Effects of ultrasonic treatment on color, carotenoid content, enzyme activity, rheological properties, and microstructure of pumpkin juice during storage

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A R T I C L E   I N F O

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A B S T R A C T

Freshly squeezed pumpkin juice (Cucurbita moschata D.) was sonicates at various power levels at a constant frequency of 25 kHz and a treatment time of 10 min. Samples were stored in the dark for 0, 4, 8, and 12 days at 4°C and were subsequently analyzed. The combined effects of power level and storage period on color parameters, carotenoid content, particle size distribution, cloud value, rheological characteristics, and microstructure were investigated. The results showed ultrasonic-treated samples had little effect on carotenoid content, cloud value, particle size distribution, and polydispersity during storage compared to those of the untreated samples. The L*, a*, b*, and C* values decreased significantly during 8–12 days of storage, resulting in a significant increase in ΔE, especially 400 W/10 min-treated samples. Meanwhile, the enzyme activity and rheological properties increased significantly on storage days 8–12. However, the microstructure of all samples did not change significantly during storage. Based on these results, during the storage period, the physical and chemical properties of 400 W/10 min-ultrasonic treated pumpkin juice were retained more than those in the untreated pumpkin juice. Therefore, ultrasonic treatment has broad application prospects in preserving bioactive substances and physicochemical properties and improving the storage life of fresh pumpkin juice.

1. Introduction

Pumpkin is an annual herb belonging to the family Cucurbitaceae. Pumpkins have significant amounts of nutrients, such as vitamin A, phenolic compounds, carotenoid polysaccharide pectin, and mineral salts [1,2], that have the ability to reduce blood sugar and lipids, anti-cancer properties, and can improve the human immunity, alleviate the pain, and other active functions [3–5]. Pumpkin fruits have been used as a part of our diet since the ancient times; however, one of the problems with eating pumpkins is that the fruit is so large that one family cannot easily consume the whole pumpkin in a day [6]. Moreover, the commercial acceptability of pumpkin pulp is limited because of the perishable nature of freshly cut pumpkin due to enzyme activity and microbial exposure [7]. Enzymatic, microbial, chemical, and physical deterioration can greatly reduce the nutritional and sensory quality and shorten the shelf life of a fresh cut pumpkin pulp, if it has not been properly processed [6-8]. Therefore, through the development and utilization of pumpkin products (pumpkin juice, pumpkin mud, pumpkin-juice compound drinks, and fermented pumpkin juice), the added value of pumpkin can be improved and the waste reduced.

For a long time, heat treatment (70–121°C) has been the preferred technique to inactivate microorganisms and enzymes, and to extend the shelf life of fruit and vegetable juices and other foods [9]. However, heating has a negative effect on ascorbic acid, which accelerates the adverse degradation of anthocyanin polyphenols and flavor components, reduces the biological acceptability of phenolic compounds and vitamin C, and further reduces the physical and chemical properties of the fruit, greatly affecting the sensory quality and nutritional value of the product [10-13]. Heat treatment has been reported to significantly reduce the antioxidant capacity (18%), total phenols (22%), flavonoids (25%), and ascorbic acid content (36%) in blueberry [14] and strawberry juice [15]. In addition, heat treatment reduces the biological...
acceptability of phenolic compounds and vitamin C in acai juice [10]. The traditional heat treatment method has great limitations in maintaining the quality of fruit and vegetable products. In contrast, ultrasonic nonthermal treatment can serve as a cheap, reliable, and environmentally friendly alternative to conventional heat treatment. Ultrasound can effectively extend the shelf life of fruit and vegetable products, maintain the original freshness and nutrient content of the product, have a low energy utilization rate, and have high sensory acceptance; thus, it has received extensive attention [16-19]. The researchers have reported that ultrasonic treatment can replace heat treatment to obtain fruit and vegetable juices with high nutritional quality and good sensory attributes [20]. Jin et al. [21] studied ultrasonic treatment (20 kHz, 400 W) for 12 min, which significantly increased the content of ascorbic acid and antioxidant activity of the total phenolic flavonoids in strawberry juice, and the color and pH remained stable. Similarly, ultrasonic (42 kHz, 240 W) treatment for 40 min significantly increased the total phenols, carotenoid content, and antioxidant activity in cape gooseberry juice [22]. Additionally, ultrasonic treatment can significantly improve the total phenols, flavonoids, and antioxidant activities in carrot-grape juice and fresh-cut quince fruit [23,24]; after 90 days of storage, compared to the control group, the ultrasonically treated group maintained better nutritional properties [24].

