



Effect of combined ultrasonic and enzymatic extraction technique on the quality of noni (*Morinda citrifolia* L.) juice

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ABSTRACT

In order to obtain noni juice with high yield and good quality, the effect of combined extraction technique of enzymatic treatment (EZ) and ultrasonication (US) on the overall quality of noni juice was investigated. Moreover, the extraction performance of the EZ-US combined extraction technique was compared with that of EZ-based extraction and the US-based extraction. Response surface methodology (RSM) was designed to optimize the parameters of ultrasonic treatment, by taking consideration of the extraction efficiency, quality parameters and bioactive ingredients of noni juice. The results indicated that combined ultrasonic and enzymatic treatment achieved a synergistic effect on promoting the quality of noni juice. The maximum juice yield of 67.95 % was obtained under ultrasonication for 10 min at 600 W after enzymatic treatment (EZU). In addition, EZU-treated juice exhibited the highest contents of total phenolic and flavonoid, which were 148.19 ± 2.53 mg gallic acid/100 mL and 47.19 ± 1.22 mg rutin/100 mL, respectively, thus contributing to better antioxidant activity. Moreover, the EZU treatment significantly reduced the particle size of noni juice, and improved its suspension stability and rheological properties. FTIR results indicated that the treatments did not bring major changes in the chemical structure and the functional groups of compounds in noni juice. Therefore, EZU treatment can be successfully applied to the extraction of noni juice with better nutritional properties and overall quality.

1. Introduction

Noni (*Morinda citrifolia* L., Rubiaceae), as a kind of perennial evergreen shrub or tree originally native to Southeastern Asia, mainly grows in tropical and subtropical regions such as Polynesia, Hawaii, Australia, India, Hainan and Taiwan Island in China [1]. Noni fruit has been used as medicinal food for treating various diseases since 2000 years ago [2]. Noni fruit is endowed with high nutritional value and contains diverse natural bioactive ingredients. So far, more than 200 bioactive phytochemicals have been identified from noni fruit, such as polyphenols, flavonoids, iridoids, coumarins, anthraquinones, lignans, terpenoids, alkaloids and glycosides [3,4]. Modern scientific studies have shown that noni fruit has bacteriostatic activity, anti-inflammatory effect, anti-thrombosis and anti-oxidation properties and shows a variety of healthcare functions such as lowering blood sugar and lipids, protecting liver, healing wound, enhancing immunity, and treating cancer,

diabetes, gout and other diseases [5–7].

Owing to rich nutrients and excellent health care effects of noni fruit, the noni products have gradually attracted increasing attention of consumers in recent years. Noni fruit is usually processed into functional products such as noni juice, noni enzyme, blended drinks and so on. However, the high fiber content in noni fruit results in low juice yield and insufficient release of nutrients. Therefore, various pretreatments on juice extraction are usually employed to improve juice yield and the content of bioactive ingredients. At present, enzymatic and ultrasonic treatment are commonly used for pretreatments of juice production. Cellulase and pectinase can degrade the cell wall components of fruit by opening the glycosidic linkages, thus increasing the juice yield and enhancing the release of target substances [8,9]. Enzymatic pretreatment is usually time-consuming and laborious. In contrast, ultrasonication is a novel non-thermal processing technology that can destroy sample tissue and cell wall by cavitation effect and mechanical

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fragmentation effect, and finally accelerates the dissolution of intracellular components [10,11]. Ultrasound treatment can achieve the extraction of target substances at low temperature, which is conducive to prevent nutrients and bioactive ingredients from being damaged [12]. However, some scholars have noticed that long-time ultrasonication or excessively high ultrasound power may cause the opposite effect on bioactive compounds retention and antioxidant capacity [13].

Since each extraction method has its own advantages and limitations, the combination of multiple extraction methods can usually achieve complementary advantages. The ultrasonication (US) combined with enzymatic treatment (EZ) can achieve higher extraction yield and better quality than either of the two. It has been reported that ultrasound-assisted enzymatic extraction method increased the yield of Sohiong juice by 20 %, with anthocyanin increased by 25 % and total phenols increased by 8 % compared with the enzymatic treatment alone [14]. Dang et al. [15] boosted the juice yield and nutritional quality of acerola mash through employing a pectinase-assisted ultrasonication method. Furthermore, it was found that pectinase-assisted ultrasonication exerted a significant effect on the yield-promotion and viscosity-reduction of mango juice [16]. In addition, the applications of combined ultrasound and enzymatic treatment on banana [17], plum juice [18] and dragon juice [19] were also reported. In short, the combined ultrasonication and enzymatic treatment can act as a superior method to obtain better quality of fruit juice by retaining most of the bioactive compounds and improving the sensory attributes.

To the best of our knowledge, there have been no reports available investigating the application of combined ultrasonication and enzymatic treatment as well as the sequence of the two methods on noni juice extraction. Hence, the objective of the present study was to explore the influence of the combination of ultrasonication and enzymatic treatment and the sequence of the two methods on noni juice yield, various physicochemical characteristics (total soluble solids, pH, titratable acidity, electrical conductivity), bioactive components content (total phenolics, total flavonoids, scopoletin and iridoids), DPPH and ABTS radicals scavenging capacity, structure properties, rheological viscosities and particle size of noni juice. This work is of practical significance in improving bioactive ingredients in noni juice, and provides reference for enhancing the extraction yield and quality of noni juice.

2. Materials and methods

2.1. Materials and chemicals

Fresh noni fruits were obtained from a fruit orchard located in Wanning (Hainan, China). Pectinase and cellulase were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Sodium hydroxide, sodium carbonate, aluminium chloride, sodium nitrite, anthrone were obtained from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Folin-phenols, DPPH, ABTS, organic acid standards were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Other chemicals and reagents in analytical or high-performance liquid chromatographic (HPLC) grade used in the experiments were provided by Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China).

2.2. Preparation of noni juice

The noni fruits were cleaned with running tap water, dried and then placed in a sterile container until becoming matured. Noni juice was extracted using a screw juicer. The enzymatic treatment of noni juice was conducted at total enzyme concentration of 0.46 %, with pectinase to cellulase ratio of 2:3, under enzymatic temperature of 50 °C for 4.2 h. Afterwards, the enzymes in the pulp were inactivated by heating at 85 °C for 15 min in a water bath. The noni pulp was filtered through double layer muslin cloth, and then centrifuged at 4 °C for 5 min at 8000 r/min to obtain the supernatant. Then, ultrasonic treatment was carried out using a temperature and time controlled ultrasonic cleaning machine

(KQ-300DE, 40KHZ, Kunshan Ultrasonic Instrument Co., Ltd. China) with a fixed frequency of 40 kHz and the maximum power of 700 W. Five different treatments of juice were conducted as follows: (I) CON group (non-enzymatic treatment) (II) EZ group (enzymatic treatment) (III) US group (ultrasound treatment) (600 W, 25 min, 45 °C) (IV) UEZ group (enzymatic treatment after ultrasonication) (600 W, 25 min, 45 °C) (V) EZU group (ultrasonication after enzymatic treatment) (600 W, 10 min, 40 °C). All sample preparations and treatments were performed in triplicate.

