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Research article

# Influence of ultrasound on the microbiological, physicochemical properties, and sensory quality of different varieties of pumpkin juice

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ABSTRACT

This study has investigated the effect of ultrasound (US) as an emerging non-thermal sterilization technique on microbial growth and quality changes in three freshly squeezed pumpkin juices (*Cucurbita maxima* Duchesne, *Cucurbita moschata* Duchesne, and *Cucurbita pepo* L.).The three pumpkin juices were ultrasonicated at different ultrasonic power (0–400 W), time (0–20 min), and temperature (0–30 °C), and the total colony counts of the treated pumpkin juices were less than 5 log CFU/mL, which complied with the food safety and consumption standards. Based on these results, we further investigated the effects of different ultrasonic power (25 kHz, 10 min, 20 °C, 0–400 W) on the physicochemical properties and sensory quality of the three pumpkin juices. The physicochemical properties (color, sugar content, organic acid content, soluble solids, and carotenoids) of treated pumpkin juice were retained or improved to some extent. The antioxidant capacity was also increased by 9.09%, 10.25%, and 16.9% compared to the untreated group. During sonication, the particle size of all samples decreased significantly, the microstructure broke down significantly, and the sensory qualities of pumpkin juice were well preserved after sonication.

## 1. Introduction

Pumpkins belong to the genus *Cucurbita* and are among the most widespread crops in the world because they are warmth-loving, short-day plants that are drought-tolerant and do not have strict soil requirements. The pumpkin is also one of the most neglected and underutilized food and medicinal plants because of the scarcity of genetically improved seeds [1]. The skin and flesh of pumpkins contain essential minerals and phytochemicals (β-carotene, total flavonoids, and total phenols) that can promote anti-aging and immunity [2]. In terms of vitamin A requirements, night blindness is more common in South Asian countries, and the long-term consumption of pumpkins could increase vitamin A intake and fortify the diet, which, in turn, provides better relief from night blindness [3]. Pumpkins are grown on approximately 30,000 ha worldwide, producing 278,320 tons. China leads the world in pumpkin production with approximately 58%, whereas India (20%), Ukraine (4%), and Russia (4%) are also important producers [3]. *Cucurbita pepo* L., *Cucurbita maxima* Duchesne, and *Cucurbita moschata* Duchesne are three economically important species of pumpkin that are widespread in agricultural areas worldwide. *C. moschata* ("Miben"), *C. maxima* ("Jinli") and *C. pepo* ("zucchini") [4] are the three most important domesticated *Cucurbita* species.

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Currently, pumpkin products are mostly sold freshly cut and processed into easy-to-carry and store foods such as juices, jellies, purees, jams, crisps, and pumpkin seed oil. To achieve a longer storage period, all products are thermally processed, mainly to sterilize microorganisms such as *Escherichia coli* and *Bacillus* in the product, while simultaneously causing certain effects on the quality, such as browning and loss of active substances such as ascorbic acid and polyphenols. The demand for freshly cut and unpasteurized pumpkin products is gradually increasing owing to the many adverse effects of thermal processing. Therefore, a clean, environmentally friendly, and effective nonthermal processing technology for pumpkins is urgently needed. Compared with the current non-thermal food processing technologies (ultra-high pressure, dense phase carbon dioxide, and pulsed electric field) [5,6], ultrasonic processing is considered the most promising.

High-intensity ultrasound is frequently employed in food processing to a frequency of 18–100 kHz and has an energy of more than 1 W/cm$^2$ [7,8]. Ultrasound-induced cavitation generates a significant amount of energy, resulting in cavity collapse [9]. This collapse generates high temperatures and pressures, which in turn produce free radicals and powerful shear forces. During the collapse process, high pressure and temperature generate microjets that aid in the removal of dust, microorganisms, and other harmful particles from the surfaces of fruits and vegetables [7]. This process also breaks down the cell wall and biofilm structure, thereby thinning the biofilm and increasing its permeability. Consequently, harmful structures disintegrate to achieve the desired cleaning effect [10]. High-power ultrasound has been shown to perform well in inactivating various pathogenic and spoilage microorganisms, either alone or in combination with other techniques, such as mango juice [11], guava juice [12], and apples [13].

In addition, in terms of the sensory quality of fruits and vegetables, ultrasound can improve their stability and lead to the maximum retention of nutrients [14,15]. For example, ultrasound can maintain the physicochemical quality of fruits and vegetables and increase the stability of cell wall polysaccharides [16]. Lepaus et al. [14] treated orange carrot juice with high-power ultrasound (110 W, 60 °C, 10 min), which increased the disruption of the cellular structure of the juice, delayed the settling of the pulp, and improved cloudiness and juice stability. Similarly, Gao et al. [17] treated tomatoes with ultrasound (20–25 kHz, 87.52 W/cm$^2$, 10 °C, 15 min) and observed a significant increase in total carotenoid content.

However, in China, local Miben pumpkin juice is consumed more frequently, whereas Jinli pumpkin and zucchini juice are consumed less frequently. Therefore, few studies have been conducted on the other two pumpkin juices. In this context, ultrasonic treatment (0–400 W, 0–20 min, 0–30 °C) was used to investigate the microbial colony counts and the effects of ultrasound technology at different powers on the color, soluble solids, organic acid content and pH, carotenoids, total antioxidant properties, polyphenol oxidase (PPO) and peroxidase (POD) activities, particle size and dispersion, and microstructure in three types of pumpkin juice, and to assess their quality. Based on the needs of manufacturers and consumers, and the innovative use of green energy, this study investigated the effects of ultrasound treatment on the physicochemical properties and bioactive compounds of pumpkin juice. The results of this study are expected to improve the quality of pumpkin juice samples and the application of ultrasound in the food and beverage sectors.

## 2. Material and methods

### 2.1. Material and chemical

*Cucurbita moschata*, *Cucurbita maxima*, and *Cucurbita pepo* were purchased at Lemeijia Supermarket (Nanchang, China). Rose bengal agar, plate count agar (PCA), ethanol, hexane, toluidine blue, sodium phosphate buffer, catechol, and NaCl were purchased from Solarbio Technology Co. (Beijing, China).

### 2.2. Experimental methods

#### 2.2.1. Sample pretreatment and sampling

Fresh pumpkin samples were selected based on their bright color and absence of spoilage. The pumpkins were cleaned, peeled, fleshed, and seeded. The resulting pieces were cut into appropriate sizes and steamed to soften them. After cooling, 500 mL of pure water was added with pumpkin pieces in a Philips juicer (HR1861; Philips, Hebei, China) for juicing.The juice was then subjected to ultrasonic treatment(Supplementary Fig. 1).

#### 2.2.2. Ultrasound-pasteurization treatment

A 100 mL sample of freshly squeezed pumpkin juice was placed in a 250 mL glass beaker, and an ultrasonic cell disrupter (200-IID Ultrasonic Cell Disrupter; Ningbo, China) with an operating frequency of 25 kHz and a probe diameter of 12.7 mm was used to subject the samples to different power (0, 100,200, 300, and 400 W), number of times (0, 5, 10, 15, and 20 min), and temperatures (0, 10, 20 and 30 °C).

The conventional pasteurization was carried out at 65 °C for 10 min (P65) in a digital temperature water bath (Changzhou Putian Instrument Manufacturing Co., Ltd, Changzhou, China). All samples were stored in brown bottles at 4 °C for 24 h until further analysis.

#### 2.2.3. Microbiological evaluation

The full-plate count method was used to detect live natural microbial cells. The experimental method and procedure were based on the National Standard GB/T 4789.2–2016 with slight modifications according to the experimental design. To measure the total number of colonies, 25 mL of pumpkin juice sample was drawn with a sterile pipette into a 225 mL sterile conical flask containing distilled water and mixed thoroughly to make a 1:10 homogenate of the sample, which was then diluted with sterilized distilled water and diluted to $10^{-1}$, $10^{-2}$ and $10^{-3}$ times dilutions according to the 10-fold dilution method. One milliliter of the dilution was pipetted and poured into 15 mL of nutrient agar base, shaken well, and allowed to set before inverting. The nutrient agar medium was then incubated at 37 °C for 48 h. The colony counts of the pumpkin juice samples under different varieties and treatment conditions were determined separately. The colony calculation formula was as follows:

$$N = \frac{\sum C}{(\mathrm{n}_1 + 0.1\mathrm{n}_2) * \mathrm{d}} \tag{1}$$

where N is the number of colonies in the sample,$\sum$C is the sum of the number of colonies on the plates (plates containing the appropriate range of colonies),$\mathrm{n}_1$ is the number of plates at the first dilution (low dilution),$\mathrm{n}_2$ is the number of plates at the second dilution (low dilution), andd is the dilution factor (first dilution).