However, there are a few reports on the processing of fresh pumpkin juice and pumpkin juice compound products using ultrasonic technology. Therefore, ultrasonic treatment (0, 200, 400, 600 W; 10 min) was used to study the color properties, carotenoid content, enzyme activity, cloud stability, particle size, rheological properties, and microstructural properties of pumpkin juice during the 4 days of storage. After ultrasonic treatment, the technology was analyzed to determine whether it is commercially practical for processing pumpkin juice and how it affects the physical and chemical properties, bioactive compounds, and rheological properties of pumpkin juice, so that it can be used in a predictable manner by the industry.

2. Material and methods

2.1. Plant material and chemical

Pumpkin (Cucurbita moschata D.) was bought at Lemeijia Supermarket (Nanchang, China). Ethanol, hexane, toluidine blue, polyvinylpolypyrrolidone (PVP), Triton X-100, catechol, hydrogen peroxide industrial, and NaCl were purchased from Solaiabao Technology Co. (Beijing, China).

2.2. Juice preparation

The pumpkin was cleaned, peeled, pared, and seeded. The pumpkin pulp was juiced by a juicer (HR1861, Philips, Hebei, China) and treated by ultrasound.

2.3. Ultrasonic treatment

Ultrasonic treatment conditions were as follows: 25 kHz frequency, 10 min, and powers of 200, 400, and 600 W (SCIENTZ-IID, Biotechnology Co. Ltd, Ningbo, China). Juice samples (100 ml) were placed in a 250 ml glass beaker. The ultrasonic treatment temperature was maintained at 25(±2) °C (water bath at 25 °C). The juice was divided into four parts: control sample (US0), sample ultrasonicated for 200 W (US200), sample ultrasonicated for 400 W (US400), and sample ultrasonicated for 600 W (US600). All samples were stored in brown bottles at 4 °C until further analysis.

2.4. Packaging and storage

Ultrasound (100 ml) and control pumpkin juice samples were separated in 50 ml sterile polypropylene tubes (NEST). The samples were stored in dark and refrigerated conditions for 12 days. The samples were analyzed on days 0, 4, 8, and 12.

2.5. Colors assessment

The color of the pumpkin juice was determined using a spectrophotometric colorimeter (X-Rite, Color i7, USA). Test condition: total transmittance, LAV, ΔE55/10°, SPIN. L*, a*, and b* values were measured. Chroma (C*), hue angle (h°), total color difference (ΔE), browning index (BI), and yellowing index (YI) were calculated using Eqs. (1)-(5) [25-27]:

\[ C^* = \sqrt{(\Delta a^* \,^2 + (\Delta b^* \,^2)} \]  

\[ h^° = \arctan (b^*/a^*) \]  

\[ \Delta E = \sqrt{[(\Delta L^*) \,^2 + (\Delta a^* \,^2 + (\Delta b^* \,^2}] \]  

\[ BI = 100\times(0.031)/0.172, \text{where } x = (a + 1.75b)/(5.645L^* + a^*-3.012b^*) \]  

\[ YI = 142.86b^*/L^* \]  

2.6. Determination of carotenoid content

The carotenoid content was determined by referring to Ordóñez-Santos et al. [22], with slight modifications according to the experimental design. Pumpkin juice (0.2 g) was weighed, ethanol (8 ml) and hexane (6 ml) were added, and the tube was covered with aluminum foil. The tube was then shaken in crushed ice for 1 h, after which 2 ml distilled water was added, and the mixture was allowed to stand for 5 min. All samples were stationary for 10 min, and then the n-hexane-phase samples were measured at 444, 450, 451, and 472 nm using a spectrophotometer (UV-5200PC Shanghai). Numerical values of β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin, and lycopene were calculated using Eq. (6) (refer to Barrett and Anthon [28]).

\[ \mu g/g = (A_{nm} \times M.wt \times 3)/(0.2 \times E_{1%1cm}) \]  

where, A_{nm} for the absorbance values, M. wt is the mass fraction of carotenoids, 3 ml is the volume of the hexane layer, 0.2 g is the weight of pumpkin juice, E_{1%1cm} is the extinction coefficient for carotenoids in hexane. Hart and Scott [29] calculated the concentration (mg/L) using the extinction coefficients E_{1%1cm} in hexane: 2560, 2800, 2460, 2480, and 3540 for β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin, and lycopene, respectively.

2.7. Polyphenoloxidase and peroxidase activities

The enzyme extract was prepared according to the methodology previously reported by Terefe et al. [30].

