Measurement of the Total Soluble Solids (°Bx), Titratable Acidity (%), pH, and Moisture Content (%)

The total soluble solids were measured using a digital refractometer (Antago RX-5000, Tokyo, Japan) and the results were presented as degree Brix (°Bx).

The titratable acidity was measured based on the titration method of Nielsen [26] and was calculated as the following formula:

$$\text{Titratable acidity}\ (\%) = \frac{(\text{mL of NaOH titrated}) \times \left(\text{N of NaOH in } \frac{\text{mol}}{\text{liter}}\right) \times (\text{Equation Wt. of acid})}{(\text{mL of sample}) \times 10} \quad (2)$$

where:

- N of NaOH = 0.1 M
- Equation wt. citric acid = 64.04
- mL of the sample = 10 mL

A digital pH meter (Hanna HI 2216–02, Padova, Italia) was used to determine the pH of the sample. The instrument was calibrated using buffer solutions of pH 4 and pH 7 prior to use. Briefly, 10 mL of the sample was continuously stirred in a beaker using a magnetic stirrer and the pH was measured at 20 ± 0.5 °C.

The moisture contents of the fresh mulberry and the juice were measured using the Association of Official Analytical Chemists(AOAC) method 976.05 [27].

2.3.3. Extraction of the Total Phenolic Content

The extraction of the total phenolic content was modified from the method of Vinson et al. [28]. Briefly, 5 mL of juice was extracted with 20 mL of 1.2 M HCl in 50% methanol (v/v) by shaking at 60 °C in the dark for 2 h (IKA KS4000ic Control, Staufen, Germany). The mixture was centrifuged at 3000× g for 15 min at 4 °C (Z326K, Wehingen, Germany). The supernatant was collected and was

stored at −40 °C until the determination of the total phenolic content and the antioxidant capacity could be commenced.

2.3.4. Measurement of the Total Phenolic Content

The total phenolic content of the juice was determined using Folin-Ciocalteu assay [16] with modifications. For this, 0.5 mL of diluted extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (2 N) and 2 mL of 7.5% sodium carbonate, was vortexed, and was incubated for 5 min at room temperature. The absorbance was measured using a UV-visible spectrophotometer (Jas.co V730, Tokyo, Japan) at 760 nm wavelength. Gallic acid (0.01–0.05 mg/mL) was used to construct a calibration curve and the results were expressed as gallic acid equivalents per 100 mL juice (mg GAE/100 mL).

2.3.5. Determination of the Antioxidant Capacity

The antioxidant capacity of the juice was measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay which was modified from a method from Lim et al. [29]. Briefly, 1 mL of the extract was mixed with 3 mL of 0.1 mM methanolic DPPH solution, it was vortexed, and the mixture was then allowed to stand in the dark at room temperature for 30 min. The absorbance was measured against a blank at 517 nm with a UV-Visible spectrophotometer (Jas.co V730, Japan, Tokyo). The experiment was performed in triplicate. The percentage of the free radical scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect } (\%) = \left(1 - \frac{A}{A_0}\right) \times 100 \quad (3)$$

where A_0 was the absorbance of the control solution.

A was the absorbance of the DPPH solution containing the sample extract at 517 nm.

2.3.6. Measurement of the Total Anthocyanin Contents

Total anthocyanin content was determined by following the method of Giusti et al. [30]. In detail, 1 mL of extract was mixed with two buffers: 0.025 M of potassium chloride (pH 1.0) and 0.4 M of sodium acetate (pH 4.5) to the volume of 10 mL and was left for 15 min at room temperature before measuring the absorbance at λ = 520 nm and λ = 700 nm. Cyanidin-3-glucoside was used as standard and the data was expressed as mg cyanidin-3-glucoside equivalents per 100 mL of juice (mg cyanidin-3-glucoside/100 mL). The total anthocyanin content was calculated as follows:

$$\text{Total anthocyanin content } (\text{mg}/100\ \text{mL}) = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times l} \quad (4)$$

where:

- $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH1.0}} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH4.5}}$
- MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside
- DF = dilution factor established in D
- l = 1 cm
- ε = 26,900 molar extinction coefficient, in L/mol·cm, for cyanidin-3-glucoside

2.3.7. Measurement of the L-Ascorbic Acid Content

Measuring L-ascorbic acid was modified from the method of Rahman et al. [31]. Mulberry juice was extracted with a mixture of 5% metaphosphoric acid and 10% acetic acid for 30 min at room temperature. Then, the extract was mixed with 3% bromine water and 10% thiourea before adding 2,4-dinitrophenylhydrazine solution. The mixture was incubated at 37 °C for 3 h and thereafter, chilled 85% sulfuric acid was added. A UV-Visible spectrophotometer (Jas.co V730, Tokyo, Japan) was used to measure the absorbance at 521 nm. The amount of AA that was present in the sample was

calculated based on the ascorbic acid standard curve (0.005–0.05 mg/mL) and was expressed as mg AA/100 mL juice (mg AA/100 mL).