### 2.2.4. Color assessment

The experimental method is based on the method described by Wang et al. [18], with slight modifications to suit the experimental design. The color of the pumpkin juice was determined using a spectrophotometric colorimeter (Color i7; X-Rite, Grand Rapids, MI, USA). L*, a*, and b* values were measured. The chroma (C*), hue angle ($\mathrm{h}^0$), total color difference (ΔE), browning index (BI), yellowing index (YI), and color index (CI) were calculated using Eqs. (2)–(7) [18,19].

$$C^* = \sqrt{(\mathrm{a}^*)^2 + (b^*)^2} \tag{2}$$

$$\mathrm{h}^0 = \arctan(\mathrm{b}^* / a^*) \tag{3}$$

$$\Delta E = \sqrt{\left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]} \tag{4}$$

$$BI = 100(\mathrm{x} - 0.31)/0.172, \;\; x = a^* + 1.75L^*/5.645L^* + a^* - 3.012b^* \tag{5}$$

$$YI = 142.86\mathrm{b}^*/L^* \tag{6}$$

$$CI = \left(180 - h^0\right) / (L^* - C^*) \tag{7}$$

### 2.2.5. Determination of reducing sugars (glucose, fructose), sucrose, and total soluble solids (TSS) content

The experimental method was based on the method described by Dhenge et al. [1] with slight adjustments. TSS content was measured using an Atago digital refractometer (Atago, Tokyo, Japan) and expressed as °Brix at 25 °C.

Sugars were analyzed by high-performance liquid chromatography with an evaporative light-scattering detector (HPLC-ELSD) (Waters, Milford, MA, America). The chromatographic system consisted of an Agilent 1100 HPLC pump coupled with a SEDERE Sedex 60 LT ELSD. A YMC - Pack Polyamine II column (4.6 * 250 mm, particle size 5 μm) was used as stationary phase, while acetonitrile: water (75:25) was used as mobile phase, with a flux of 1.0 mL/min, and the column oven at 25 °C. Carbohydrates in pumpkin samples were quantified using calibration curves of glucose, fructose, and sucrose, built with standard solutions of each sugar in water in concentration ranges of 5–500 μg/mL.

### 2.2.6. Determination of pH, and titratable acidity

The experimental method described by Anda et al. [20]. The pH of the pumpkin was measured using a digital pH meter (Metrohm, Herisau, Switzerland) at room temperature (25 °C). Titratable acidity (TA) was measured with sodium hydroxide (0.1 N) up to a pH of 8.2 ± 0.1. It was expressed as grams of citric acid per 100 mL of sample.

$$TA(\%) = \frac{ml\ NaOH \times Normality\ of\ NaOH \times Acid\ factor \times 100}{Sample\ volume(mL)} \tag{8}$$

### 2.2.7. Determination of carotenoid content

Carotenoid content was determined according to Suo et al. [21], 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 on crushed ice for 1 h, after which 2 mL of distilled water was added, and the mixture was allowed to stand for 5 min. All the samples were stationary for 10 min, and the n-hexane-phase samples were measured at 444, 450, 451, and 472 nm using a spectrophotometer (UV–5200PC; Unocal Corporation, Shanghai, China). The numerical values of β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin, and lycopene were calculated using Eq. (9)

$$\mu g / g = (A_{nm} \times M.Wt \times 3) / \left(0.2 \times E_1^{1\%}\right) \tag{9}$$

where $\mathrm{A}_{\mathrm{nm}}$ is the absorbance value, 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, and $E_1^{1\%}$ is the extinction coefficient for carotenoids in hexane. Hart and Scott [22] calculated the concentrations (mg/L) using the extinction coefficient $E_1^{1\%}$ in hexane as 2560, 2800, 2460, 2480, and 3540 for β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin, and lycopene, respectively.

### *2.2.8. Determination of total antioxidant activity*

The antioxidant capacity of pumpkin juice was carried out as previously reported by Chen et al. [23] with slight modifications. 1.0 g supernatant of juice was mixed with 1.0 mL DPPH ethanolic solution (0.3 mM) for 30 min in darkness, then the absorbance value at 517 nm was recorded. The ethanol instead of the juice was subjected to the same procedure and measured as blank control.

### *2.2.9. Determination of enzyme activity (PPO, POD)*

The UV spectrophotometric method was used according to Chen et al. [24] with slight modifications based on the design of the present experiment. The enzyme extract solution was 0.2 M sodium phosphate buffer (pH 6.5, 4 % [w/v] polyvinyl polypyrrolidone [PVPP], 1 % [v/v] TritionX-100, and 1 M NaCl). After mixing pumpkin juice: extract = 1:1 (v/v), each sample was vortex shaken for 1 min, centrifuged at 11,000 g (4 °C, 30 min) and the supernatant collected. The supernatant was centrifuged under the same conditions. All extracts were stored at 4 °C in the dark and used immediately for activity determination.

To measure polyphenol oxidase (PPO), 200 μL of the substrate solution was mixed with 250 μL of 0.2 mol/L phosphate buffer (pH 6.5) and 500 μL of 0.02 mol/L o-phenol diphenyl solution. The absorbance was measured at 420 nm at ambient temperature for 1 min.

To measure peroxidase (POD), 200 μL of the substrate solution was mixed with with 1.5 mL of 0.05 M phosphate buffer (pH 6.5), and 200 μL of 1% *p*-phenylenediamine was added with 1.5% hydrogen peroxide. The absorbance at 485 nm was then measured by standing at ambient temperature for 1 min. Residual enzyme activity (RT) was calculated as follows:

$$RT(\%) = \frac{Specific\ activity\ after\ treatment}{Specific\ activity\ before\ treatment} \times 100 \tag{10}$$

### *2.2.10. Particle size distribution analyses*

The particle size distribution of the juice was determined using the method described by Yildiz et al. [25] with minor modifications. The pumpkin juice was filtered through gauze to remove larger particles and diluted 50 times with distilled water for measurement. The particle size of the pumpkin juice samples was determined using a particle size analyzer (BIC Brookhaven Instruments Corporation, Austin, TX, USA), and the effective particle size of the pumpkin juice samples was determined at room temperature.

### *2.2.11. Microstructure*

The procedure described by Stratakos et al. [26] was modified based on the experimental design. After mixing 2 mL of different varieties of pumpkin juice with 2 mL of distilled water, 20 μL of the mixture was transferred onto slides and stained with an equal volume of 0. 1% Toluidine blue solution for 90 s. Images were captured under an inverted light microscope (IX-71; Olympus, Tokyo, Japan) fitted with a digital camera. Images were taken at 10x magnification.