Polyphenoloxidase (PPO): 50 μL of the enzyme extract was added to 3 ml of 0.05 M phosphate buffer (pH 6.5) containing 0.07 M catechol. The absorbance was measured at λ = 420 nm at 25 °C for 10 min using a UV-visible spectrophotometer (UV-5200PC, Shanghai, China). The blank sample was prepared in the same way; however, the supernatant was replaced with 0.05 M phosphate buffer (pH 6.5).

Peroxidase (POD): The enzyme extract (50 μL) was added to 3 ml of 0.05 M phosphate buffer (pH 6.5). To start the reaction, 50 μL of 1% p-phenylenediamine (w/v) in 0.05 M phosphate buffer (pH 6.5) and 50 μL of 1.5% hydrogen peroxide (v/v) were added. The absorbance was measured at λ = 485 nm at 25 °C for 10 min (UV-5200PC, Shanghai, China). A blank sample was prepared in the same way, but the supernatant was replaced with 0.05 M phosphate buffer (pH 6.5). The residual
activity for both the analyzed enzymes (PPO and POD) was calculated according to Eq. (7):

$$\text{Residual activity (\%)} = \frac{A}{A_0}$$  \hspace{1cm} (7)

where $A$ is the activity of the US-treated juice and $A_0$ is the activity of the untreated juice.

2.8. Cloud value determination

The determination method was based on that of Jin et al. [25], and some modifications were made according to the experimental design. 6 ml of juice samples from each treatment were centrifuged at 5000 $\times$ g for 20 min at 4 $^\circ$C, then collected 2 ml supernatant and added 2 ml distilled water to mix. The absorbance of the mixture was immediately measured at 660 nm using a spectrophotometer (UV-5200PC, Shanghai, China), and distilled water was used as blank.

2.9. Particle size distribution analyses

All pumpkin juice samples were filtered through four layers of gauze to remove large particles and then diluted 50 times with distilled water to measure the particle size. The particle sizes of the pumpkin juice samples were measured using a particle size analyzer (BIC Brookhaven Instruments Corporation, USA). The effective diameter of particles in pumpkin juice samples was determined at room temperature.

2.10. Rheological characteristics

The determination method was referred to Jin et al. [25] and slightly modified according to this experiment. Rheological analyses were performed using an AR2000 rheometer (TA Instruments, USA) with a cone-plate (40 mm diameter).

2.11. Microstructure

Referring to Stratakos et al. [31], some modifications were made according to the experimental design. One milliliter of pumpkin juice was mixed with 1 ml of distilled water, and 20 $\mu$L of the mixture was transferred to a glass slide, stained with 0.1% toluidine blue solution of equal volume for 90 s, and viewed under an inverted light microscope (IX-71, Olympus, Japan) equipped with a digital camera. Images were taken at 10 $\times$ magnification.

2.12. Statistical analysis

All experimental data and pictures were analyzed by Origin 2019, SPSS 21, Illustrator2020, and ImageJ.

3. Results and discussion

3.1. Color attributes

Ultrasonic power has been considered a key variable in ultrasonic
processing in terms of ultrasonic cavitation effects generated by energy [32]. Therefore, gradient power of 200, 400 and 600 W were used for 10 min, and the changes in the color parameters of the pumpkin juice on days 0, 4, 8 and 12 were studied. The color parameters L*, a*, b*, h°, C*, ΔE, BI, and YI can be used to describe the color characteristics of the pumpkin juice [22,26,33].

The effect on the color attributes in pumpkin juice by ultrasound treatment and during storage are shown in Fig. 1 (A-H). Ultrasound treatment (US200, US400, US600) did not induce changes (P > 0.05) in the L*, a*, b*, h°, C*, ΔE, BI, and YI values of pumpkin juice (Fig. 1). This may be the result of the absence of browning reactions during processing. During storage, the color values of the four groups of samples showed no change (P > 0.05) on the fourth day. However, the L*, a*, b*, C*, h°, ΔE, BI, and YI values in the four groups decreased significantly (P < 0.01) on days 8 and 12. It should be noted that h° showed no change (P > 0.05) either by ultrasonic treatment or during storage. The BI value of US0 decreased gradually throughout the storage period, while the BI values of US200, US400, and US600 decreased significantly on day 8 (P < 0.01), increased significantly on day 12 compared to day 8 (P < 0.01), and overall increased significantly as compared with day 0 and 4 (P < 0.05). Laghika et al. studied the combination of ultrasonic and chemical treatment of white mushroom and found that the L° of white mushroom decreased and the ΔE increased during the whole storage period [36]. Similarly, Pinheiro et al. ultrasonically treated tomatoes, after 8 days of storage at 10°C, the a° of all samples decreased [37]. During storage, the color of pumpkin juice constantly changed, and these changes may be closely related to chemical, biochemical, enzymatic, and physical changes during the ultrasonic processing [23,37]. The results illustrate that it is possible to produce pumpkin juice with good sensory properties using ultrasound treatment and hence, can be a novel nonthermal alternative to the thermal method.