2.3. Single factor experiment

The ranges of three factors affecting the yield of US-treated noni juice (U1), including ultrasonic power (300 W, 400 W, 500 W, 600 W, 700 W), ultrasonic temperature (30 °C, 35 °C, 40 °C, 45 °C, 50 °C) and ultrasonic time (10 min, 15 min, 20 min, 25 min, 30 min) were determined preliminarily through single factor experiments. By contrast, the same three factor experiments were also performed on EZ-treated juice (U2), in which the ranges of ultrasonic power and ultrasonic temperature were set the same as that of U1 sample, except for the extraction time increasing from 5 min to 25 min.

2.4. Design of response surface methodology (RSM)

Based on the preliminary result of single factor experiment, the RSM was conducted using Design-Expert v.11.0 software (Stat-Ease, Inc., Minneapolis, USA) to optimize the ultrasonic extraction parameter of noni juice. Central composite experiment design with three factors and three levels was adopted for construction of response surfaces. The independent variables were coded at three levels (-1, 0, 1), and the complete design consisted of 17 experimental points with three replicates at the center points.

2.5. Determination of physicochemical indexes

Total soluble solids (TSS), pH and titratable acid (TA) of juice samples were determined according to the method described by Abid et al. [20]. A hand-held refractometer (SJ-FNV32, Suijing, Henan, China) with a range of 0–32 °Brix was used to determine TSS. pH was measured using a pH meter (pHS-3E, INESA Electron Co., Ltd. Shanghai, China). TA was determined by titration with 0.1 M NaOH until the end point (pH 8.2 ± 0.1), and the results was expressed as per gram of malic acid equivalent per liter of juice sample (g/L). Electrical conductivity (EC) of juice sample was recorded by a DDS-307A conductometer (INESA Electron Co., Ltd. Shanghai, China). The yield of extracted juice was calculated according to the following equation [17]:

$$\text{Juicelyield}(\%) = [\text{juiceweight}/\text{pulpweight}] \times 100 \quad (1)$$

2.6. Total sugar content and reducing sugar content

Total sugar content of noni juice was determined by anthrone sulphuric acid method as depicted by Wang et al. [21]. Sample or glucose standard solution was mixed with 975 μ L of anthrone solution and then incubated in a boiling water bath for 10 min before cooling in an ice bath for 10 min. Subsequently, the absorbance at 625 nm was recorded using a microplate reader (Synergy LX, Biotek, USA). The reducing sugar content was assessed using 3, 5-dinitrosalicylic acid (DNS) method slightly modified from Ma et al. [22]. The juice sample or glucose standard solution was added to 200 μ L of DNS reagent and maintained in boiling water for 5 min, followed by rapid cooling in an ice bath. After filling the final volume of solution to 10 mL, the absorbance of reacted solution was measured at 540 nm. The final results were expressed as per gram of glucose equivalent per liter of juice sample (g /L).

2.7. Total phenolic content (TPC) and total flavonoids content (TFC)

The determination of TPC and TFC was carried out according to the method described by Wu et al. [23]. Briefly, juice sample or standard solution was reacted with 50 μL of Folin-Ciocalteu reagent for 10 min in the dark, after which 200 μL of sodium carbonate solution (20 %, m/v) was added for 20 min of incubation before recording the absorbance of mixture at 765 nm. The results of TPC were expressed as mg of gallic acid equivalents (GAE) /100 mL of sample. The TFC analysis procedure was conducted as follows: noni juice was mixed with 110 μL of sodium nitrite solution (0.066 M) to incubate for 5 min, and then 15 μL of 0.75 M aluminum chloride solution was added. After 6 min, 100 μL of 0.5 M sodium hydroxide solution was added for reaction for 10 min before measuring the absorbance at 510 nm. The TFC results were expressed as mg of Rutin equivalent contained in 100 mL of juice sample.

2.8. HPLC analysis of scopoletin and iridoids content

Scopoletin and iridoids contents were quantified by referring to the method of Wang et al. [24] using the HPLC system (Agilent 1260, MA, USA) equipped with an Agilent diode array detector (Agilent 1260, MA, USA) an Agilent Zorbax SB C18 column (4.6 mm \times 250 mm, 5 μm , CA, USA). The isocratic gradient elution of scopoletin was carried out using 70 % solvent A (0.1 % acetic acid in ultrapure water) and 30 % solvent B (acetonitrile) with a flow rate of 1.0 mL/min at 35 $^{\circ}\text{C}$ for 20 min. The detection wavelength was set at 346 nm and the injection volume was 10 μL . For the iridoids analysis, the mobile phases consisted of 0.1 % phosphoric acid in ultrapure water (solvent A) and acetonitrile (solvent B). The elution gradient was programmed as follows: 0–10 min, 96 % A; 10–22 min, 85 % A; and 22–40 min, 96 % A. Chromatographic analysis was performed under injection volume of 10 μL at a flow rate of 0.5 mL/min and column temperature of 35 $^{\circ}\text{C}$. The detection wavelength was 237 nm. The HPLC results were qualitatively analyzed by peak retention time and quantified by peak area using the external standard method.

2.9. DPPH and ABTS radicals scavenging activity

DPPH free radical scavenging activity was estimated based on the method reported by Abid et al. [20]. Briefly, juice sample was mixed with 500 μL of 0.2 mM ethanolic DPPH solution and incubated for 30 min in the dark. The absorbance at 517 nm was assayed. The results were calculated using the equation (2):

$$\text{DPPHradicalscavengingactivity}(\%) = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}}\right) \times 100 \quad (2)$$

where A_{sample} and A_{control} represent the absorbance of the DPPH radical solution reaction with and without samples, respectively. A_{blank} represents the absorbance of sample.

ABTS radical scavenging activity was analyzed based on the previous method [25]. The fresh ABTS stock solution was prepared by mixing 7 mM ABTS solution with potassium persulfate solution (2.45 mM) in the dark for 16 h before use. The juice sample was added to 500 μL of diluted ABTS solution (0.7 \pm 0.02 at 734 nm). The absorbance at 734 nm was recorded after incubation for 6 min avoiding light. The ABTS radical scavenging activity (%) can be calculated using the following formula:

$$\text{ABTSradicalscavengingactivity}(\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \quad (3)$$

where A_0 and A_1 represent the absorbance of the ABTS⁺ radical solution reaction without and with samples, respectively. A_2 represents the absorbance of sample.

2.10. The cloud value and color

The cloud value of noni juice was determined using a UV160U

spectrophotometer (Shimadzu, Kyoto, Japan) at 660 nm [26]. The color change of noni juice was evaluated using a portable colorimeter (CR-10 Plus, Konica Minolta, Japan), which was calibrated with a white standard. The color parameters L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellow/blueness) were measured. In addition, the total color difference (ΔE^*), in comparison to CON, was calculated according to the equation (4) [22]:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

where $\Delta L^* = L^*_{\text{standard}} - L^*_{\text{sample}}$; $\Delta a^* = a^*_{\text{standard}} - a^*_{\text{sample}}$; $\Delta b^* = b^*_{\text{standard}} - b^*_{\text{sample}}$.