2.4. Statistical Analysis

All of the values were presented as the mean of 3 determinations ± S.D. The data was analyzed by a one-way analysis of variance (ANOVA) and the differences among the means were determined using a Tukey test with a level of significance of $p < 0.05$. The statistical analysis was conducted using the software Minitab® version 16 (Minitab Ltd., Coventry, UK).

3. Results and Discussion

3.1. The Effects of Different Sonication Time on the Yield and Quality of Mulberry Juice

3.1.1. Juice Yield, Total Soluble Solids (TSS), Titratable Acidity (TA), pH, and Moisture Content (MC)

The initial quality parameters of the raw juice are shown in Table 1.

Table 1. The initial quality parameters of the raw juice.

Parameters	Content
Total soluble solids (°Brix)	10.94 ± 0.08
Titratable acidity (%)	1.11 ± 0.07
pH	3.32 ± 0.03
Moisture content	89.06 ± 0.08
L-Ascorbic acid (L-AA) (mg/100 mL)	18.02 ± 1.20
Total phenolic content (TPC) (mg GAE/100 mL)	67.01 ± 1.62
Total anthocyanin content (TAC) (mg cy-3-glc/100 mL)	50.01 ± 1.52
Antioxidant capacity (AC) (%DPPH inhibition)	55.14 ± 2.48

As shown in Table 2, the extraction yield of mulberry juice that was treated at different sonication times at a fixed temperature of 60 °C ranged from 81.80–90.21%. The values were significantly higher than that of the pressed juice. Among four periods of sonication, the 60 min treatment resulted in the highest yield. However, thereafter, the yield started to decrease (Table 2). Besides, all four periods of the sonicated samples were carried out at a fixed temperature of 60 °C, meanwhile the pressed juice was sampled without a treating temperature. This might be a potential factor that creates the difference between the two treatments.

Total soluble solids, titratable acidity, pH, and moisture content are major quality parameters of the mulberry juice which affect the sensory characteristics of the juice. Similar trends for the extraction yield, shown in Table 2, are the total soluble solids which ranged from 11.44 to 11.95 °Brix, and the titratable acidity increased from 1.31% to 1.50%, respectively. As compared to the untreated one, the ultrasonic treatment clearly proved to be more efficient. Nguyen and Le [32] also observed a decrease in the acidity of the pineapple mash that was treated with ultrasound. Moreover, the total soluble solids (TSS) and titratable acidity (TA) values reached their maximum after 60 min of sonication. The increases of TSS and TA might be ascribed to the increase in the extraction efficacy due to the ultrasound treatment, causing the destruction of cell walls [33]. This resulted in more water being able to enter the cells and the more soluble solids could permeate cell membranes. Nevertheless, when the time extended, there was a reduction in these values. Consequently, 60 min was chosen as the suitable sonication time for processing mulberry juice.

The result also showed that different ultrasound durations caused significant changes ($p < 0.05$) in the pH and moisture content between the non-sonicated and sonicated juice samples (Table 2). Due to the cell degradation that occurred by pressing as well as the cavitation phenomenon, more soluble substances were released. Consequently, the moisture content of the pressed and the ultrasonic juice were increased compared to the raw one.

Table 2. Juice yield, TSS, TA, pH, and moisture content (mean ± SD) of mulberry juice at different ultrasonic times at a fixed temperature of 60 °C.