3.2. Carotenoid content

The effects of ultrasonic treatment of pumpkin juice on carotenoid content are shown in Table 1. In this study, a significant increase in carotenoid content was observed in the juice processed by ultrasound when compared to that at US0 (Table 1). Similarly, ultrasonic treatment also significantly increased the carotenoid content in kiwi juice, tomato sauce, and apple juice [22,38,39]. After US200, US400, and US600, a significant increase of 22.83–32.28% in β-carotene, 23.57–32.52% in α-carotene, 22.55–32.33% in β-cryptoxanthin, 23.70–32.59% in zeaxanthin and 24.14–34.48% in lycopene content was observed. The
increase in carotenoid content in pumpkin juice processed by ultrasound could be explained by the mechanical rupture of the cell wall and organelles, resulting in the release of these compounds into the food matrix [40,41].

On days 4, 8, and 12 of the storage period, the carotenoid content of all samples decreased compared with day 0, but the carotenoid content of US200, US400, and US600 was still higher than that of US0. Moreover, the carotenoid content of US400 was always the highest after ultrasonic treatment and during the storage period. These results show that ultrasonic processing is beneficial for improving and maintaining carotenoid content in pumpkin juice. Wu et al. [42] showed that the increase in carotenoid content in pumpkin juice processed by ultrasound could be explained by the mechanical rupture of the cell wall and organelles, resulting in the release of these compounds into the food matrix [40,41].

On days 4, 8, and 12 of the storage period, the carotenoid content of all samples decreased compared with day 0, but the carotenoid content of US200, US400, and US600 was still higher than that of US0. Moreover, the carotenoid content of US400 was always the highest after ultrasonic treatment and during the storage period. These results show that ultrasonic processing is beneficial for improving and maintaining carotenoid content in pumpkin juice. Wu et al. [42] showed that the table provides the carotenoid content of pumpkin juice under different ultrasonic power during storage.

### Table 1: Carotenoid content of pumpkin juice under different ultrasonic power during storage.

| Carotenoid | Treatments | Storage period (days) | 0 | 4 | 8 | 12 |
|------------|------------|-----------------------|---|---|---|----|
| β-carotene | US0        | 1.27 ± 0.06           | 1.13 ± 0.08 | 1.11 ± 0.08 | 0.98 ± 0.05 |
|            | US200      | 1.40 ± 0.07           | 1.33 ± 0.08 | 1.14 ± 0.06 | 0.95 ± 0.04 |
|            | US400      | 1.47 ± 0.07           | 1.46 ± 0.08 | 1.42 ± 0.06 | 0.93 ± 0.04 |
|            | US600      | 1.38 ± 0.07           | 1.30 ± 0.08 | 1.18 ± 0.06 | 0.93 ± 0.04 |
| α-carotene | US0        | 1.23 ± 0.06           | 1.10 ± 0.08 | 1.09 ± 0.08 | 0.94 ± 0.05 |
|            | US200      | 1.37 ± 0.07           | 1.30 ± 0.08 | 1.12 ± 0.06 | 0.92 ± 0.04 |
|            | US400      | 1.47 ± 0.07           | 1.43 ± 0.08 | 1.39 ± 0.06 | 0.93 ± 0.04 |
|            | US600      | 1.34 ± 0.07           | 1.19 ± 0.08 | 1.19 ± 0.06 | 0.92 ± 0.04 |
| β-cryoxanthin | US0      | 1.39 ± 0.06           | 1.18 ± 0.08 | 1.16 ± 0.06 | 1.14 ± 0.06 |
|            | US200      | 1.47 ± 0.07           | 1.42 ± 0.08 | 1.22 ± 0.06 | 1.09 ± 0.05 |
|            | US400      | 1.54 ± 0.07           | 1.52 ± 0.08 | 1.49 ± 0.06 | 1.08 ± 0.05 |
|            | US600      | 1.45 ± 0.07           | 1.28 ± 0.08 | 1.24 ± 0.06 | 1.06 ± 0.05 |
| Zeaxanthin | US0        | 1.35 ± 0.06           | 1.21 ± 0.08 | 1.19 ± 0.06 | 1.16 ± 0.07 |
|            | US200      | 1.48 ± 0.07           | 1.31 ± 0.08 | 1.22 ± 0.06 | 1.15 ± 0.07 |
|            | US400      | 1.57 ± 0.07           | 1.55 ± 0.08 | 1.52 ± 0.06 | 1.09 ± 0.05 |
|            | US600      | 1.48 ± 0.07           | 1.31 ± 0.08 | 1.26 ± 0.06 | 1.06 ± 0.05 |
| Lycopene   | US0        | 0.87 ± 0.07           | 0.78 ± 0.08 | 0.76 ± 0.05 | 0.75 ± 0.05 |
|            | US200      | 0.98 ± 0.07           | 0.93 ± 0.08 | 0.80 ± 0.05 | 0.80 ± 0.05 |
|            | US400      | 1.02 ± 0.07           | 1.02 ± 0.08 | 1.00 ± 0.05 | 0.99 ± 0.05 |
|            | US600      | 0.96 ± 0.07           | 0.85 ± 0.08 | 0.83 ± 0.05 | 0.83 ± 0.05 |