2.11. Particle size distribution (PSD) and zeta-potential

All noni juice samples were filtered through layers of gauze to get rid of large particles. Samples were diluted in distilled water until reaching optimal obscuration level for the laser (10–15 %). The particle size distribution (PSD) and zeta-potential of noni juice were measured using a laser particle size analyzer (Zetasizer Nano ZS90, Malvern Instrument, UK). Laser light diffraction was used to measure particles with size ranging from 0 to 2000 μm . The PSD, the area mean diameter $D_{3,2}(\mu\text{m})$ and volume mean diameter $D_{4,3}(\mu\text{m})$ were determined in triplicate. Meanwhile, the data measured by the instrument included d_{10} , d_{50} and d_{90} values for the particles in noni juice.

2.12. Rheological viscosities

Based on the method described by Shen et al. [27], the viscosity of the extracted juice was determined using a HAAKE MARS40 rheometer (Thermo Fisher Scientific Co., Ltd., USA). The viscosity of juice sample was recorded in the range of 0–100 s^{-1} shear rate. The test temperature was maintained constant at 25 $^{\circ}\text{C}$. The distance between the plate and the sample was 1 mm. Each sample was analyzed in triplicate.

2.13. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples were obtained using a Frontier FTIR spectrometer (PerkinElmer, USA). The freeze-dried samples were incorporated with KBr powder at a ratio of 1:50 and then pressed into thin pellets. The results were recorded in the range from 4000 to 400 cm^{-1} [28].

2.14. Sensory evaluation

The sensory evaluation was performed by quantitative descriptive analysis (QDA) [27]. The sensory attributes of juice samples were evaluated by a well-trained team of 10 members. Each sample shall be randomly coded at room temperature and determined three times in parallel. Comparison was performed in terms of color, acidity, taste, cloudiness, noni flavour, and overall acceptability of samples with results rating from 0 (poor)-10 points (strong) for each index. The average score of each sensory attribute given by 10 members is presented in a radar diagram (Fig. 5d).

2.15. Statistical analysis

All analyses were carried out in triplicate. The results were expressed as mean values \pm standard deviation (SD). Statistical analyses were conducted using SPSS statistics 25 (SPSS-IBM Chicago, IL., USA) with one-way analysis of the variance (ANOVA) and the Duncan's multiple range test at a 95 % confidence level.

3. Results and discussion

3.1. Optimal extraction parameters obtained from the single-factor experiment and RSM

Suitable ultrasonic extraction conditions can not only improve the yield of juice, but also help to retain the nutrients and bioactive ingredients in juice [20]. The effects of ultrasonic power, ultrasonic temperature and ultrasonic time on the extraction efficiency of noni juice were investigated. As shown in Fig. 1S, the extraction rate of noni juice increased with the increase of ultrasonic power, but the increase in yield was no longer significant when the power exceeded 500 W under either U1 or U2. In order to obtain higher juice yield and reduce machine loss, the optimal ultrasonic power was set to be 600 W.

Similarly, as shown in Fig. 1S, for U1-treated juice samples, the yield of juice displayed an upward trend with the increase of ultrasonic temperature, and reached the maximum yield of 58.12 % at 50 °C, but this yield was not significantly higher than that obtained at 45 °C. For U2-treated juice samples, juice yield first gradually increased with ultrasonic temperature, and then tended to be stable when the ultrasonic temperature surpassed 40 °C. Therefore, 45 °C for U1 and 40 °C for U2 were adopted as the central points of RSM.

As ultrasonic time went on, the variation trend of juice yield under U1 was slightly different from that obtained under U2 treatment (Fig. 1S). The yield of U1-treated juice first increased, then maintained stable and finally reached the maximum yield of 58.02 % after extraction for 30 min. However, the yield at the extraction time of 30 min was statistically insignificant in comparison with that at the extraction time of 25 min. By contrast, the yield under U2 treatment first displayed an increasing trend within 10 min, afterwards, the yield decreased slightly. Therefore, 25 min and 10 min were considered the optimal ultrasonic time for U1 and U2, respectively.

In the variance analysis of U1 and U2 treated juice samples (Table 1S and Table 2S), the p values of two models (0.0085 and 0.0005) were both less than the significant level of 95 %, indicating that the models had reached the significant level. The p values for lack of fit were relatively high (0.1136 and 0.1413), implying that the relationship between these values and the pure error was not significant. Comparing the F values and p values, the contribution of the factors affecting the response values (the yield of noni juice) could be ranked as follows: ultrasonic time > ultrasonic temperature > ultrasonic power. The mutual interactions between different factors on the extraction rate of noni juice were investigated using RSM, which is manifested in three-dimensional response surfaces and two-dimensional elliptical contour diagrams