Times (min)	Extraction Yield (%)	Total Soluble Solids (°Brix)	Titratable Acidity (%)	pH	Moisture Content (%)
0 (Pressed juice)	71.03 ± 0.21 ^{d}	11.12 ± 0.05 ^{d}	1.17 ± 0.03 ^{bc}	3.24 ± 0.02 ^{b}	88.88 ± 0.05 ^{b}
30	83.74 ± 0.27 ^{b}	11.81 ± 0.08 ^{ab}	1.42 ± 0.10 ^{a}	3.21 ± 0.04 ^{b}	88.19 ± 0.08 ^{de}
60	90.21 ± 0.22 ^{a}	11.95 ± 0.03 ^{a}	1.50 ± 0.09 ^{a}	3.23 ± 0.03 ^{b}	88.05 ± 0.03 ^{e}
90	82.41 ± 0.28 ^{c}	11.66 ± 0.05 ^{b}	1.35 ± 0.09 ^{ab}	3.20 ± 0.02 ^{b}	88.34 ± 0.05 ^{d}
120	81.80 ± 0.12 ^{d}	11.44 ± 0.07 ^{c}	1.31 ± 0.08 ^{ab}	3.18 ± 0.03 ^{b}	88.56 ± 0.07 ^{c}

The values are mean ± SD (n = 3). Mean values within a column with the same superscript are not significantly different ($p < 0.05$).

3.1.2. L-Ascorbic Acid Content, Total Phenolic Content, and Total Anthocyanin Content

The effect of different ultrasonic durations on the ascorbic acid content of the juice is shown in Table 3. The results indicate a significantly higher ($p < 0.05$) ascorbic acid content in the sonicated juice compared to the non-ultrasonic one. The ascorbic acid content reached its peak after 60 min of sonication (37.38 ± 1.53 mg/100 mL) and reduced as the time extended. This could be explained by the release of ascorbic acid from the cell fluid during cavitation which was produced by ultrasound treatment together with the elimination of dissolved oxygen, leading to an increase in L-ascorbic acid in the juice [15]. Besides, Valdramidis et al. [12] and Petrier et al. [34] found that the loss of ascorbic acid during the sonication process is due to oxidative processes in aerobic and anaerobic environments that are associated with the production and the use of hydroxyl radicals.

Table 3. L-ascorbic acid, total phenolics, total anthocyanin contents, and antioxidant capacities (mean ± SD) in mulberry juice at different sonicating times.

Times (min)	L-AA (mg/100 mL)	TPC (mg GAE/100 mL)	TAC (mg cy-3-glc/100 mL)	AC (%DPPH inhibition)
0 (Pressed juice)	20.73 ± 1.22 ^{c}	73.74 ± 1.34 ^{d}	55.33 ± 1.71 ^{d}	65.08 ± 3.02 ^{c}
30	31.08 ± 2.27 ^{b}	159.84 ± 2.32 ^{b}	118.01 ± 2.59 ^{b}	76.02 ± 2.94 ^{ab}
60	37.38 ± 1.53 ^{a}	183.29 ± 2.01 ^{a}	132.48 ± 1.68 ^{a}	83.67 ± 3.82 ^{a}
90	36.04 ± 2.03 ^{a}	164.96 ± 2.38 ^{b}	129.03 ± 1.51 ^{a}	72.58 ± 2.34 ^{bc}
120	27.05 ± 1.67 ^{b}	131.18 ± 3.33 ^{c}	109.14 ± 2.06 ^{c}	69.15 ± 2.65 ^{bc}

The values are mean ± SD (n = 3). Mean values within a column with the same superscript are not significantly different ($p < 0.05$).

Table 3 also shows the effect of ultrasonic time on the level of the phenolic compounds and the anthocyanin content of mulberry juice. Applying ultrasonic treatment for 60 min significantly increased the TPC and TAC of the mulberry juice by more than two times (Table 3) compared to the pressed juice only. The antioxidant concentrations increased significantly when the ultrasonic times ranged from 30–60 min ($p < 0.05$). The maximum of these values was achieved after 60 min of sonication. However, when the ultrasonic time was prolonged, these values started to reduce. According to Escarpa et al. [35], antioxidant compounds are present in the vacuole in soluble form or are bound to the cell wall such as pectin, cellulose, hemicellulose, and lignin traces. It is possible that the use of the ultrasound enhanced the disruption of the biological cell walls, and facilitated the release of their contents [36] via cavitational collapse in the surroundings of colloidal particles [15].

The mechanism of the ultrasound treatment is ascribed to the acoustic cavitation, which includes the formation, growth, and implosive collapse of bubbles in liquid [37]. The implosion of the cavitation bubbles generates severe turbulence, high velocity inter-particle collisions, and perturbation in microporous particles of the materials, which accelerates the eddy diffusion and the internal diffusion. As a result, the amounts of extractable antioxidants were increased and were higher than those in the non-treated samples.