All data were the means ± SD, n = 3. a, b and c: Different letters in the same column indicate a significant difference; A, B and C: Different letters in the same row indicate a significant difference (P < 0.05).
combined treatment of ClO₂ and ultrasound could reduce the degradation of chlorophyll and vitamin C in cabbage during storage. In addition, Ordonez-Santos et al. [39] reported that the presence of vitamin C, vitamin E, and phenolic bioactive compounds in fruit juices was beneficial for maintaining carotenoid stability. Pumpkin is rich in carotenoids; there is evidence that carotenoids have anti-cancer activity, the ability to prevent chronic diseases, enhance immunity and antioxidant function, and can inhibit the development of heart diseases [43-45]. Epidemiological studies have also shown an inverse relationship between the presence of various cancers and carotenoid levels in the diet and blood [46]. Studies have also shown that increasing the intake of carotenoids can help reduce the risk of cancer and heart diseases [47].

3.3. PPO and POD activities

The influence of ultrasonication on the activity of PPO and POD, expressed as the residual activity, is shown in Fig. 2. There was a significant decrease in the activity of PPO (Fig. 2a) and POD (Fig. 2b), in all the ultrasound pumpkin juice samples, as compared to that in US0. A power of 200 W caused the inactivation of this enzyme by 24.40%, whereas the application of a higher power (400 W) resulted in lower inactivation (42.73%). The best results for PPO inactivation (58.80% reduction) were obtained when 600 W was applied. The POD inactivation rate was 31.66% at 200 W and 45.37% at 400 W. When the power was increased to 600 W, the inactivation effect of POD was optimal, with a decrease of 63.75%. With the increase in ultrasonic power, the residual activity of PPO and POD in pumpkin juice samples decreased, and the POD inactivation rate was higher than that of PPO. Moreover, it was observed that the highest power (600 W) was the most effective for

Fig. 4. Effects of different ultrasonic power on cloud value of pumpkin juice during storage at 4°C.

Fig. 5. Changes of rheological properties of pumpkin juice during ultrasonic treatment and storage at 4°C (A-P).
inactivating PPO and POD. Similarly, Yildiz et al. [23], Ok et al. [48], Yeoh et al. [49], and Jang et al. [50] showed that ultrasonic treatment could inhibit the production of PPO and POD in cynonia oblonga mill, banana fruit, fresh-cut pineapple, and fresh-cut apple. It has also been reported that the increase of ultrasonic treatment time and power can inactivate pectin methylesterase (PME) and superoxide dismutase (SOD) in fruit juice [51,52]. The inactivation of enzymes in pumpkin juice may be caused by chemical and physical changes during ultrasonic treatment. The local high temperature and pressure generated by the cavitation effect in the ultrasonic treatment process cause the spatial structure of the enzyme to change, so that the biological activity of the enzyme is lost, and water can be decomposed into free radicals (H• and OH•), which combine with the enzyme, thereby affecting enzyme activity [51,53,54].