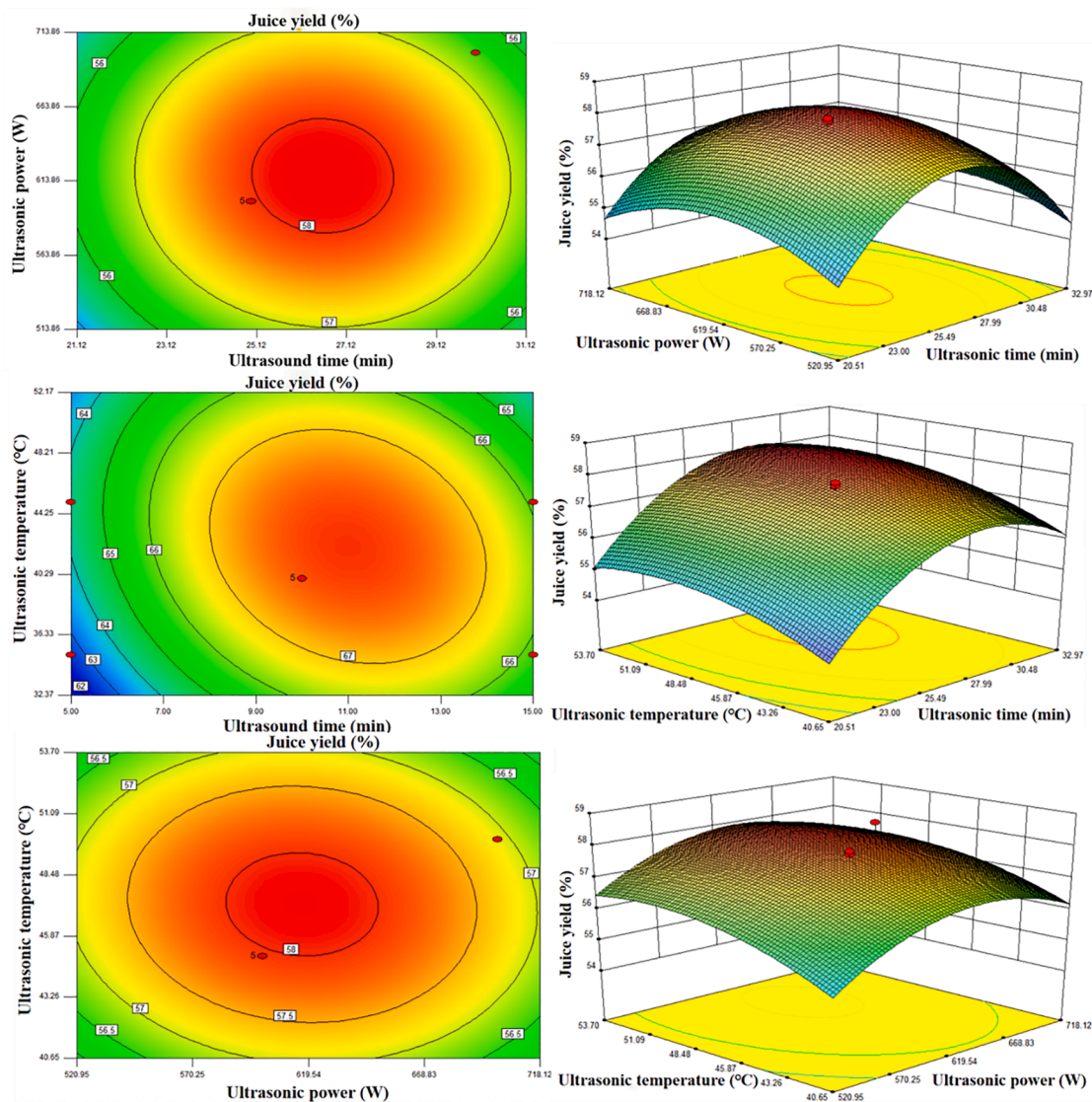


Fig. 1. Three-dimensional (3D) response surfaces plots of different extraction parameters (X1: ultrasonic time; X2: ultrasonic power; X3: ultrasonic temperature) on the yield of noni juice for U1. U1 represents individual ultrasound treatment condition of noni juice.

Table 1

The color change of noni fruit juice obtained by different treatment methods.

	CON	US	EZ	UEZ	EZU
L*	69.37 ± 2.01 ^a	70.73 ± 2.21 ^a	67.45 ± 1.57 ^{ab}	63.35 ± 2.07 ^{bc}	60.90 ± 2.65 ^c
a*	-4.17 ± 0.21 ^d	-4.60 ± 0.10 ^c	-2.53 ± 0.16 ^b	-1.60 ± 0.11 ^a	-1.83 ± 0.16 ^a
b*	20.20 ± 1.20 ^c	16.67 ± 1.71 ^d	27.75 ± 0.87 ^b	31.23 ± 1.32 ^a	27.60 ± 1.10 ^b
Δ E*	0.00 ± 0.00 ^d	4.39 ± 0.33 ^c	8.09 ± 0.47 ^b	12.99 ± 0.23 ^a	11.69 ± 1.22 ^a

All results are specified as mean ± SD. Results with different alphabets in the same row are significantly different ($p < 0.05$) from each other.**Table 2**

Particle size and zeta-potential of noni juice from different treatments.

	CON	US	EZ	UEZ	EZU
d ₁₀ (μm)	58.90 ± 4.32 ^a	52.30 ± 4.36 ^b	42.30 ± 3.32 ^c	44.50 ± 3.26 ^c	38.80 ± 2.30 ^c
d ₅₀ (μm)	255.00 ± 10.00 ^a	203.00 ± 13.36 ^b	109.00 ± 8.00 ^d	127.00 ± 9.00 ^c	93.00 ± 4.00 ^d
d ₉₀ (μm)	960.00 ± 33.00 ^a	814.00 ± 36.36 ^b	341.00 ± 12.36 ^d	410.00 ± 12.36 ^c	303.00 ± 16.32 ^d
D _{3,2} (μm)	144.00 ± 12.36 ^a	127.00 ± 10.33 ^b	91.50 ± 8.12 ^c	96.10 ± 5.36 ^c	83.90 ± 6.36 ^c
D _{4,3} (μm)	452.00 ± 13.23 ^a	386.00 ± 15.36 ^b	193.00 ± 9.36 ^{cd}	212.00 ± 10.33 ^c	183.00 ± 11.36 ^d
Span value	3.53 ± 0.24 ^a	3.75 ± 0.11 ^a	2.74 ± 0.12 ^b	2.87 ± 0.13 ^b	2.84 ± 0.22 ^b
Zeta-potential (mv)	-5.23 ± 0.36 ^a	-7.69 ± 0.55 ^b	-13.26 ± 0.45 ^d	-11.64 ± 0.64 ^c	-15.69 ± 0.32 ^e

All results are specified as mean ± SD. Results with different alphabets in the same row are significantly different ($p < 0.05$) from each other.

(Fig. 1 and Fig. 2S).

The optimal U1 conditions of noni juice predicted by RSM of Design Expert software were as follows: ultrasonic time of 25 min; ultrasonic power of 600 W; ultrasonic temperature of 45 °C. Meanwhile, the optimal U2 ultrasonic conditions were generated: ultrasonic time of 10 min; ultrasonic power of 600 W; ultrasonic temperature of 40 °C. Under

these conditions, the extracted yields of noni juice predicted by RSM were 57.96 % and 67.37 %, respectively. In order to validate the model, the additional confirmation tests were carried out under the optimal conditions. The mean values of noni juice yield obtained under optimum U1 and U2 condition were 58.35 % and 67.95 %, respectively, which matched well with the predicted results and thus indicated the high accuracy and feasibility of the models.

3.2. Physicochemical properties of juice

The influences of different treatments of noni juice on total soluble solids (TSS), pH, total acid (TA), and electrical conductivity (EC) were investigated, as shown in Fig. 2. Obviously, pH and TA of noni juice were not affected by either individual treatment (US or EZ) or their combined treatment (Fig. 2a). US processing did not significantly ($p < 0.05$) change the TSS content in noni juice, while EZ treatment significantly ($p < 0.05$) increased TSS content in noni juice (Fig. 2b), which was consistent with the research of Olawuyi et al. [18] on plum juice. This result could be owed to the fact that cellulase and pectinase effectively decomposed structural polysaccharides in tissues and thus bound soluble sugars could be released from the complex molecules [29]. When the ultrasonication was combined with enzymatic treatment, the TSS content in noni juice was further increased, indicating that the combined ultrasonic and enzymatic method could exert a synergistic effect on the extraction of noni juice. It was worth noting that the TSS content of samples varied depending on the sequence of ultrasonication and