The total phenolic and total anthocyanin contents decreased over the sonication time, and this may be due to longer exposure to ultrasonic waves. Jahouach-Rabai et al. [38] reported that the degradation of polyphenolic compounds was due to excessive cavitations and cell disruption of the product.

3.1.3. Antioxidant Capacity (AC)

The antioxidant capacity of mulberry juice in the ultrasonic treatment was examined by % DPPH inhibition (Table 3). Similar to TPC and TAC, DPPH scavenging activity obtained the highest value after sonication for 60 min (83.67 ± 3.82%) and decreased thereafter. This may be attributed to the fact that the cavitation bubbles may grow too big to collapse or may collapse weakly which could cause the reduction in the cavitation effect. Also, many bubbles may hamper the propagation of the ultrasound wave [39].

As the sonication time extended at 60 °C fixed, the level of antioxidants reduced. This finding was in agreement with [40] who ascribed this phenomenon to the relation between sonication time and the number of cavitation bubbles. When the time increased, the number of cavitation bubbles increased, thereby it may be inferred as reducing antioxidant compounds and decreasing DPPH-free radical scavenging rates in the mulberry juice.

Therefore, among four sonication times, the appropriate sonication time for processing mulberry juice was 60 min due to achieving high results in the yield and antioxidant levels.

According to Nguyen et al. [41], mulberry fruits which were sonicated at 63 °C with the time varied from 0 to 8 min had higher results in total phenolic, anthocyanins contents, and antioxidant capacity as compared to the non-sonicated samples. However, in the current study with a longer sonication time—60 min at a fixed temperature of 60 °C, the obtained mulberry juice contained much higher amounts of bioactive compounds than that of the non-sonicated samples (Table 3), and the results were also greater than the mulberry fruits that were sonicated within 0 to 8 min [41]. In addition, the reported study used an ultrasonic probe, meanwhile an ultrasonic bath was used in the present study. It has been known that an ultrasonic bath and an ultrasonic probe are common systems that are used in ultrasound-assisted extraction. However, two main negative properties that are related to experimental repeatability and reproducibility were found in using ultrasonic probe [42]. Therefore, an ultrasonic bath was chosen for the current work. Moreover, to prevent the oxidation of the juice, glass containers were used in this study to contain the mulberry mass for the ultrasonic treatments instead of directly pouring the mass into the ultrasonic bath. This could also be a potential reason causing the prolonged ultrasonic duration to gain the highest results.

3.2. Effects of Different Sonication Temperatures on the Yield and Quality of Mulberry Juice

3.2.1. Juice Yield, Total Soluble Solids (TSS), Titratable Acidity (TA), pH, and Moisture Content (MC)

From Table 4, the extraction yield of the mulberry mash which was treated at different sonication temperatures ranged from 83.62 ± 0.23% to 92.07 ± 0.13%. The maximum yield was obtained at 45 °C. As temperature was rising to over 45 °C, a reduction trend of the yield was observed.

Total soluble solids and the titratable acidity also reached their peak at 45 °C. The reduction of TSS and TA is also shown in Table 4, as the temperature increased from 60 °C to 75 °C. The increase in soluble solids was possibly due to the enhancement of the extraction efficacy. Previous studies of Zafra-Rojas et al. [43] and Zou et al. [44] have shown that the mass transfer effects, shear, and shock waves that were generated during the acoustic cavitation process can damage fruit tissues and cell walls, resulting in the diffusion of water into fruit cells. This would ultimately result in the solubilization of more soluble solids [24]. There were no significant changes in the pH as the sonicated temperature of the mulberry juice samples increased (Table 4). However, the moisture content result of the ultrasonic sample at 45 °C showed that there was a significant difference ($p < 0.05$) between 45 °C and the other temperatures.

According to Vilkhu et al. [45], in solid-liquid extraction, cavitation can cause surface erosion and particle breakdown. This phenomenon provided new surfaces and increased mass transfer. Hence, the extraction yield of the mulberry juice was higher when it was treated with ultrasound.

Table 4. Juice yield, TSS, and TA (mean ± SD) of mulberry juice at different ultrasonic temperatures.