The significant changes in PPO and POD were observed during the storage of samples at 4°C in Fig. 2a-b. In this study, the reduction in residual PPO and POD activity appears to be reversible, with enzyme activity increasing again from day 4 of storage. This change is consistent with Amaral et al. [55], the changes in PPO activity during storage of fresh cut potato treated by ultrasound. On days 12 of storage, the residual activity of PPO and POD increased by 25.21 and 24.67% in US200, 51.75 and 22.54% in US400, and 65.75 and 22.90% in US600, respectively, compared to those on day 0. The PPO activity during storage by ultrasonic treatment was significantly higher than that of POD, and the POD activity of US600 was 44.55% after 12 days of storage. Although the results of this study showed that ultrasonic treatment was more conducive to inactivating and inhibiting POD activity in pumpkin juice, it alone could not completely inactivate PPO and POD in pumpkin juice under the experimental conditions of this study.

Ultrasoundation is a promising technique for processing fruit and vegetable products, but pumpkin juice still has high enzyme activity during storage, which can shorten the half-life of nutrient compounds to less than six weeks [56]. PPO and POD can reduce the sensory quality and content of bioactive ingredients, such as polyphenols, in freshly carrot and celery juices [57]. Therefore, it is necessary to further optimize the inhibition and inactivation conditions of ultrasound on the enzymatic activity in pumpkin juice and other fruit and vegetable juices in future research. Moreover, Ultrasonic can also be collaborative with heat, hydrostatic pressure, pressure and heat preservation techniques together in fruit and vegetable juice processing for microbial inactivation [58-61].

3.4. Particle size distribution

The influence of ultrasonic treatment (0–600 W, 10 min) on the particle size distribution (PSD) and polydispersity of pumpkin juice is shown in Fig. 3 and Table 2. Ultrasonic processing significantly reduced the particle size of pumpkin juice. The particle size of the sample decreased from 584.1 nm for US0 to 298.9 nm for US200, and gradually decreased to 256.8 nm for US400 and 253.9 nm for US600. The change
in the particle size between US400 and US600 was significantly smaller than the change between US0 and US200. Similarly, significant reduction in the particles size was also reported in mango nectar, peach juice, diluted avocado puree, and tomato juice [62-65]. The mechanical damage both due to cavitation and the shear stress produced by ultrasonic waves could have contributed to the particle size reduction [66].

In addition, the polydispersity of pumpkin juice samples decreased from 0.335 for US0 to 0.260 for US200 and gradually decreased to 0.245 for US400 and 0.212 for US600 (Table 2). The variation in polydispersity between 400 W and 600 W was significantly smaller than that between 0 W and 200 W, as was the case with particle size. This result was contrary to the results of Jin et al. [24], who showed that ultrasonic processing significantly increased the polydispersity of kiwi fruit juice. Previous studies have confirmed that the significant reduction in fruit and vegetable juice particle size is related to the destruction of the fruit tissue microstructure caused by the cavitation effect in the ultrasonic treatment process. Because of cell wall rupture of fruit juice samples, they are cut into smaller fragments [63,67].

During the entire storage period, from 0 to 12 d (Fig. 3), the particle size of all the pumpkin juice samples increased. For US200, the particle size of pumpkin juice increased by 21.95% during storage, followed by US400 (18.46%), US600 (14.85%), and US0 (2.02%). In Table 2, the polydispersity of all pumpkin juice samples showed interesting changes during storage: US0 decreased and US200, US400, and US600 increased. The polydispersity of US0 decreases from 0.335 to 0.310, US200 increases from 0.260 to 0.288, US400 increases from 0.230 to 0.270, and US600 increases from 0.212 to 0.257. The changes in grain size and polydispersity during storage may be caused by the sedimentation and agglomeration of pumpkin juice particles.

3.5. Cloud value

The influence of ultrasonic treatment at 25 kHz, 0–600 W for 10 min, and storage period on the cloud stability of pumpkin juice, as well as the change in cloud stability of pumpkin juice by ultrasonic treatment for 0, 4, 8 and 12 days, is shown in Fig. 4. Compared to the untreated sample, the cloud stability of pumpkin juice treated with US was significantly increased (P < 0.01), and the cloud value increased from 0.75 to 0.98. Among all the treatments, the cloud values of US200, US400, and US600 were significantly different, and the cloud values of pumpkin juice increased with increasing ultrasonic power. This result is consistent with those of Rojas et al. [63], Tiwari et al. [68], Jin et al. [24], and Anda et al. [69], who studied the effect of sonication on the cloud stability of peach juice, orange juice, kiwi juice, and fresh orange and celery juice blends. On days 4, 8 and 12 of storage, the cloud value of the US0 sample significantly decreased, while the cloud values of US200, US400, and US600 decreased slowly and remained more stable with the increase in storage time.