### *2.2.12. Sensory evaluation*

Sensory analyses were performed according to the method described by Wang et al. [27]. Assessments were performed in the laboratory by 43 participants (semi-trained), including 23 women and 20 men, aged 19–60 years. Approximately 50 mL of pumpkin

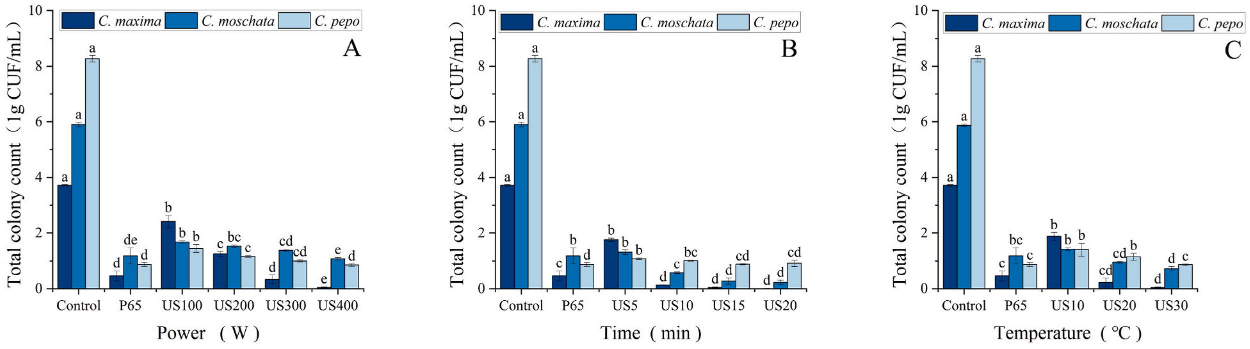


**Fig. 1.** Effect of pasteurization and different sonication treatments on microbial colonisation of three pumpkin juices (A–C) Figure A: Effect of pasteurization and different power (0–400 W) of ultrasound on the microbial colonies of *C. maxima* Duchesne, *C. moschata* Duchesne and *C. pepo* juice. P65, Pasteurization at 65 °C for 10 min. US100, Sonication at 100 W, 20 °C for 10 min. US200, Sonication at 200 W, 20 °C for 10 min. US300, Sonication at 300 W, 20 °C for 10 min. US400, Sonication at 400 W, 20 °C for 10 min. Figure B: Effect of pasteurization and different time (0–20 min) of ultrasound on the microbial colonies of *C. maxima*, *C. moschata* and *C. pepo* juice. P65, Pasteurization at 65 °C for 10 min. US5, Sonication at 200 W, 20 °C for 5 min. US10, Sonication at 200 W, 20 °C for 10 min. US15, Sonication at 200 W, 20 °C for 15 min. US20, Sonication at 200 W, 20 °C for 20 min. Figure C: Effect of pasteurization and different temperature (0–30 °C) of ultrasound on the microbial colonies of *C. maxima*, *C. moschata* and *C. pepo* juice. P65, Pasteurization at 65 °C for 10 min. US10, Sonication at 200 W, 10 °C for 10 min. US20, Sonication at 200 W, 20 °C for 10 min. US30, Sonication at 200 W, 30 °C for 10 min.
Data with different lowercase letters indicate significant differences ($P < 0.05$).

juice was poured into a colourless, clear 100 mL glass beaker prior to the sensory test. Pumpkin juice samples were covered with a glass cover, marked with a unique three-digit random code, and supplied in a random order. The products were rated from very unattractive (1) to very attractive (10). The following qualities were evaluated: overall acceptability, color, aroma, taste, acidity, and turbidity. Based on the scores obtained by the panelists, each quality was expressed as the mean ± standard deviation (SD).

### 2.2.13. Statistical analysis

The results of the microbiological analysis and chemical properties were expressed as the average of five and three measurements for each sample, respectively. Results are presented as mean ± standard deviation. Multigroup analyses were performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$. Origin 2021 software (OriginLab, Northampton, MA, USA) was used for the drawing.

## 3. Results and discussion

### 3.1. Effect of ultrasound on the sterilization of three types of pumpkin juice

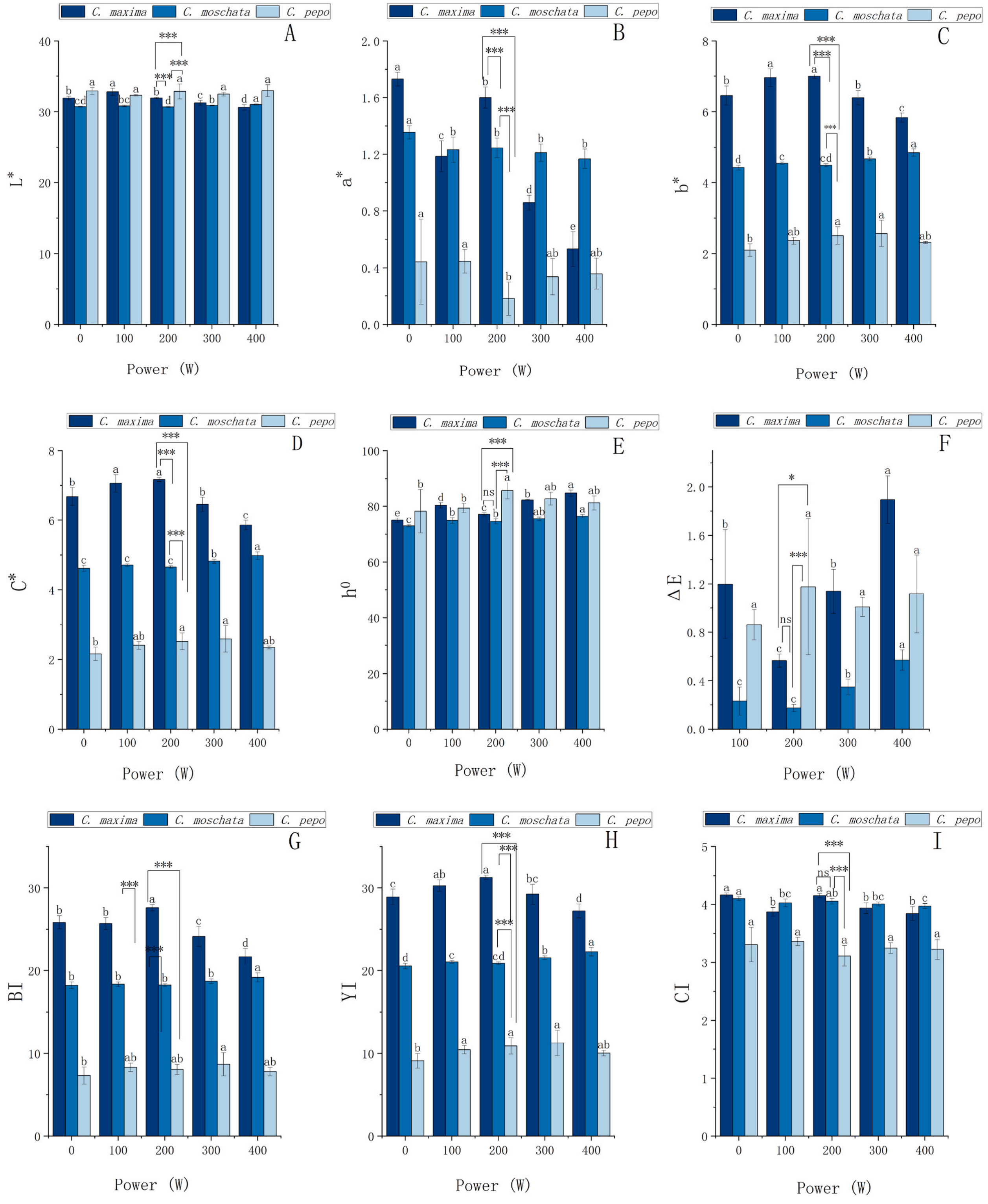
The sterilization effects of pasteurization and different ultrasound treatments on the three types of pumpkin juice (*C. maxima, C. moschata*, and *C. pepo*) are shown in Fig. 1 (A-C). The number of colonies contained in different samples varied under the initial conditions. The number of colonies after treatment under the same treatment conditions also varied considerably, and the degree of treatment determined the value of the reduction in the number of colonies. In this experiment, we found that the total number of colonies in the samples decreased significantly with a gradual increase in power, time, and temperature under ultrasonic treatment. In Fig. 1(A), untreated *C. pepo* pumpkin juice contained the highest total number of microorganisms (8.27 log CFU/mL), followed by *C. moschata* (5.90 log CFU/mL) and *C. maxima* (3.71 log CFU/mL). After US100 treatment, the microbial colony counts in *C. moschata*, *C. maxima*, and *C. pepo* pumpkin juices were reduced to 2.42, 1.68, and 1.45 log CFU/mL, respectively, with a significantly lower number of microbial colonies in the samples as the power was increased ($P < 0.05$). When the power was increased to 400 W, the microbial counts in each sample were reduced by 98.83%, 81.71%, and 89.76%, respectively. Fig. 1(B) shows the effect of ultrasonic treatment time on microorganisms. The number of microbial colonies was significantly reduced after ultrasonic treatment (5, 10, 15, and 20 min) compared with that in the control group. At a treatment time of 20 min, the sterilization rates of the samples were 99.82%, 96.27%, and 88.99%, respectively. We observed that the number of colonies in *C. maxima* pumpkin juice with a treatment time of more than 15 min tended to be close to zero, and the sterilization rate was above 99% in all cases. This indicates that sonication time was the most beneficial factor affecting the sterilization of *C. maxima* pumpkin juice. For *C. moschata* and *C. pepo* pumpkin juices, there was no significant change ($P > 0.05$) in the sterilization effect after more than 15 min of treatment, and the ultrasonic power could be appropriately increased to improve the sterilization rate. Fig. 1(C) depicts the effect of sonication temperature on the sterilization of the three pumpkin juices. The total number of colonies in the samples was reduced after the temperature treatment. At a temperature of 10 °C, the colony counts of *C. maxima, C. moschata*, and *C. pepo* pumpkin juice were 1.88, 1.42, and 1.40 log CFU/mL, respectively. When the temperature was raised to 30 °C, the colony counts decreased to 0.04, 0.72, and 0.86 log CFU/mL, and the sterilization rates reached 98.92%, 87.73%, and 89.60%, respectively. This finding is consistent with the results reported by Keasvan et al. [28] for treated kutkura (*Meyna spinosa*) juice.