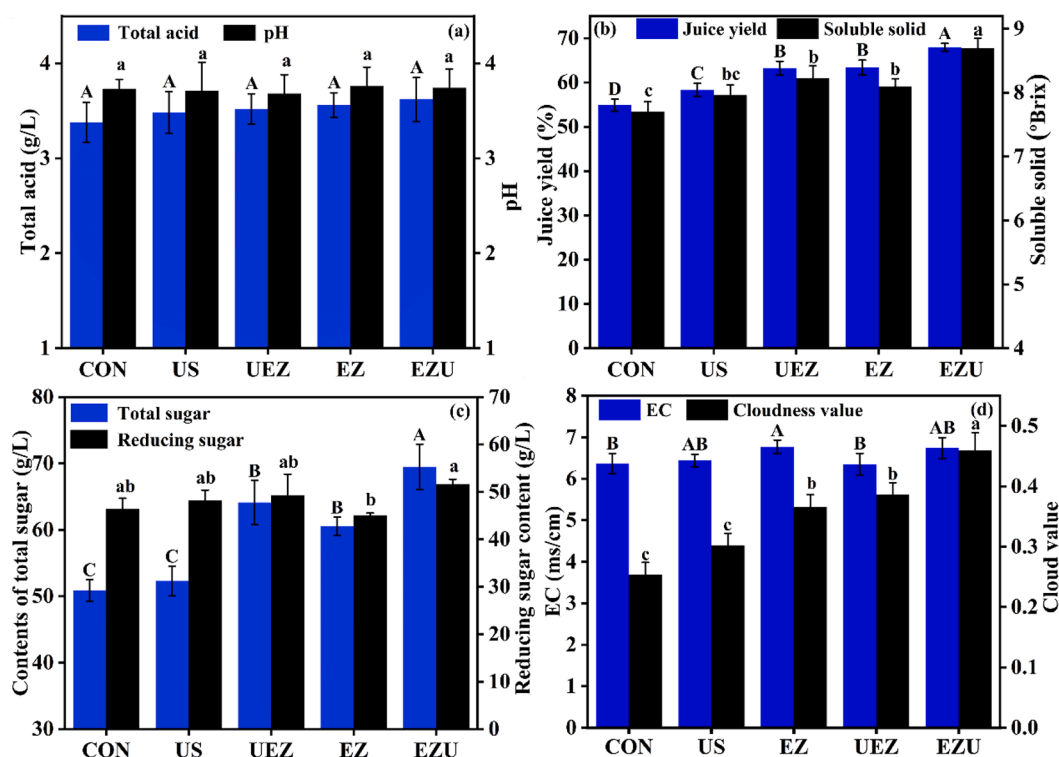


Fig. 2. The influence of different treatments of noni juice on (a) pH and total acid (TA), (b) total soluble solids (TSS) and juice yield, (c) reducing sugar and total sugar content, and (d) electrical conductivity (EC) and cloud value.

enzymatic treatment. It can be observed from Fig. 2b that the TSS content in noni juice extracted by the EZU treatment was evidently higher than that of UEZ-treated juice. Actually, the rise in TSS could be partially ascribed to the increase of soluble sugars which were derived from the conversion of insoluble pectin by pectinolytic enzymes and the action of cellulase on cellulose, and subsequent US treatment contributed to partial degradation of the cell wall components, leading to the further increase of the TSS content in noni juice [30,17]. Electrical conductivity (EC) of juice is generated due to the existence of various nutrients including vitamins, minerals, fatty acids and proteins [26]. Either US or EZ method did not exert much effect on increasing the EC of noni juice, while their combined treatment triggered significant change of EC in comparison with the single method. In addition, no significant difference in EC was observed between the EZU treatment and UEZ treatment (Fig. 2d).

The juice yield of noni juice was significantly ($p < 0.05$) affected by treatment method. Fig. 2b exhibits the noni juice extraction efficiency under different treatments. Compared with the CON, US treatment and EZ treatment increased the juice yield by 10.32 % and 21.74 %, respectively. It was observed that the yield of noni juice extracted by the combined ultrasound and enzymatic method was higher than that of juice sample treated by the individual method. Similar results were observed for the extraction of acerola mash [15] and mango juice [16]. In addition, the sequence of enzyme-assisted ultrasonication significantly ($p < 0.05$) effected the juice yield. The juice yield under EZU treatment was still higher 5.88 % than that under UEZ treatment. This result could be attributed to the fact that the cellulase and pectinase degraded the cell wall component, thus contributing to the release of intracellular contents. Furthermore, the ultrasonic cavitation effect led to the disruption of fruit cell walls, surface erosion and breakdown of some particles, which could help in the increase in mass transfer and juice yield [31].

3.3. Total sugar content and reducing sugar content

The effects of various treatments on total sugar content and reducing sugar content in noni juice were analyzed, as shown in Fig. 2c. It can be observed that the contents of total sugar and reducing sugar in noni juice treated by US alone were not significantly changed, which was in agreement with the change of TSS content after ultrasonication. Compared with the CON, the total sugar content of noni juice sample treated by EZ alone increased by 18.94 %, but the reducing sugar content was not significantly changed. The contents of total sugar and reducing sugar in noni juice extracted by combined treatment were higher than those in juice treated by single methods (either US or EZ method). For the two combined methods, EZU treatment obtained higher level of sugar content as compared with UEZ treatment. The higher sugar content of EZU-treated juice samples may be ascribed to the improved hydrolysis of cell wall polysaccharides after ultrasound and enzymatic treatment, which further enhanced the release of sugars from

the tissues into the juice.

3.4. Total phenolic content (TPC) and flavonoids content (TFC)

Phenolic compounds are closely related to the appearance (pigments accumulation), influence on taste (astringency) and mainly on antioxidant ability of juice [18]. As shown in Fig. 3a, significant differences in TPC and TFC were observed between noni juice treated by ultrasonication and that treated by enzymatic method. It was found that the TPC and TFC in US-treated juice were 2.67 % and 22.06 % higher than those of untreated juice, respectively, whereas enzymatic treatment did not cause any significant changes in TPC and TFC. Related researches have reported that cavitation energy induced by ultrasonication could facilitate the release of polyphenols from intracellular structures by virtue of destructing the binding of polyphenols with the polysaccharides and cell wall proteins [32]. The combination of ultrasound and enzymatic treatment, especially the EZU treatment, further promoted the TPC and TFC in noni juice. Interestingly, although UEZ treatment could significantly increase the TFC in juice, but it did not cause any significant changes in TPC. The TFC and TPC of the EZU-treated juice were 16.75 % and 5.53 % higher than those of UEZ-treated juice, respectively. The high TPC and TFC in EZU-treated juice may be attributed to the destruction of the tissue cell wall of noni fruit under enzymatic treatment and ultrasonic treatment, which effectively contributed to the release of phenolic compounds [33]. In line with the present study, Osete-alcaraz et al. [34] not only observed that the combination of pectinase and ultrasonic treatment increased the phenolic content in grape juice, but also revealed that ultrasonic time caused great differences in phenolic composition of wine.