Temperature (°C)	Extraction Yield (%)	Total Soluble Solids (TSS–°Brix)	Titratable Acidity (%)	pH	Moisture Content (MC-%)
30	86.44 ± 0.20 ^{c}	11.67 ± 0.07 ^{c}	1.41 ± 0.04 ^{bc}	3.23 ± 0.02 ^{b}	88.32 ± 0.07 ^{b}
45	92.07 ± 0.13 ^{a}	12.09 ± 0.04 ^{a}	1.63 ± 0.08 ^{a}	3.25 ± 0.03 ^{b}	87.91 ± 0.04 ^{d}
60	90.18 ± 0.17 ^{b}	11.91 ± 0.05 ^{b}	1.51 ± 0.09 ^{ab}	3.22 ± 0.04 ^{ab}	88.09 ± 0.05 ^{c}
75	83.62 ± 0.23 ^{d}	11.78 ± 0.04 ^{bc}	1.31 ± 0.07 ^{cd}	3.18 ± 0.02 ^{b}	88.22 ± 0.04 ^{bc}

The values are mean ± SD (n = 3). Mean values within a column with the same superscript are not significantly different ($p < 0.05$).

3.2.2. L-Ascorbic Acid Content (L-AA), Total Phenolic Content (TPC), and Total Anthocyanin Content (TAC)

From Table 5, the ascorbic acid content at different temperatures ranges from 28.11 mg/100 mL to 40.28 mg/100 mL. Among the studied temperatures, the highest ascorbic acid content was produced at 45 °C (40.28 mg/100 mL). There was degradation in the ascorbic acid content as the temperature increased to 75 °C due to the sensitivity of the heat of the ascorbic acid compounds.

Table 5. L-ascorbic acid, total phenolics, total anthocyanin contents, and antioxidant capacities (mean ± SD) in mulberry juice at different sonicating temperatures.

Temperature (°C)	L-AA (mg/100 mL)	TPC (mg GAE/100 mL)	TAC (mg cy-3-glc/100 mL)	AC (%DPPH Inhibition)
30	31.62 ± 1.87 ^{bc}	164.92 ± 2.45 ^{c}	118.12 ± 1.51 ^{d}	80.18 ± 2.16 ^{b}
45	40.28 ± 1.77 ^{a}	202.15 ± 1.25 ^{a}	142.15 ± 1.73 ^{a}	91.58 ± 3.32 ^{a}
60	36.08 ± 1.73 ^{ab}	183.32 ± 1.97 ^{b}	132.37 ± 1.84 ^{b}	83.75 ± 3.66 ^{ab}
75	28.11 ± 1.98 ^{c}	126.42 ± 3.49 ^{d}	124.84 ± 2.81 ^{c}	67.32 ± 3.63 ^{c}

The values are mean ± SD (n = 3). Mean values within a column with the same superscript are not significantly different ($p < 0.05$).

The effect of the ultrasonic temperature on the antioxidant compounds of the mulberry juice in terms of total phenolic and total anthocyanin contents were also evaluated (Table 5). As can be seen from Table 5, 45 °C was shown to be an appropriate temperature that gave high phenolics and anthocyanin contents by 202.15 mg GAE/100 mL and 142.15 mg cy-3-glc/100 mL, respectively. Due to the moderate sonochemical hydroxylation of the phenolic compounds, which was caused by hydroxyl radicals that were produced during the sonolysis of water, the antioxidant properties could improve [46].

As the temperature increased, TPC was lower due to the fact that the phenolic compounds were highly susceptible to heat [47]. As shown in Table 5, the increasing sonicating temperature led to degradation of these bioactive compounds ($p < 0.05$). The reduction of anthocyanin contents could be explained by the fact that anthocyanin compounds were more sensitive to thermal damage and process exposure time than colorless phenolic compounds [48].

3.2.3. Antioxidant Capacities (AC)

The antioxidant capacities of mulberry juice at different ultrasonic temperatures were evaluated (Table 5). Similar to L-AA, TPC, and TAC, the antioxidant capacities that were measured based on the % DPPH scavenging effect showed the highest result at 45 °C (91.58%). As the temperature increased, there was a reduction in the antioxidant activity. During the ultrasound treatment, there was

a formation of bubbles. The implosion of cavitation bubbles generates a great amount of heat. This phenomenon might help to produce a higher yield of antioxidants, however, when the samples had already been sonicated at a high temperature, such as 60 °C to 75 °C as in this study, this led to the reduction of antioxidant properties due to the vulnerability of the antioxidant compounds to heating [49]. As a result, 45 °C was chosen as the most suitable temperature for the ultrasound treatment in this study.

Applying ultrasound on the mulberry juice processing in the current work resulted in a higher juice yield and antioxidants compared to using the enzymes only in the reported study [37].