In addition, the pasteurization of *C. maxima, C. moschata*, and *C. pepo* pumpkin juice were found to be 87.87%, 80.10%, and 89.60%, respectively. Conventional pasteurization significantly reduced the number of microbial colonies in pumpkin juice compared to the control group. But still not as effective as ultrasound for sterilization. For *C. maxima* pumpkin juice, ultrasonic treatment at 300 W, 15 min, 30 °C sterilization effect is the best. *C. moschata* pumpkin juice is best treated at 400 W, 15 min, 20 °C. For *C. pepo* pumpkin juice, the sterilization rate can reach 89.84% at 400 W, 15 min, 30 °C. According to National Standard GB 7101-2022, the total number of colonies in fruit and vegetable juices should be less than 5 log CFU/mL, which indicates that the pumpkin juice processed by ultrasonication reached the standard of safe for consumption, and ultrasonication can effectively improve the microbial sterilization rate compared with the conventional heat treatment.

Tahi et al. [29] studied the survival of *Staphylococcus aureus* cell viability in orange juice by sonication and showed that sonication at 20, 30, and 40 °C resulted in 52.3 ± 80.0, 49.4 ± 30.0, and 74.90 ± 60.60 logarithmic cycles of reduction in colony counts after 90, 60, and 60 min of treatment, respectively. In the present study, the reduction in the number of microorganisms in pumpkin juice may be related to the fragmentation of microbial cell membranes as a result of the ultrasonic treatment. Ultrasound causes cavity collapse through the cavitation effect, resulting in high temperature and pressure, both of which act synergistically to disintegrate the cell wall and membrane structure for sterilization. In conclusion, the total number of colonies in the ultrasonically treated pumpkin juice complied with the food safety standards. Therefore, ultrasound can be used as a clean and effective non-thermal processing technique to replace the traditional pasteurization method.

### 3.2. Effect of ultrasound on the color of three types of pumpkin juice

Color is the primary reference standard for consumers when selecting and buying fruits and vegetables and is also an important benchmark for evaluating the quality of fruits and vegetables [30]. During the storage of fruits and vegetables, various natural pigments such as anthocyanins, chlorophylls, and beetroot red pigments gradually degrade or isomerize over time, resulting in browning during storage and affecting the color of the fruits and vegetables [31]. Wang et al. [11] used ultrasound to treat mango juice and found



*(caption on next page)*

**Fig. 2.** Effect of different ultrasonic powers on the colour parameters of three pumpkin juices (A–I) Figure A–I: Effect of different power (0–400 W, 10 min, 20 °C) treatments of ultrasound on *C. maxima*, *C. moschata* and *C. pepo* juice L* (A), a* (B), b* (C), C* (D), h0 (E), ΔE (F), BI (G), YI (H), CI (I). Data with different lowercase letters indicate significant differences in juice colour parameters of the same pumpkin species at different power levels ($P < 0.05$). *** indicates significant differences in juice colour parameters of different pumpkin species at the same power level (200 W) ($P < 0.001$), ** indicates significant differences in juice colour parameters of different pumpkin species at the same power level (200 W) ($0.001 < P < 0.01$), * indicates significant differences in juice colour parameters of different pumpkin species at the same power level (200 W) ($0.01 < P < 0.05$), ns indicates that the difference in juice colour parameters between pumpkin species at the same power (200 W) was not significant ($P > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that as the power increased, the color of the juice deepened (L* increased from 36.40 to 35.16), the color became greener (a* increased from −2.04 to −2.13), and the yellowness decreased (b* increased from 13.92 to 12.63). The absolute values of C* and YI increased with increasing ultrasound duration. In this study, gradient powers of 100, 200, 300, and 400 W were used to treat different varieties of pumpkin juice for 10 min to study the changes in the color parameters of different pumpkin juices after ultrasound. Changes in the apparent color of the three pumpkin juices treated with ultrasound are shown in Fig. 2 (A-I). In *C. maxima* pumpkin juice, compared with untreated fresh pumpkin juice, the difference in L* values was not significant ($P > 0.05$) with increasing treatment power, a* values gradually decreased, and after 400 W ultrasound treatment, a* values decreased from the original 1.73 to 0.532, with a significant decrease in redness; b*, C*, browning index (BI), and yellowing index (YI) were not significantly different after low-power treatment, while these values decreased slightly after high-power treatment. In contrast, $h^0$ increased slightly from 74.98° to 84.80°, whereas ΔE tended to decrease and then increase, with the lowest value at 200 W (0.566) and the highest value at 400 W (1.9). As previously reported, ΔE between 0 and 1 is indistinguishable to the naked eye, and in the interval 1–2 is slightly detectable to the naked eye but still meets the requirements. The differences in L*, b*, C*, $h^0$, ΔE, BI, YI, and CI values before and after ultrasound treatment were not significant ($P > 0.05$) in *C. maxima* and *C. pepo* juices. The larger flesh particles in the untreated pumpkin juice were partially precipitated, increasing the a* and b* values. With an increase in ultrasonic power, the unstable suspended particles were broken into fragmented particles under the cavitation effect of ultrasonic waves and dispersed into the solution, resulting in lower a* and b*. This is consistent with the observations of Wang et al. [19] who treated strawberry juice with ultrasound.

Of the three pumpkin juices, *C. pepo* juice had the highest L* value (32.93), followed by *C. maxima* (31.92) and *C. moschata* (30.74). *C. maxima* had the highest redness and yellowness among the three varieties, more than three times that of *C. pepo*. For *C. maxima* and *C. moschata* juice, the highest color retention and best results were obtained with sonication at 200 W (10 min, 20 °C). For *C. pepo* juice, the lowest color change was obtained at a sonication intensity of 100 W; all color parameters were closer to those of fresh pumpkin juice, and the color sensation was superior. Under optimal treatment conditions (200 W, 10 min, 20 °C), significant differences were found between *C. maxima, C. moschata*, and *C. pepo* juice, for all parameters except for $h^0$, ΔE, and CI. After 100 W treatment, for a* and CI, the differences between *C. maxima,* and *C. moschata* juice were not significant, and for $h^0$, the differences between *C. maxima* and *C. pepo* juice were not significant. In addition, the variability between the pumpkin juices of each variety at different power levels was significant. During the production process, different treatment intensities can be selected according to the characteristics of the varieties to achieve the best quality. Ornelas-Paz et al. [32] reported elevated a* results and small Hue angle values in mango, which were corresponded with a high β-carotene results in mango flesh. However, in the present study, because of the slight changes in the color parameters, it was not possible to investigate this behavior. In this study, the ΔE values of the three varieties of pumpkin juice decreased slightly after ultrasound treatment and then increased, and the BI and YI also decreased slightly compared with the untreated condition. The above results showed that ultrasonic treatment at a certain power can better preserve the color of the juice, reduce the degradation of natural pigments in pumpkin juice, delay aging, and maintain the fruit and vegetable juice in a better color during storage.