3.5. Analysis of scopoletin and iridoids contents

Scopoletin, as one of the most important coumarins in noni fruits, has the antioxidative, anti-inflammatory, immunomodulatory, ameliorated hyperglycemia and hepatoprotective properties [35]. Deacetyl asperulosidic acid (DAspA) and asperulosidic acid (AspA) are found to be the major iridoids in noni fruit. Fig. 4 shows the content changes of scopoletin and iridoids (including DAspA and AspA) in noni juice after different treatments. In general, US treatment was superior to enzymatic treatment in increasing the bioactive components of noni juice. Compared with the CON, the contents of scopoletin, DAspA and AspA were increased by 6.17 %, 10.90 % and 27.71 % after ultrasonic treatment. Surprisingly, the contents of all these three bioactive components after EZ treatment were slightly decreased. This was probably due to the heating process during inactivated enzymes. Compared with US treatment, the combination of EZ and US treatment further improved the contents of bioactive ingredients in noni juice, and the effect of EZU treatment was more remarkable than UEZ treatment. Scopoletin content in noni juice treated with EZU was 14.25 % higher than that of UEZ-treated juice. Furthermore, the contents of DAspA and AspA in noni

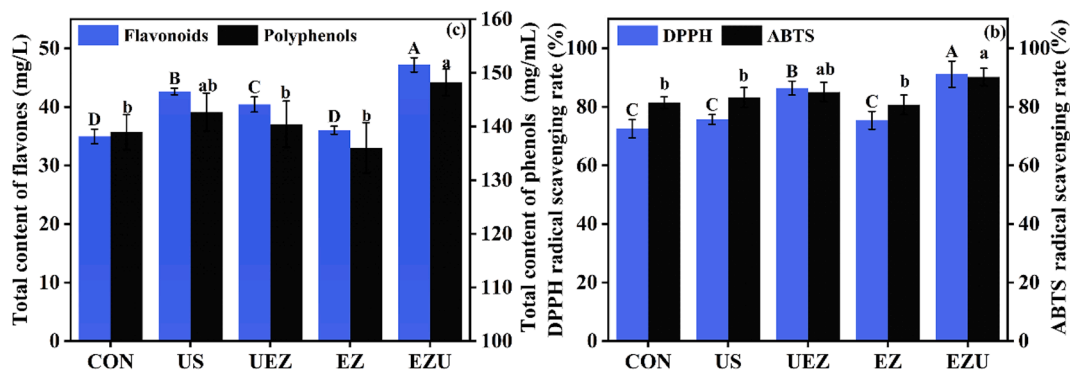


Fig. 3. (a) Total phenol content (TPC) and total flavonoids content (TFC), and (b) antioxidant capacity of noni juice under different treatments.

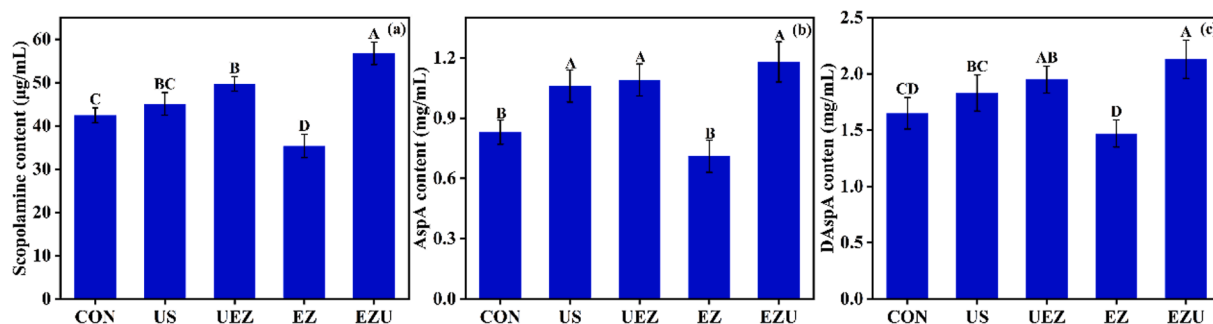


Fig. 4. The contents of scopoletin (a) and iridoids including (b) asperulosidic acid (AspA) and (c) Deacetyl asperulosidic acid (DAspA) in noni juice after various treatments.

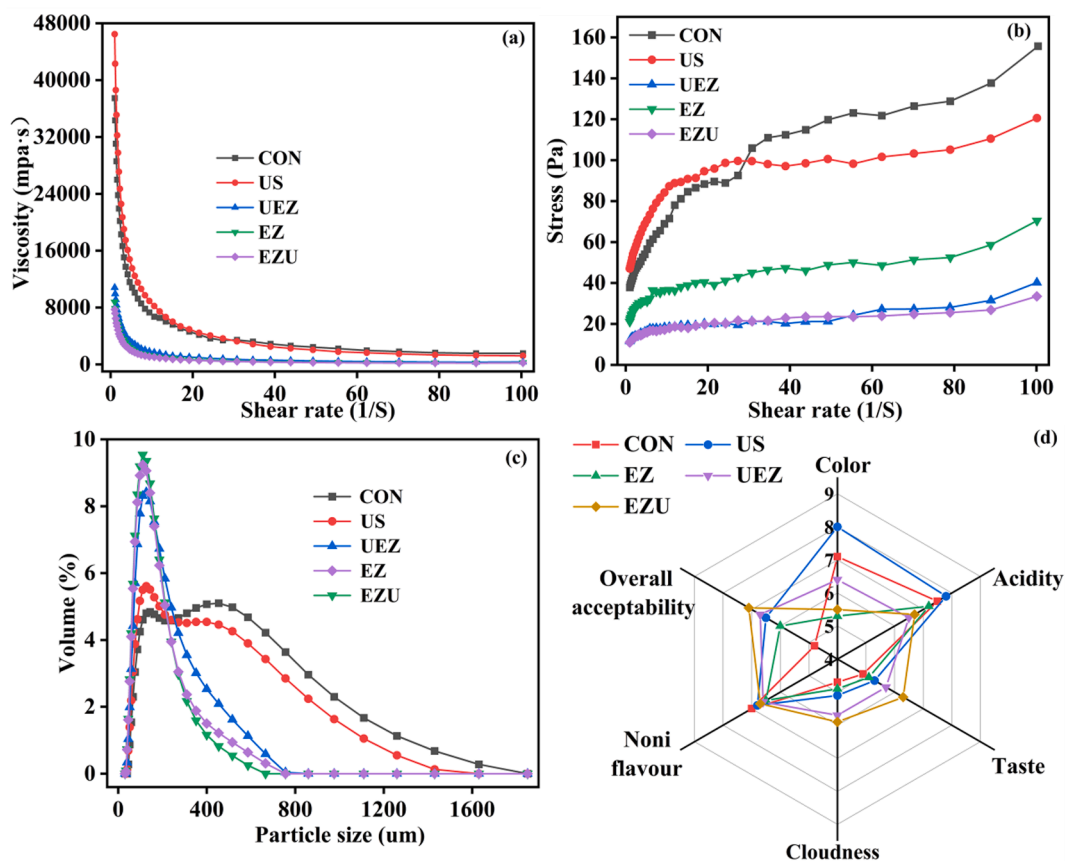


Fig. 5. The change of (a) rheological viscosities, (b) flow curve, (c) particle size distribution (PSD) and (d) sensory evaluation of noni juice under different treatments.

juice treated by EZU were 9.23 % and 8.26 % higher than those in UEZ-treated juice, respectively, but the difference between the two treatment methods was of no statistical significance ($p > 0.05$).