It can be seen from Table 5 that there was a strong correlation between the total phenolic content and the antioxidant capacity measured by the percentage of DPPH inhibition (r^2 = 0.891, $p < 0.05$), while the ascorbic acid content showed less of a correlation with antioxidant capacity (r^2 = 0.596, $p < 0.05$). Similarly, there was a weak correlation between the total anthocyanin content and the antioxidant capacity (r^2 = 0.412, $p < 0.05$). Hence, it could be suggested that polyphenols accounted for a large part of the antioxidants in the mulberry juice in this study.

It is known that ultrasonic treatment causes the collapse through cavitation in the surroundings of colloidal particles and then releases bioactive compounds from the cell wall. In addition, there is a significant strong correlation between TPC and antioxidant activity (Table 6). This means that the treatment helped extract more of the bound polyphenols into the juice, which thereby increased the total antioxidant activity of the mulberry juice [50].

Table 6. Correlation coefficients (r^2) between antioxidant capacities (% DPPH inhibition), total phenolic, total anthocyanin, and ascorbic acid contents in the mulberry juice.

Correlation Coefficients	L-AA	TPC	TAC
AC (% DPPH inhibition)	0.596 *	0.891 *	0.412 *

* $p < 0.05$.

4. Conclusions

In this study, ultrasonic treatment showed significantly increased extraction efficiencies for processing an antioxidant-rich fruit juice from mulberry fruit. Moreover, this treatment exhibited some advantages, such as shorter extraction time and higher extraction yield for total ascorbic acid contents, phenolics, and anthocyanins in comparison to normal processing. The positive correlations between TPC and the antioxidant capacities of the mulberry juice suggested polyphenols to be the main antioxidants in this product. It is suggested from the current work that the ultrasound treatment should be considered as a positive alternative technique in the extraction of antioxidant-rich juices.

Author Contributions: Methodology: C.L.N. and H.V.H.N.; Writing-Original Draft Preparation: C.L.N.; Writing-Review & Editing: H.V.H.N.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bae, S.-H.; Suh, H.-J. Antioxidant activities of five different mulberry cultivars in Korea. *LWT Food Sci. Technol.* **2007**, *40*, 955–962. [CrossRef]
2. Huang, H.P.; Ou, T.T.; Wang, C.J. Mulberry (Sang Shèn Zǐ) and its bioactive compounds, the chemoprevention effects and molecular mechanisms in vitro and in vivo. *J. Tradit. Complement. Med.* **2013**, *3*, 7–15. [CrossRef] [PubMed]
3. Kim, Y.R.; Lee, J.S.; Lee, K.R.; Kim, Y.E.; Baek, N.I.; Hong, E.K. Effects of mulberry ethanol extracts on hydrogen peroxide-induced oxidative stress in pancreatic β-cells. *Int. J. Mol. Med.* **2014**, *33*, 128–134. [CrossRef] [PubMed]

4. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive compounds and antioxidant activity in different types of berries. *Int. J. Mol. Sci.* **2015**, *16*, 24673–24706. [CrossRef] [PubMed]
5. Salih, N.D.; Hazir, N.S.M.; Hamid, M.H.A. The Effect of Mulberry (*Morus* sp.) Tea Supplement on Acetaminophen Induced Renal Failure in Rats. *World J. Pharm. Pharm. Sci.* **2015**, *4*, 111–125.
6. Lin, J.Y.; Tang, C.Y. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* **2007**, *101*, 140–147. [CrossRef]
7. Yuan, Q.; Zhao, L. The Mulberry (*Morus alba* L.) Fruit—A Review of Characteristic Components and Health Benefits. *J. Agric. Food Chem.* **2017**, *65*, 10383–10394. [CrossRef] [PubMed]
8. Valero, M.; Recrosio, N.; Saura, D.; Muñoz, N.; Martí, N.; Lizama, V. Effects of ultrasonic treatments in orange juice processing. *J. Food Eng.* **2007**, *80*, 509–516. [CrossRef]
9. Shirsath, S.; Sonawane, S.; Gogate, P. Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. *Chem. Eng. Process. Process Intensif.* **2012**, *53*, 10–23. [CrossRef]
10. Bermudez-Aguirre, D. *Ultrasound: Advances in Food Processing and Preservation*; Academic Press: London, UK, 2017; pp. 215–230.
11. Jerman, T.; Trebše, P.; Vodopivec, B.M. Ultrasound-assisted solid liquid extraction (USLE) of olive fruit (*Olea europaea*) phenolic compounds. *Food Chem.* **2010**, *123*, 175–182. [CrossRef]
12. Valdramidis, V.P.; Cullen, P.J.; Tiwari, B.K.; O'donnell, C.P. Quantitative modelling approaches for ascorbic acid degradation and non-enzymatic browning of orange juice during ultrasound processing. *J. Food Eng.* **2010**, *96*, 449–454. [CrossRef]
13. Dolatowski, Z.J.; Stadnik, J.; Stasiak, D. Applications of ultrasound in food technology. *Acta Sci. Pol. Technol. Aliment.* **2007**, *6*, 88–99.
14. Bhat, R.; Kamaruddin, N.S.B.C.; Min-Tze, L.; Karim, A. Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrason. Sonochem.* **2011**, *18*, 1295–1300. [CrossRef] [PubMed]
15. Cheng, L.; Soh, C.; Liew, S.; Teh, F. Effects of sonication and carbonation on guava juice quality. *Food Chem.* **2007**, *104*, 1396–1401. [CrossRef]
16. Feng, H.; Yang, W.; Hielscher, T. Power ultrasound. *Food Sci. Technol. Int.* **2008**, *14*, 433–436. [CrossRef]
17. Herrera, M.; De Castro, M.L. Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. *J. Chromatogr. A* **2005**, *1100*, 1–7. [CrossRef] [PubMed]
18. Rodrigues, S.; Pinto, G.A. Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder. *J. Food Eng.* **2007**, *80*, 869–872. [CrossRef]
19. Ma, Y.Q.; Chen, J.C.; Liu, D.H.; Ye, X.Q. Simultaneous extraction of phenolic compounds of citrus peel extracts: Effect of ultrasound. *Ultrason. Sonochem.* **2009**, *16*, 57–62. [CrossRef] [PubMed]
20. Khan, M.K.; Abert-Vian, M.; Fabiano-Tixier, A.-S.; Dangles, O.; Chemat, F. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. *Food Chem.* **2010**, *119*, 851–858. [CrossRef]
21. Knorr, D.; Zenker, M.; Heinz, V.; Lee, D.-U. Applications and potential of ultrasonics in food processing. *Trends Food Sci. Technol.* **2004**, *15*, 261–266. [CrossRef]
22. Oms-Oliu, G.; Odriozola-Serrano, I.; Soliva-Fortuny, R.; Elez-Martínez, P.; Martín-Belloso, O. Stability of health-related compounds in plant foods through the application of non thermal processes. *Trends Food Sci. Technol.* **2012**, *23*, 111–123. [CrossRef]
23. Lieu, L.N. Application of ultrasound in grape mash treatment in juice processing. *Ultrason. Sonochem.* **2010**, *17*, 273–279. [CrossRef] [PubMed]
24. Zou, Y.; Jiang, A. Effect of ultrasound treatment on quality and microbial load of carrot juice. *Food Sci. Technol. (Camp.)* **2016**, *36*, 111–115. [CrossRef]
25. Lee, W.; Yusof, S.; Hamid, N.; Baharin, B. Optimizing conditions for hot water extraction of banana juice using response surface methodology (RSM). *J. Food Eng.* **2006**, *75*, 473–479. [CrossRef]
26. Nielsen, S.S. *Food Analysis Laboratory Manual*; Springer: New York, NY, USA, 2003; pp. 95–102.
27. AOAC. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gathersberg, MD, USA, 2002.
28. Vinson, J.A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: Vegetables. *J. Agric. Food Chem.* **1998**, *46*, 3630–3634. [CrossRef]