**Table 1**
Effect of different ultrasonic power on the sugar content and organic acid content of three types of pumpkin juice.

| Samples | Treatment conditions | Sucrose (g/100 g FW) | Glucose (g/100 g FW) | Fructose (g/100 g FW) | TSS (°Brix) | pH | Titratable acidity (g/100g) |
|---|---|---|---|---|---|---|---|
| *C.maxima* | US0 | $4.04 \pm 0.02^{a}$ | $1.55 \pm 0.01^{a}$ | $2.44 \pm 0.01^{a}$ | $7.6 \pm 0.24^{a}$ | $6.83 \pm 0.01^{a}$ | $0.24 \pm 0.01^{a}$ |
| | US100 | $4.02 \pm 0.02^{a}$ | $1.56 \pm 0.02^{ab}$ | $2.45 \pm 0.01^{a}$ | $7.2 \pm 0.10^{a}$ | $6.83 \pm 0.01^{a}$ | $0.22 \pm 0.02^{a}$ |
| | US200 | $4.05 \pm 0.02^{a}$ | $1.59 \pm 0.02^{ab}$ | $2.43 \pm 0.02^{a}$ | $7.4 \pm 0.19^{a}$ | $6.85 \pm 0.01^{a}$ | $0.23 \pm 0.02^{a}$ |
| | US300 | $4.03 \pm 0.01^{a}$ | $1.56 \pm 0.03^{ab}$ | $2.41 \pm 0.01^{a}$ | $7.7 \pm 0.03^{a}$ | $6.84 \pm 0.02^{a}$ | $0.19 \pm 0.02^{a}$ |
| | US400 | $4.06 \pm 0.01^{a}$ | $1.50 \pm 0.03^{b}$ | $2.40 \pm 0.02^{a}$ | $7.5 \pm 0.21^{a}$ | $6.83 \pm 0.01^{a}$ | $0.23 \pm 0.03^{a}$ |
| *C. moschata* | US0 | $2.14 \pm 0.02^{a}$ | $1.08 \pm 0.02^{a}$ | $1.72 \pm 0.02^{a}$ | $4.5 \pm 0.07^{ab}$ | $6.06 \pm 0.02^{a}$ | $0.27 \pm 0.01^{a}$ |
| | US100 | $2.10 \pm 0.02^{a}$ | $1.06 \pm 0.02^{a}$ | $1.74 \pm 0.03^{a}$ | $4.3 \pm 0.10^{b}$ | $6.03 \pm 0.01^{b}$ | $0.24 \pm 0.02^{a}$ |
| | US200 | $2.13 \pm 0.02^{a}$ | $1.05 \pm 0.02^{a}$ | $1.76 \pm 0.02^{a}$ | $4.7 \pm 0.09^{a}$ | $6.04 \pm 0.01^{a}$ | $0.24 \pm 0.01^{a}$ |
| | US300 | $2.11 \pm 0.02^{a}$ | $1.06 \pm 0.04^{a}$ | $1.74 \pm 0.03^{a}$ | $4.8 \pm 0.06^{a}$ | $6.03 \pm 0.00^{a}$ | $0.26 \pm 0.02^{a}$ |
| | US400 | $2.12 \pm 0.03^{a}$ | $1.04 \pm 0.03^{a}$ | $1.73 \pm 0.02^{a}$ | $4.7 \pm 0.17^{a}$ | $6.05 \pm 0.02^{a}$ | $0.23 \pm 0.01^{a}$ |
| *C. pepo* | US0 | $1.00 \pm 0.03^{a}$ | $0.57 \pm 0.01$a | $0.62 \pm 0.01^{ab}$ | $2.5 \pm 0.12^{a}$ | $5.92 \pm 0.02^{a}$ | $0.33 \pm 0.02^{a}$ |
| | US100 | $0.99 \pm 0.01^{a}$ | $0.57 \pm 0.03^{a}$ | $0.59 \pm 0.02^{b}$ | $2.4 \pm 0.20^{a}$ | $5.95 \pm 0.01^{a}$ | $0.32 \pm 0.03^{a}$ |
| | US200 | $1.01 \pm 0.06^{a}$ | $0.58 \pm 0.03^{a}$ | $0.63 \pm 0.01^{a}$ | $2.5 \pm 0.09^{a}$ | $5.96 \pm 0.01^{a}$ | $0.33 \pm 0.01^{a}$ |
| | US300 | $1.01 \pm 0.05^{a}$ | $0.57 \pm 0.03$a | $0.65 \pm 0.00^{a}$ | $2.5 \pm 0.15^{a}$ | $5.92 \pm 0.01^{a}$ | $0.33 \pm 0.03^{a}$ |
| | US400 | $1.02 \pm 0.04^{a}$ | $0.55 \pm 0.01^{b}$ | $0.63 \pm 0.01^{a}$ | $2.5 \pm 0.13^{a}$ | $5.94 \pm 0.01^{a}$ | $0.34 \pm 0.01^{a}$ |

All data are mean ± SD, n = 3. a, b, c: different letters in the same column indicate significant differences.

### 3.3. *Effect of ultrasound on the sugar content and organic acid content of three types of pumpkin juice*

There were no significant differences ($P > 0.05$) in the physicochemical properties (TSS, pH, and TA) of the three pumpkin juices (*C. maxima*, *C. moschata*, and *C. pepo*) treated at different ultrasonic powers (Table 1). Among the three pumpkin juices, *C. maxima* juice had the highest glucose content (4.04 g/100 g FW), *C. moschata* juice the second highest (2.14 g/100 g FW), and *C. pepo* juice the lowest (1.00 g/100 g FW), with glucose having the highest content of the three sugars in all three juices, followed by sucrose and fructose. The TSS content was also highest in *C. maxima* juice and lowest in *C. pepo* juice. Similarly, there was no significant difference in the TSS content when turbid apple juice was treated with ultrasound compared with the untreated group [33]. Changes in sugar content are also related to fermentation; for example, Sun et al. [34] showed that during the fermentation of pumpkin juice using lactic acid bacteria, the sucrose content was significantly reduced at the end of fermentation. Fructose, the main sugar component used by lactic acid bacteria in mulberry juice, was reduced by 13.3 mg/mL by the end of fermentation [27].

*C. maxima* juice had a neutral pH, whereas *C. moschata* and *C. pepo* juices were more acidic and had higher levels of titratable acids than *C. maxima* juice. *C. maxima* juice had the lowest TA value (0.24 g/100 g), *C. moschata* juice had a slightly higher value (0.27 g/100 g), and *C. pepo* juice had the highest acid content (0.33 g/100 g). The sugar and acid contents of the three pumpkin juices treated with ultrasound did not change significantly compared with those of the control group, indicating that the physicochemical properties of the pumpkin juices were stable and the taste remained better after ultrasound treatment. These results are consistent with those reported previously by Yikmiş et al. [35] for functional sirkencubin syrup, and Ma et al. [36] for prune juice during different storage periods. It has also been found that when red wines are treated with high-power ultrasound, the pH decreases with increasing treatment time [37]. In conclusion, there was no significant difference in the sugar-acid content of the three pumpkin juices after ultrasonic treatment in this study. This indicated that ultrasonic treatment was beneficial to maintain the physicochemical properties of pumpkin juice, which could better retain the inherent organoleptic qualities of pumpkin juice and reduce the risk of losing thermally unstable key flavour substances.