3.6. DPPH and ABTS radicals scavenging activity

The changes of antioxidant capacity of noni juice are closely associated with the concentration of antioxidant ingredients. Previous studies have suggested there exists a positive correlation between the content of phenolic compounds and antioxidant activity. Fig. 3b shows the effect of different treatments on antioxidant activity of noni juice. It can be observed that there was a slight enhancement in DPPH and ABTS scavenging capacity of noni juice under US and EZ treatments but statistically insignificant ($p > 0.05$) as compared to the CON. Compared with US or EZ method, the combined treatment could significantly

improve the DPPH and ABTS radicals scavenging activity, indicating that the combination of ultrasonication and enzymatic treatment could exert a synergistic effect on enhancing antioxidant capacity of noni juice. The EZU-treated juice sample exhibited the highest level of DPPH and ABTS radical scavenging activity among all treatments. This result might be ascribed to the increase of phenolic compounds in noni juice due to the cavitations produced by ultrasonication and the enzymatic cell wall degradation [36,37].

3.7. The cloud value and color

Color is an important factor which contributes to the quality attributes of juice. Color parameters of noni juice after different treatments are presented in Table 1. The increased L^* values and decreased $-a^*$ and $+b^*$ values of juice were observed in US treatment, while other

treatments brought about opposite trends. Similar results were also reported in orange [38] and strawberry juice [39] treated with ultrasound. The brightness of noni juice after EZ treatment dropped significantly compared with the CON, which may be due to the enzymatic browning caused by long-time enzymatic treatment and non-enzymatic browning induced by the heating process of enzyme elimination [40,22]. Under combined enzymatic and ultrasonic treatment, the greenness of juice sample decreased obviously, while the yellowness increased significantly. The total color difference of juice after various treatments exhibited a significant upward trend. In particular, the US-treated juice sample exhibited the lowest total color difference. Meanwhile, the total color difference of juice after combined treatment was generally higher than that of juice treated by single method, which revealed the application of combined ultrasonication and enzymatic treatment could make greater impact on the color changes of noni juice than individual treatment.

The cloudiness of juice is depended on the dispersed insoluble particles in juice such as pectin, protein, lipids, cellulose and hemicelluloses [41]. The degree of homogeneity of fruit juices can be evaluated by the cloud value. Fig. 2d displays that the cloudiness change of noni juice after various treatments. Compared with the CON, the cloud values of all treated juice samples increased. The juice treated by combined ultrasound with enzymatic treatment was observed to have higher cloud values than that treated by single method, which was in agreement with the study of Vivek et al [14]. The cloud value of EZU treated- juice was the highest, indicating that the juice system was the most stable due to the uniform dispersion of small particles in noni juice. The increase in cloud values of treated juice may be due to the hydrolysis of pectin led by the pectinase, as pectin substances can act as a natural colloidal suspension in the juice [14]. In addition, the ultrasonic cavitation effect could also contribute to the colloidal disintegration and breakdown of macromolecules into smaller substances, increasing the number of suspended particles and enhancing the homogeneity and consistency of juice [32]. In previous study, it was reported that the release of intracellular components such as sugars caused by cavitation during ultrasonic treatment also led to the increase in cloudiness of juice [42].

3.8. Particle size distribution (PSD) and zeta-potential

The particle size of suspension solution plays an important role in maintaining its stability against phase separation. Smaller particle size effectively inhibits coalescence and thus contributes to the stability of system against sedimentation [43]. The changes of area-mean diameter $D_{3,2}$ and volume-mean diameter $D_{4,3}$ of particles from different treatments are shown in Table 2. As can be seen from the table, $D_{3,2}$ and $D_{4,3}$ values of CON group were the highest ($p < 0.05$). Compared with the CON, the $D_{3,2}$ values of juice samples treated by US and EZ method were reduced by 11.80 % and 36.46 %, respectively, and $D_{4,3}$ values were reduced by 14.60 % and 57.30 %, respectively, indicating that enzymatic hydrolysis was more effective than ultrasonic treatment in reducing particle size of juice system. With regard to combined treatment, the $D_{3,2}$ value and $D_{4,3}$ value of EZU-treated sample as well as the d_{10} , d_{50} and d_{90} values were further reduced, but the difference was not statistically significant compared with the EZ-treated sample. Unlike the EZU-treated juice sample, the particle size of juice treated by UEZ was significantly smaller than that of juice treated by ultrasound method, but larger than that of juice treated by enzymatic method alone. Moreover, the $D_{4,3}$ values of all treated samples were higher than the $D_{3,2}$ values. However, the reduction of the $D_{4,3}$ values of all treated juice was more significant than the reduction of the $D_{3,2}$ values, which indicated that the number of larger particles in noni juice was evidently reduced after US and/or EZ treatment. Previous researches have suggested that the $D_{3,2}$ is highly affected by smaller particles, while the $D_{4,3}$ is more affected by larger particles, which might account for the greater reduction in $D_{4,3}$ values [44,45].

The distribution of particle size (PSD) of juice under different

treatments in the range of 0–2000 μm was investigated. It was observed from the Fig. 5c that a significant difference appeared between the PSD of noni juice under enzymatic treatment and that without enzymatic treatment. The PSD of the CON was the widest and showed a bimodal distribution, with particles size ranging from 7.64 to 2000 μm and the highest volume peak appearing at about 450 μm . US treatment effectively decreased the particle size of noni juice because the particle size of its PSD volume peak was notably different from that of CON. Similar result was reported for ultrasound-treated pumpkin juice where suggested the mechanical damage due to cavitation and the shear stress produced by ultrasonic waves could contribute to the particle size reduction [46]. However, ultrasonic treatment had a less significant effect on the particle size of noni juice than enzymatic treatment. The particle size of noni juice under enzymatic treatment and its combination with ultrasonic treatment was significantly lower than that under non-enzymatic treatment (CON and US), and their volume peak shifted left to smaller particle size, generating a narrower PSD ranging from 5.92 to 800 μm . EZU-treated juice displayed the most concentrated PSD with the volume peak appearing at about 110 μm , thereby showing the highest uniformity of juice system. In summary, the EZU treatment exerted the most significant effect on the reduction of juice particle size.