The decrease in cloud stability during storage after the ultrasonic treatment may be caused by the inactivation of PME during the
ultrasonic treatment, which leads to pectin de-esterification [68,70]. Overall, the results showed that the stability of pumpkin juice treated with ultrasound was improved. This may be due to the high-pressure gradient generated by cavitation bubble rupture during ultrasonic treatment, which causes macromolecules to break down into smaller sizes [69]. In addition, the destruction of tissue cells caused by cavitation leads to the release of intracellular compounds (such as carotenoids) during ultrasound treatment, which may also contribute to the increase in the cloud value of the juice [21].

3.6. Rheological properties

Rheology is the science of the deformation and flow behavior of matter [71]. Rheological properties of food are important for unit operation design, process optimization, and high-quality product assurance. Therefore, studying the effects of processing on the rheological properties of foods is crucial for efficient product and process design [72]. The rheological properties (shear stress, apparent viscosity, storage modulus, and loss modulus) of pumpkin juice treated with US were studied.

3.6.1. Flow behavior of pumpkin juice

Fig. 5 (A-P) presents the rheological properties of pumpkin juice at US0, US200, US400, US600, as well as during storage (0, 4, 8 and 12 d), which showed that the shear stress and apparent viscosity significantly changed during US-treatment and storage. On day 0, when the shear rate increased from 0.1 to 100 s$^{-1}$ by ultrasonic treatment, the change trend of the shear stress of US200, US400, and US600 was not obvious, and the apparent viscosity of all samples showed a downward trend. The shear stress of pumpkin juice at US400 and US600 was significantly lower than at US0, and there was no significant difference between US200 and US0 (Fig. 5A). Consistent with the shear stress results, the apparent viscosities at US400 and US600 were significantly lower than at US0, and there was no significant change between US200 and US0 (Fig. 5B). Earlier studies by Jin et al. [24], Bi et al. [63], Wang et al. [64], and Roja et al. [73] showed that the viscosity of tomato pectic, avocado puree, kiwi juice and peach juice was significantly enhanced. They speculated that the increase in viscosity may be related to the promotion of hydrogen bonding and hydrophobic interactions between molecules of de-esterified pectin by ultrasound or that cavitation destroys particles or molecules and enhances interactions between particles, which may also be related to the solubilization of juice cell wall material and the presence of polysaccharides in juice. However, the results of this study showed that the viscosity of pumpkin juice treated by ultrasound did not increase but decreased, which was consistent with the results of the study by Bhat et al. [74]. US also significantly reduced the viscosity of strawberry juice. The decrease in viscosity of pumpkin juice and strawberry juice may be attributed to the fragmentation of polymer structures (usually pectin) in fruit and vegetable juice owing to the cavitation effects induced by ultrasound, resulting in a decrease in
viscosity [75]. On day 4, the shear stress and apparent viscosity of US0 did not change significantly but were still higher than those of the sample treated by US. On days 8 and 12, the shear stress of all samples increased with the increase in shear rate and was significantly higher than that on days 0 and 4. All apparent viscosities decreased with the increase in shear rate, but the viscosity on day 8 was lower than that on day 0, 4 and 12. Notably, on day 12, both shear stress and apparent viscosity were significantly higher in US200 than in US0, US400, or US600 (Fig. 5, M, N), and US600 exhibited the lowest shear stress and apparent viscosity throughout the storage period (Fig. 5, A, B, E, F, I, J, M, N). These results indicate that low power ultrasonic treatment may be beneficial for the maintenance of the rheological properties of pumpkin juice, whereas high power is unfavorable for the maintenance of the rheological properties of pumpkin juice. During storage, the effects of shear stress and apparent viscosity on fruit and vegetable juice may be related to changes in the cell structure, particle size, polydispersity, and cloud stability of fruit and vegetable juice damaged by ultrasonic treatment [62,63,76].

3.6.2. Dynamic rheological characteristics of pumpkin juice

The changes in the storage modulus and loss modulus of pumpkin juice during storage under ultrasonic treatment are shown in Fig. 5 (C, D, G, H, K, L, O, P). In this study, a frequency scanning model was used to determine the behavior of the storage modulus and loss modulus of pumpkin juice within a frequency range of 0.1–10 Hz. As shown in Fig. 5 (C, D), the storage modulus of pumpkin juice samples that were subjected to ultrasonic processing US200, US400, and US600, compared with untreated samples, were significantly reduced at the same frequency storage on day 0. The storage modulus of all samples significantly increased at a frequency of 0.1–0.4 Hz, and the increasing trend was not obvious after 0.4 Hz. Compared with US0, the loss modulus of the other pumpkin juice samples did not increase significantly with an increase in frequency. At the same frequency, the storage modulus and loss modulus were the highest in US0, followed by US200, US400, and US600. The storage and loss moduli of US200, US400, and US600 were not significantly different from each other. The effect of ultrasonic treatment on the storage modulus and loss modulus of pumpkin juice is different from that of kiwifruit juice and mango juice according to Wang et al. [27] and Huang et al. [62].