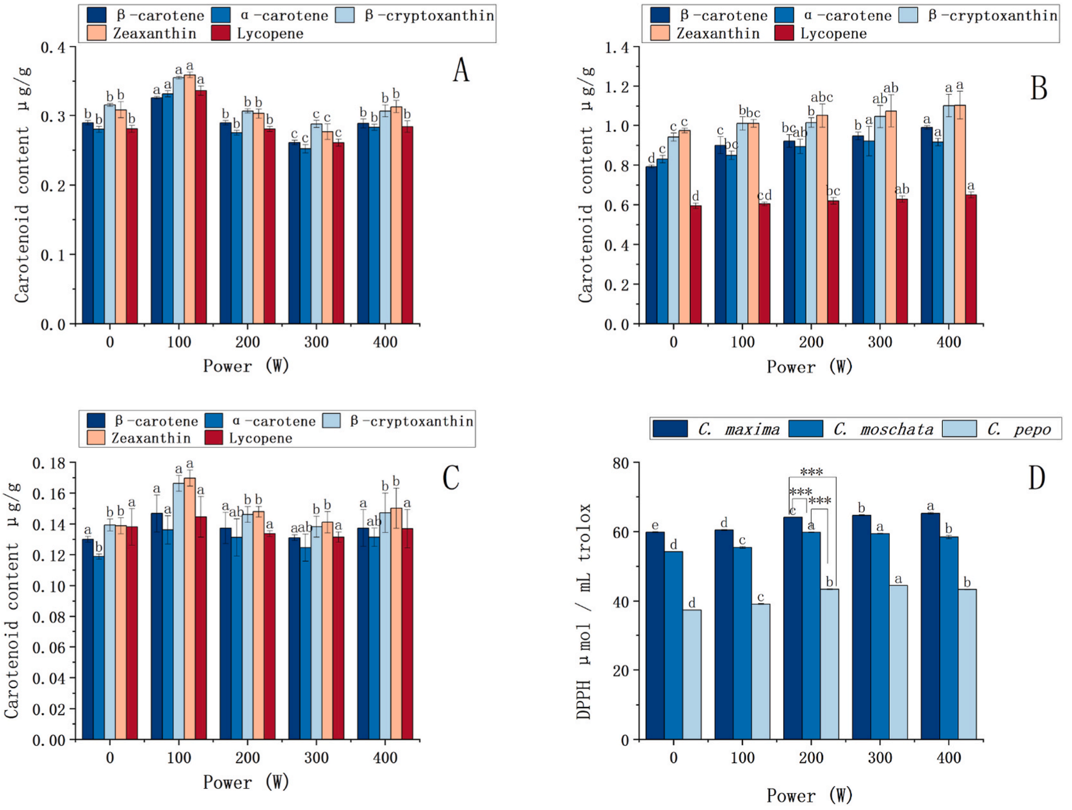


**Fig. 3.** Effect of different ultrasound powers on carotenoids and the antioxidant properties in three types of pumpkin juice (A–D) Figure A: Effect of ultrasonic treatment at different power levels (0–400 W, 10 min, 20 °C) on carotenoids in *C.maxima* juice. Figure B: Effect of ultrasonic treatment at different power levels (0–400 W, 10 min, 20 °C) on carotenoids in *C. moschata* juice. Figure C: Effect of ultrasonic treatment at different power levels (0–400 W, 10 min, 20 °C) on carotenoids in *C. pepo* juice. Figure D: effect of different power (0–400 W, 10 min, 20 °C) of ultrasound on the antioxidant properties of *C. maxima*,*C. moschata* and *C. pepo* juice. Data with different lowercase letters indicate significant differences ($P < 0.05$). *** indicates significant differences in antioxidant properties of the juice of different pumpkin species at the same power level (200 W) ($P < 0.001$).

### 3.4. Effect of ultrasound on carotenoids in three types of pumpkin juice

The changes in the carotenoid content of the ultrasound-treated samples of different pumpkin juice varieties are shown in Fig. 3(A-C). Fig. 3(A) represents the contents of various types of carotenoids in *C. moschata* juice. Fig. 3(B) shows these values for *C.maxima* juice and Fig. 3(C) for *C. pepo* juice. In the present study, only *C. moschata* juice showed an increase in carotenoid content with power, while the *C.maxima* and *C. pepo* juices showed an increase and then a decrease in carotenoid content with ultrasound power. β-carotene and α-carotene are precursors of vitamin A and are important for normal growth and development in humans. β-cryptoxanthin has strong antioxidant properties and protects against oxidative DNA damage. β-cryptoxanthin may protect against non-alcoholic fatty liver disease (NAFLD) by suppressing liver inflammation and subsequent fibrosis by inhibiting the differentiation of macrophages towards a pro-inflammatory phenotype [38]. Zeaxanthin is a lutein-like carotenoid that cannot be synthesized by humans and must be obtained from the diet; its main sources include pumpkin, maize, and egg yolk. Lutein, zeaxanthin, and meso-zeaxanthin are macular pigments that protect the retina and act as antioxidants and blue light filters to protect vision [39]. Lycopene is a deep red carotenoid that has been shown to reduce the risk of prostate cancer, other cancers and cardiovascular disease [40]. Because of their antioxidant activity, carotenoid consumption may reduce the risk of diseases, such as atherosclerosis, cancer, and macular degeneration.

Factors such as the stage of maturity and the growing environment of different varieties could affect the carotenoid composition of pumpkins. Studies have shown that β-carotene and α-carotene are the main carotenoids in *C. moschata, C. maxima* is dominated by β-carotene, lutein, and violet xanthin, while *C. pepo* is dominated by lutein and β-carotene as the main carotenoids [41]. As shown in Fig. 3(A), the carotenoid content of *C. maxima* juice was significantly increased by 12.68%, 18.5%, 12.48%, 16.36%, and 19.93% under different low power ultrasound treatments. This is consistent with the results of the ultrasonic treatment of grapefruit juice [42]. However, increasing the ultrasound power further to 400 W resulted in a slight decrease in carotenoid content compared with that in the control group, with no significant change ($P > 0.05$). In the ultrasonic treatment of *C. moschata* juice, the carotenoid content increased in all treatment groups compared with that in the untreated control group. Under US100-400 treatment, β-carotene content was significantly increased by 13.56–24.79%, α-carotene by 2.32–10.33%, β-cryptocanthin by 7.25%–16.97%, zeaxanthin by 3.67–13.32%, and lycopene by 1.51–9.11%. Carotenoid content in *C. pepo* juice increased and then decreased compared with that in the untreated group, with all types of carotenoids being higher after treatment than in the treated group, except for lycopene, which was lower in the US300 treatment group than in the untreated group. Therefore, in order to obtain a higher carotenoid content, pumpkin juice can be treated with 200 W of ultrasonic power for 10 min at room temperature (20 °C). It has been reported that carotenoids are more stable in the presence of other antioxidants. In our study, however, the increase in carotenoid content after ultrasound treatment may be due to the mechanical breakdown of the cell wall and cell membrane structure by ultrasound, allowing the contents of the cells to flow out. This indicated that ultrasonic treatment was beneficial for enhancing and maintaining carotenoids in pumpkin juice samples. In summary, carotenoids presented better bioaccessibility for treatments using higher ultrasound energy power. Consequently, we can conclude that the ultrasound processing can improve the bioaccessibility of pumpkin juices since the compounds retained in plant cells can be released after processing. Furthermore, the consumption of food products presenting higher levels of bioactive compounds can bring several benefits to consumer.