Zeta-potential can be used to judge the stability of colloidal suspensions so as to indicate the degree of electrostatic repulsion between adjacent particles with the same charge in suspension [45]. As shown in Table 2, the zeta-potential values of noni juice under all treatments were negative values, which could be ascribed to the fact that the particles in the juice samples were surrounded by pectin layer with negative charges [44]. In comparison with the CON, various pretreatments had significant impacts on zeta-potential of noni juice. It was found that all treated samples exhibited an increase trend of absolute zeta-potential values, which reflected the partial enhancement of juice system stability. The EZU-treated juice sample exhibited the most significant increase in the absolute value of zeta-potential, indicating that the system stability of juice treated by combined enzyme and ultrasonication treatment was significantly higher than that of juice treated by single method. Silva et al. [47] revealed that the change of zeta-potential values after ultrasonication was caused by a rupture of molecular chain induced by the high shear forces of ultrasonic cavitation. Moreover, smaller particle size resulted in an increase of interfacial area and a reduction of the average distance between particles, which led to stronger inter-particle interactions to generate higher zeta-potential and thus improved the stability of suspended system [44].

3.9. Rheological behavior

Rheological characteristics of processed noni juice under different treatments are illustrated in Fig. 5a and 5b. Obviously, with the increase of shear rate from 0.1 to 100 s^{-1} , the shear stress of treated noni juice increased gradually (Fig. 5b), and the apparent viscosity of all samples showed a downward trend (Fig. 5a), indicating that noni juice conformed to the shear thinning properties of pseudoplastic fluid. In the present study, US did not significantly ($p > 0.05$) alter the viscosity of noni juice, while the EZ treatment remarkably reduced the apparent viscosity of noni juice, which indicated that the rheological properties of noni juice extracted by enzymatic method were effectively improved. The apparent viscosities of noni juice treated by UEZ and EZU were significantly lower than the CON sample over the entire shear rate, but there was no significant difference in apparent viscosity reduction between UEZ and EZU treatment. This result was consistent with the early study of Olawuyi et al. [18] who reported that the apparent viscosity of plum juice treated by EZ or UEZ was evidently lower than that of US and CON samples. Previous study indicated that the reduction in viscosity could be ascribed to the degradation of polysaccharide components (e. g. pectin, cellulose) [8], especially, the pectin could act as a gelling agent to make the juice viscous [17]. The pectinase played a considerable role in degradation of the pectin in fruit tissue, thus decreasing the viscosity

of juice. Moreover, the cavitation effect of ultrasound treatment lowered the molecular weight of carbohydrates via breaking the pectin molecules, leading to the reduction of juice viscosity [26]. Furthermore, the reduction of particle size accounted for the decrease in apparent viscosity of noni juice [48]. In short, the rheological results suggested the noni juice treated by enzymatic method was more fluid and less viscous than that treated by non-enzymatic method. Meanwhile, the combined ultrasound and enzymatic method have a more significant effect on reduction of apparent viscosities as compared with individual US or EZ treatment.

3.10. FTIR analysis

FTIR is a technique to characterize and identify the functional groups of biochemical composition of plant or fruit tissues, so as to reflect the changes of chemical composition based on structural information. Fig. 6 displays the FTIR spectra of noni juice extracted by different treatments. It was found that all FTIR spectra of all treated noni juice sample had similar absorption pattern while some characteristic peaks slightly differed in the wavenumbers and/or absorption intensity, suggesting that different treatment methods had no noticeable effect on the fundamental structure of tissue compounds such as polysaccharide or polyphenols in the noni juice. This result was in line with the study of Bai et al [49]. The broad absorption band at 3600–3000 cm^{-1} was attributed to O–H stretching vibration of carbohydrates such as sugars, cellulose and pectin present in noni tissues [28]. The characteristic peaks about 2930 cm^{-1} and 1413 cm^{-1} corresponded to the C–H bonds of aliphatic groups. The absorption peaks around 1623 cm^{-1} were ascribed to –C=O stretching vibrations of saccharide carbonyls and the carboxylic acid [50]. The characteristic peak between 1200 and 900 cm^{-1} was considered the fingerprint region corresponding to the typical signals of C–O and C–C bonds present in sugars and organic acids [51], and the absorption peaks around 1076 cm^{-1} in the Fig. 6 represented the stretching vibration of these bonds. Furthermore, the signals peaks about 772 cm^{-1} were due to D-glucopyranose ring vibrations [49]. In short, the FTIR results indicated that there were no major changes in the chemical structure and the presence of functional groups of the compounds in noni juice treated by different treatment methods.

3.11. Sensory evaluation

Fresh noni juice has a strong pungent odor similar to butyric acid. After various treatments, the sensory quality of noni juice was improved. Sensorial attributes of noni juice samples (color, acidity, taste, noni flavour, cloudness and overall acceptability) are shown in Fig. 5d. The US-treated juice had excellent color property, but still showed obvious odor of pungent acidity. The juice treated by EZ method alone was slightly darker in color. However, when ultrasonic treatment was used in combination with enzymatic treatment, the acidity and taste of the juice were further improved. The general acceptability was obtained through comprehensively analyzing the scores of above six sensory parameters, and results showed that EZU-treated sample obtained the highest score of the general acceptance. Sensory evaluation results showed that EZU treatment improved the odor, taste and overall quality of noni juice.

4. Conclusion

The present study was the first to investigate the influence of combined ultrasound and enzymatic extraction technique on the quality of noni juice. It was observed that the yield of noni juice showed a significant increase under combined treatment than that under single treatment. The EZU-treated noni juice not only achieved the maximum juice yield and the highest content of soluble sugars, but also preserved the most of phenols, flavonoids and other bioactive ingredients, which contributed to increased antioxidant ability. Besides, EZU treatment significantly reduced the particles size and rheological viscosity, and

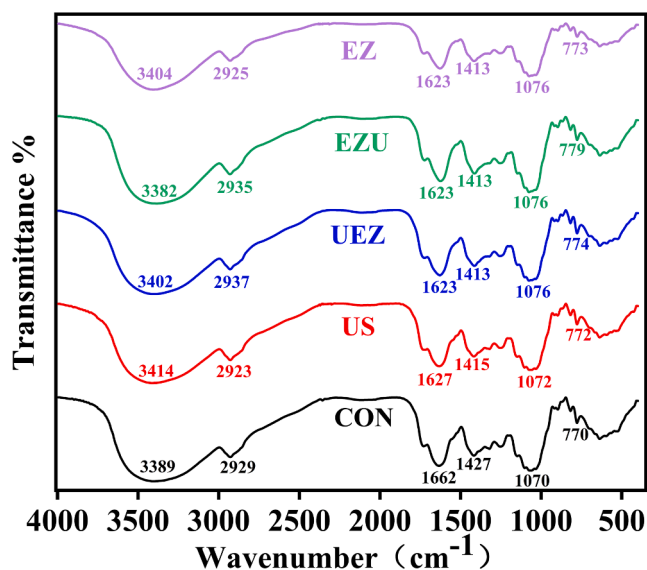


Fig. 6. The FTIR spectra of noni juice under different treatments.

enhanced the suspension stability of noni juice. FTIR spectra indicated that different treatment methods did not cause major changes in the chemical structure and functional groups of compounds in noni juice. From the results of this study, it can be concluded that EZU method is more effective than individual method in extraction of noni juice, and can achieve the production of noni juice with higher yield and better quality.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ultsonch.2022.106231>.

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