At 4, 8, and 12 days, the storage modulus and loss modulus changed significantly. On day 4 (Fig. 5, G, H), the increasing trend of the storage modulus and loss modulus with increasing frequency was consistent with the change in storage modulus on day 0. On days 8 and 12 (Fig. 5, K, L, O, P), the storage and loss moduli of US0, US200, and US400 all showed an increasing trend with increasing frequency. On day 8, US0 was the highest at the same frequency, followed by US400, US200, and US600. On day 12, US0 had the highest frequency, followed by US400, US200, and US600. The rheological properties of fruit and vegetable juice processing and complex changes in the process of storage to a large extent also depend on the species and concentration of fruit and vegetable juice [71]. However, current studies on the effect of ultrasonic treatment on rheological properties of fruit and vegetable juices are limited, and further studies are needed to confirm the principle of
change.

3.7. Microstructure

An optical microscope (IX–71, Olympus, Japan) was used to observe the microstructure of pumpkin juice after ultrasonic treatment at 0, 200, 400, and 600 W, at 10 min, and during the storage period (Fig. 6). The results showed that, with an increase in ultrasonic power, the cell wall of the tissue was significantly damaged by ultrasonic treatment. Specifically, the microstructure at US0 exhibited intact cell walls. The cell structure at US200 was not completely destroyed, and it was observed that the edge of the cell wall began to blur, and the phenomenon of plasma-wall separation appeared. It is assumed that low-power ultrasound treatment causes movement of intracellular substances but does not cause significant damage to the tissue of pumpkin juice. In contrast, cell fracture and tear were significantly increased in the US400 sample owing to the increase in ultrasonic power, although some intact cells could still be observed in the sample. The microstructure of US600 was different from that of US0, US200, and US400, and the cell wall was broken, and resulted in the cell structure was disintegrated.

With an increase in ultrasonic power, the destruction of the US600 cell structure was the most obvious, which may result in the complete release of intracellular components into pumpkin juice. In the early stages, Campoli et al. [77], Rojas et al. [63], and Starek et al. [55] showed similar trends in guava juice, peach juice and tomato juice. After 4–12 days of storage, no significant changes were observed in the cells of all treated pumpkin juice samples compared to that on day 0. While it is assumed that the physiological and biochemical changes in pumpkin juice during storage do not further damage the cell structure, any damage to the cell structure in fruit and vegetable juice caused by ultrasonic processing is irreversible [78].

The changes in cell structure may have been caused by the shock wave due to the cavitation effect in the ultrasonic process, which destroys the cell structure, or by the microflow related to shear stress and cytoplasmic viscosity changes, which leads to changes in the cell finally destroying the cell structure [67,78,79]. Microanalysis showed that ultrasonic treatment at different powers had different effects on the microstructure of the pumpkin juice. However, the exact causes of these changes have not yet been proven.

4. Conclusion

In conclusion, compared to the untreated samples, ultrasonic treatment had no negative effect on the color index of pumpkin juice, whereas the carotenoid content was significantly increased. Moreover, the color index (L*, a*, b*, h°, YI) and carotenoid content of pumpkin juice were better maintained during the storage period at 400 W/10 min. At the same time, there was a correlation between the color change and natural pigment content (carotenoid content). During the storage period, the PPO and POD residual activities of pumpkin juice increased but were still lower than those at US0. The higher the ultrasonic power, the stronger was the inhibition of enzyme activity (600 W/10 min). Ultrasonic treatment significantly reduced the particle size of pumpkin juice, resulting in higher uniformity of pumpkin juice, better taste, and no significant increase during the entire storage period. Ultrasonic treatment increased the damage to the tissue and cell structure of pumpkin juice and significantly increased the carotenoid content and cloud value of pumpkin juice, especially at 600 W/10 min. changed the rheological properties (flow and viscoelastic behavior) of pumpkin juice during storage. In addition, previous studies have reported that, compared to the untreated samples, high-intensity ultrasonic treatment can significantly improve the physical and chemical properties, nutritional composition, and rheological properties of fruit juice. Therefore, ultrasonic processing can be considered as a potential new processing technology to improve the quality of pumpkin juice.

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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