### 3.5. Effect of ultrasound on the antioxidant properties of three types of pumpkin juice

The effect of ultrasonic treatment (US100, US200, US300, and US400) on the total antioxidant capacity (expressed as the antioxidant capacity in DPPH) of *C. maxima*, *C. moschata*, and *C. pepo* juices is shown in Fig. 3(D). The antioxidant properties of all three pumpkin juices differed significantly after ultrasound treatment ($P < 0.05$). The antioxidant properties of the pumpkin juice increased with increasing ultrasound power. The antioxidant activity of *C. maxima* juice increased from 59.85 μmol/mL to 60.47 μmol/mL after US100 treatment, 64.17 μmol/mL (US200), 68.75 μmol/mL (US300) and 65.29 μmol/mL (US400), with a maximum growth rate of 9.09%. The antioxidant properties of *C. moschata* juice increased by 2.21%, 10.25%, 9.55%, and 7.75%, respectively, after different power treatments compared with the untreated group, with the free radical scavenging rate reaching a maximum at US200 and then gradually decreasing but remaining higher than the untreated pumpkin juice. The resistance of *C. pepo* juice also showed an increasing trend, increasing to 43.35 μmol/mL after a maximum power of 400 W and 10 min of treatment, an increase of 15.87%, and the maximum resistance of *C. pepo* juice (44.47 μmol/mL) under US300 treatment, an increase of 18.84%. Of the three pumpkin juices, *C. maxima* juice had the greatest antioxidant activity and *C. pepo* juice had the highest growth rate at the same treatment gradient. The growth rate of pumpkin juice slowed under high-power ultrasound treatment, and the antioxidant properties of *C. moschata* and *C. pepo* juice first increased and then decreased with increasing power, but remained higher than those in the untreated samples. *C. maxima*, *C. moschata*, and *C. pepo* juices showed peak antioxidant activity at US400, US200, and US300, respectively. The three pumpkin juices differed significantly under the same power treatment, with the US200 treatment group showing a highly significant difference between *C. maxima*, *C. moschata*, and *C. pepo* juices ($P < 0.01$). This result is similar to that reported by Cheng et al. [43], who ultrasonicated strawberry juice for 20 min. Similarly, Wang et al. [44] also treated cherry tomatoes with 66.64 or 106.19 W/L ultrasound to effectively reduce the presence of spoilage microorganisms, delay fruit ripening, and maintain total antioxidant capacity.

Yildiz et al. [25] found that the antioxidant properties of peach juice improved when treated with different methods, however, the US-treated group showed the highest antioxidant values compared with high-pressure homogenization (HPH) and high temperature-short time (HTST). This finding aligns with our previous observations, which demonstrated that the occurrence of cavitation during sonication improves the extraction and utilization of phenolic compounds, resulting in heightened antioxidant properties of pumpkin juice. In addition, appropriate temperature increases could help the extraction of phenolic compounds by increasing the solubility and diffusion rate of solutes, which may lead to denaturation of phenolic compounds if the temperature is too high (>60 °C) [28]. Simultaneously, some oxidation-related enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD), which are associated with enzymatic browning, are inactivated by cavitation and thus contribute to the antioxidant properties [45]. In conclusion, ultrasonic detection of antioxidant activity in pumpkin juice showed that with the increase in ultrasonic power, the antioxidant components increased significantly. A significant increase ($P < 0.05$) of DPPH free radical scavenging activity of pumpkin juices was observed, which may be related to the considerable release of phenolic compounds into the juice serum after ultrasound processing. The results indicated that ultrasonic pretreatment can effectively improve the antioxidant capacity of pumpkin juice and enhance the nutritional quality of pumpkin juice.

### 3.6. *Effect of ultrasound on the enzyme activity of three types of pumpkin juice*

In addition to bioactive compounds, a range of endogenous enzymes play a vital role in the quality of pumpkin juice. Examples include PPO and POD for enzymatic browning, pectin methylesterase (PME) and polygalacturonase (PG) for fruit stability and viscosity, and lipoxygenase (LOX) and ascorbate peroxidase (APx) for food flavor. The effects of ultrasound treatment of *C. maxima*, *C. moschata* and *C. pepo* juice on enzyme activity are shown in Fig. 4. As the ultrasonic power increased, endogenous enzyme activity decreased. Fig. 4(A) shows the effect of ultrasonic power on PPO activity in the three pumpkin juices, while Fig. 4(B) shows its effect on POD activity. As shown in Fig. 4(A), the PPO residual enzyme activity was significantly reduced ($P < 0.05$) in the ultrasound-treated (US100, US200, US300, and US400) samples compared with that in the untreated (US0) pumpkin juice samples. The residual enzyme activities of *C. maxima* juice after treatment were 1.37, 1.09, 0.94, and 0.82 u/mL, respectively. *C. moschata* juice showed decreases in enzyme activity of 9.02%, 24.13%, 29.95%, and 39.83% after sonication. The enzyme activity of *C. pepo* juice also showed the same downward trend, decreasing by 16.39% after US100 treatment, 38.80% after US200 treatment, 48.09% at US300, and 51.92% at US400. The rate of decline in pumpkin juice slowed down after US300 treatment, indicating that the inhibitory effect of ultrasound on the PPO activity of pumpkin juice has some limitations and that the treatment temperature could be appropriately increased at a later stage to further inhibit enzyme activity. As shown in Fig. 4(B), the POD activity of all three pumpkin juices treated with ultrasound decreased significantly. The activity of *C. maxima* juice decreased by 13.99%, 40.97%, 58.78%, and 59.29%, respectively after treatment. The activity of *C. moschata* juice decreased by 0.76, 0.59, 0.40, and 0.35 u/mL and the POD activity of *C. pepo* juice decreased by 25.58%, 45.35%, 53.48%, and 47.09% respectively, after 10 min of US treatment. The enzymatic activity of the three pumpkin juices more than doubled after high-power ultrasound treatment, but none were significantly inactivated.

Significant differences were observed among the three pumpkin juices under ultrasonic treatment, with all three showing highly significant p-values of less than 0.01, according to the experimental data. Of the three pumpkin juices, *C. maxima* juice had the highest PPO and POD activities, followed by *C. moschata*, and *C. pepo* juice had the lowest activity. *C. maxima* juice showed the greatest reduction in PPO activity. The rate of reduction was fastest in *C. pepo* juice, and the rate of reduction in enzyme activity of the three pumpkin juices gradually slowed with increasing ultrasonic power. The enzyme activity of POD increased slightly in *C. pepo* juice under US400 treatment compared with US300, but was still lower than that of the untreated group. The reduction in enzyme activity may be due to the disruption of the enzyme structure by the high temperature and pressure generated by the sonication process, resulting in hydrogen bonding and van der Waals force interactions in the polypeptide chains, resulting in the inactivation or inhibition of the enzyme. The faster rate of POD inactivation compared to PPO inactivation indicates a higher resistance to ultrasound than that of PPO. This contrasts with the results reported by Kar and Sutar et al. [46]. In Chen et al. [24] who treated carrot juice with ultrasound, PPO activity decreased while POD activity increased, and PME activity showed an increase at 0.95 W/mL and a decrease at 2.38 W/mL and 3.80 W/mL. PME is an endogenous pectinase present in the cell wall, and the increased activity was probably because of the cavitation effect leading to cell breakage, causing the release of cell wall material. Inhibition of PPO and POD activities facilitates the

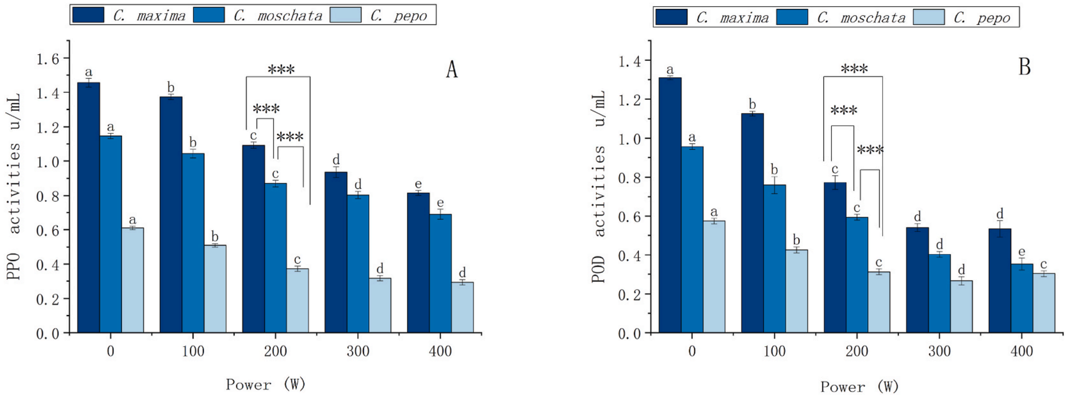


**Fig. 4.** Effect of different sonication powers on the enzymatic activity of three pumpkin juices (Fig. A, B)Figure A: Effect of different power (0–400 W, 10 min, 20 °C) of ultrasonic waves on PPO of *C. maxima*,*C. moschata* and *C. pepo* juice. Figure B: Effect of different power (0–400 W, 10 min, 20 °C) of ultrasonic waves on POD of *C. maxima*,*C. moschata* and *C. pepo* juice. Data with different lowercase letters indicate significant differences in enzyme activities of the same pumpkin juice at different power ($P < 0.05$). *** indicates significant differences in enzyme activities of pumpkin juice of different pumpkin species at the same power (200 W) ($P < 0.001$).

maintenance of color in pumpkin juice and prevents the impact of browning on juice quality.