29. Lim, Y.Y.; Lim, T.T.; Tee, J.J. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.* **2007**, *103*, 1003–1008. [CrossRef]
30. Giusti, M.M.; Wrolstad, R.E. Characterization of red radish anthocyanins. *J. Food Sci.* **1996**, *61*, 322–326. [CrossRef]
31. Rahman, M.M.; Khan, M.M.R.; Hosain, M.M. Analysis of Vitamin C (ascorbic acid) Contents in Various Fruits and Vegetables by UV-spectrophotometry. *Bangladesh J. Sci. Ind. Res.* **2007**, *42*, 417–424. [CrossRef]
32. Nguyen, T.P.; Le, V.V.M. Application of ultrasound to pineapple mash treatment in juice processing. *Int. Food Res. J.* **2012**, *19*, 547–552.
33. Zou, Y.; Xie, C.; Fan, G.; Gu, Z.; Han, Y. Optimization of ultrasound-assisted extraction of melanin from *Auricularia auricula* fruit bodies. *Innov. Food Sci. Emerg. Technol.* **2010**, *11*, 611–615. [CrossRef]
34. Petrier, C.; Combet, E.; Mason, T. Oxygen-induced concurrent ultrasonic degradation of volatile and non-volatile aromatic compounds. *Ultrason. Sonochem.* **2007**, *14*, 117–121. [CrossRef] [PubMed]
35. Escarpa, A.; González, M. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. *Anal. Chim. Acta* **2001**, *427*, 119–127. [CrossRef]
36. Mason, T.; Paniwnyk, L.; Lorimer, J. The uses of ultrasound in food technology. *Ultrason. Sonochem.* **1996**, *3*, S253–S260. [CrossRef]
37. Chowdhury, P.; Viraraghavan, T. Sonochemical degradation of chlorinated organic compounds, phenolic compounds and organic dyes—A review. *Sci. Total Environ.* **2009**, *407*, 2474–2492. [CrossRef] [PubMed]
38. Jahouach-Rabai, W.; Trabelsi, M.; Van Hoed, V.; Adams, A.; Verhé, R.; De Kimpe, N.; Frikha, M. Influence of bleaching by ultrasound on fatty acids and minor compounds of olive oil. Qualitative and quantitative analysis of volatile compounds (by SPME coupled to GC/MS). *Ultrason. Sonochem.* **2008**, *15*, 590–597. [CrossRef] [PubMed]
39. Sun, Y.; Liu, D.; Chen, J.; Ye, X.; Yu, D. Effects of different factors of ultrasound treatment on the extraction yield of the all-trans-β-carotene from citrus peels. *Ultrason. Sonochem.* **2011**, *18*, 243–249. [CrossRef] [PubMed]
40. Wang, X.; Wu, Y.; Chen, G.; Yue, W.; Liang, Q.; Wu, Q. Optimisation of ultrasound assisted extraction of phenolic compounds from *Sparganii rhizoma* with response surface methodology. *Ultrason. Sonochem.* **2013**, *20*, 846–854. [CrossRef] [PubMed]
41. Nguyen, T.; Phan, L.; Le, V. Enzyme-assisted and ultrasound-assisted extraction of phenolics from mulberry (*Morus alba*) fruit: Comparison of kinetic parameters and antioxidant level. *Int. Food Res. J.* **2014**, *21*, 1937.
42. Corrales, M.; Toepfl, S.; Butz, P.; Knorr, D.; Tauscher, B. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 85–91. [CrossRef]
43. Zafra-Rojas, Q.Y.; Cruz-Cansino, N.; Ramírez-Moreno, E.; Delgado-Olivares, L.; Villanueva-Sánchez, J.; Alanís-García, E. Effects of ultrasound treatment in purple cactus pear (*Opuntia ficus-indica*) juice. *Ultrason. Sonochem.* **2013**, *20*, 1283–1288. [CrossRef] [PubMed]
44. Zou, Y.; Hou, X. Sonication enhances quality and antioxidant activity of blueberry juice. *Food Sci. Technol. (Camp.)* **2017**, *37*, 599–603. [CrossRef]
45. Vilkhu, K.; Mawson, R.; Simons, L.; Bates, D. Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 161–169. [CrossRef]
46. Ashokkumar, M.; Sunartio, D.; Kentish, S.; Mawson, R.; Simons, L.; Vilkhu, K.; Versteeg, C.K. Modification of food ingredients by ultrasound to improve functionality: A preliminary study on a model system. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 155–160. [CrossRef]
47. Masibo, M.; He, Q. Major mango polyphenols and their potential significance to human health. *Compr. Rev. Food Sci. Food. Saf.* **2008**, *7*, 309–319. [CrossRef]
48. Cheok, C.Y.; Chin, N.L.; Yusof, Y.A.; Talib, R.A.; Law, C.L. Optimization of total monomeric anthocyanin (TMA) and total phenolic content (TPC) extractions from mangosteen (*Garcinia mangostana* Linn.) hull using ultrasonic treatments. *Ind. Crops Prod.* **2013**, *50*, 1–7. [CrossRef]

49. Chemat, F.; Khan, M.K. Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835. [CrossRef] [PubMed]
50. Nadeem, M.; Ubaid, N.; Qureshi, T.M.; Munir, M.; Mehmood, A. Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrason. Sonochem.* **2018**, *45*, 1–6. [CrossRef] [PubMed]