It was found that ultrasonic treatment alone did not completely inhibit enzymatic activity, and that the ultrasonic treatment conditions could be further optimized or combined with other techniques at a later stage. In a study by Szczepańska et al. [47], UHP treatment had an inhibitory effect on PPO and POD in carrot juice, and the inhibition of PPO was stronger than that of POD. Liang et al. [48] showed a logarithmic decrease in PPO activity after combined ultrasonic and UV treatment of fruit and vegetable juices. It has also been shown that ultrasound treatment does not significantly alter enzyme activity, possibly due to changes in protein structure caused by ultrasound to promote substrate-enzyme binding [30]. Based on the results of the current study, it was found that mild sonication does not inactivate the PPO and POD enzymes, and that even sonication in combination with other techniques only results in some inhibition of their activity. If the enzyme is not completely inactivated, there will be some effects on the quality of pumpkin juice, including the production of an undesirable colour, off-flavor, and off-odor. Therefore, how to deal with the enzyme that can be inactivated in pumpkin juice needs to be further explored.

### *3.7. Effect of ultrasound on the particle size distribution of three types of pumpkin juice*

The effect of ultrasonic treatment (0–400 W, 10 min) on the particle size distribution (PSD) of different pumpkin juices is shown in Table 2. When the ultrasonic power was gradually increased from 0 to 400 W, the particle sizes of the three varieties of pumpkin juice samples decreased significantly, similarly to the results observed by Wang et al. [18] for kiwi (400 W, 20 kHz, 0–16 min), Yildiz [25] for peach juice (20 kHz, 4 °C, storage 0–28 days), and Suo et al. [21] for pumpkin juice (0–600 W for 10 min). The particle size of *C. maxima* juice samples decreased from 769.7 for US0 to 527.8 for US100, and gradually decreased with increasing power to 504.8 for US200, 467.1 US300 and 461.6, US400. The *C. moschata* juice sample also showed a gradual reduction in particle size from an initial 1249.5 to 457.5 for US200 and 405.2 for US400. The particle size of the *C. pepo* juice was reduced by 29.32%, 37.57%, 40.87%, and 44.16%, respectively, compared with the untreated pumpkin juice samples. The particle size varied more in the 0–200 W treatment interval than in the 300–400 W interval. In addition, the polydispersity of pumpkin juice samples was reduced to varying degrees. The polydispersity of *C. maxima* juice decreased from 0.361 for US0 to 0.298 for US400, whereas that of *C. pepo* juice changed differently, increasing by 10.47% and 1.16% under the US100 and US200 treatments, while decreasing by 2.33% under US300 and 3.10%. This may be related to the sink agglomeration of pumpkin juice particles [21]. The effect of ultrasound on juice particle size was consistent with the results of the HPH treatment of juice particle size, and both treatments were effective in reducing the size of suspended particles in juice. This result is in contrast with that of Yildiz et al. [25] who found that the particle size of peach juice significantly increased in size and polydispersity after both thermal (HTST) and nonthermal (US and HPH) treatments.

*C. maxima* juice exhibited the smallest particle size (769.7 nm) of all species. After ultrasound treatment, *C. moschata* juice showed the highest particle size reduction, with the greatest reduction and the most significant effect at 200 W. The reduction in effective particle size was due to the shear forces generated by ultrasonic cavitation, which caused large suspended particles to break up into smaller fragments. A significant reduction in the effective diameter of the cellular debris can be clearly observed in the microstructure image, and it has been found that a significant reduction in particle size correlates with the disruption of the microstructure, owing to the cavitation effect during sonication [49]. This also provides some evidence of increased bioactives in ultrasonically treated pumpkin juice. At the same time the reduction of particles during the sonication process improved the homogeneity and stability of the juice.

### *3.8. Effect of ultrasound on the microstructure of three types of pumpkin juice*

An optical microscope (LX-71, Olympus) was used to observe the ultrasonic treatment (0, 100, 200, 300, and 400 W, 10 min) of the three pumpkin juices (Fig. 5). This study shows that ultrasonic treatment leads to significant changes in the microstructure of pumpkin juice, and this change increases in destructive strength with increasing ultrasonic power. The intracellular fractions of pumpkin juice

**Table 2**

Effect of different ultrasonic power on the particle size of three types of pumpkin juice.

| Samples | Treatment conditions | particle size | polydispersity |
|---|---|---|---|
| *C. maxima* | US0 | $769.7 \pm 13.4^{a}$ | $0.361 \pm 0.008^{a}$ |
| | US100 | $527.8 \pm 14.9^{b}$ | $0.315 \pm 0.003^{b}$ |
| | US200 | $504.8 \pm 0.6^{b}$ | $0.304 \pm 0.007^{c}$ |
| | US300 | $467.1 \pm 8.3^{c}$ | $0.275 \pm 0.007^{d}$ |
| | US400 | $461.6 \pm 3.4^{c}$ | $0.298 \pm 0.003^{cd}$ |
| *C. moschata* | US0 | $1249.5 \pm 78^{a}$ | $0.344 \pm 0.019^{c}$ |
| | US100 | $480.5 \pm 11.2^{b}$ | $0.351 \pm 0.005^{a}$ |
| | US200 | $457.5 \pm 9.9^{b}$ | $0.342 \pm 0.012^{a}$ |
| | US300 | $447.9 \pm 9.8^{b}$ | $0.315 \pm 0.003^{b}$ |
| | US400 | $405.2 \pm 3.2^{b}$ | $0.311 \pm 0.004^{b}$ |
| *C. pepo* | US0 | $818.9 \pm 33.2^{a}$ | $0.258 \pm 0.031^{a}$ |
| | US100 | $578.8 \pm 12.1^{b}$ | $0.285 \pm 0.015^{a}$ |
| | US200 | $511.2 \pm 5.6^{c}$ | $0.261 \pm 0.016^{a}$ |
| | US300 | $484.2 \pm 8.1^{c}$ | $0.252 \pm 0.002^{a}$ |
| | US400 | $457.3 \pm 6.1^{c}$ | $0.250 \pm 0.008^{a}$ |

All data are mean ± SD, n = 3. a, b, c: different letters in the same column indicate significant differences.

from all three varieties were clearly visible in the untreated cells, and the cell wall microstructure was intact. The cell structure of the US100 sample was more complete than that of the untreated group; the cell wall had not yet ruptured but was bent to some extent, probably because of the rapid movement of intracellular material caused by ultrasound and a small amount of content flowing out. In contrast, the cells of the US200-treated pumpkin juice sample showed an irregular, distorted shape, and the cell walls began to break down, with large cell fragments still clearly present in the pumpkin juice. After the US300 process, the cells in the tissue lost their shape, and the cell walls were heavily broken and aggregated into larger cell wall fragments, whereas in *C. moschata* juice, the cell walls were folded into thin strips, and all intracellular material was precipitated. When the power reached a peak of 400 W, maximum damage to the cell structure was observed. Specifically, the US400 treatment completely released the cell contents, and high-power ultrasonic treatment cut large cell fragments into smaller pieces, resulting in the release of a large number of small particles from the pumpkin juice sample. This is consistent with the abovementioned reduction in particle size and increase in carotenoid content.

Du et al. [50] reported a decrease in particle size with increasing power after high-intensity ultrasonic treatment of pumpkin seed isolates. Qiu et al. [51], studied the effect of ultrasonic pretreatment on the structural integrity of sweet potato crisps, and found that with increasing ultrasonic power and pretreatment time, the integrity of fried sweet potato crisps was significantly reduced as the cells fell off and the cell walls were disrupted. Lepaus et al. [14] showed less dense particles, ruptured cell membranes and release of cell

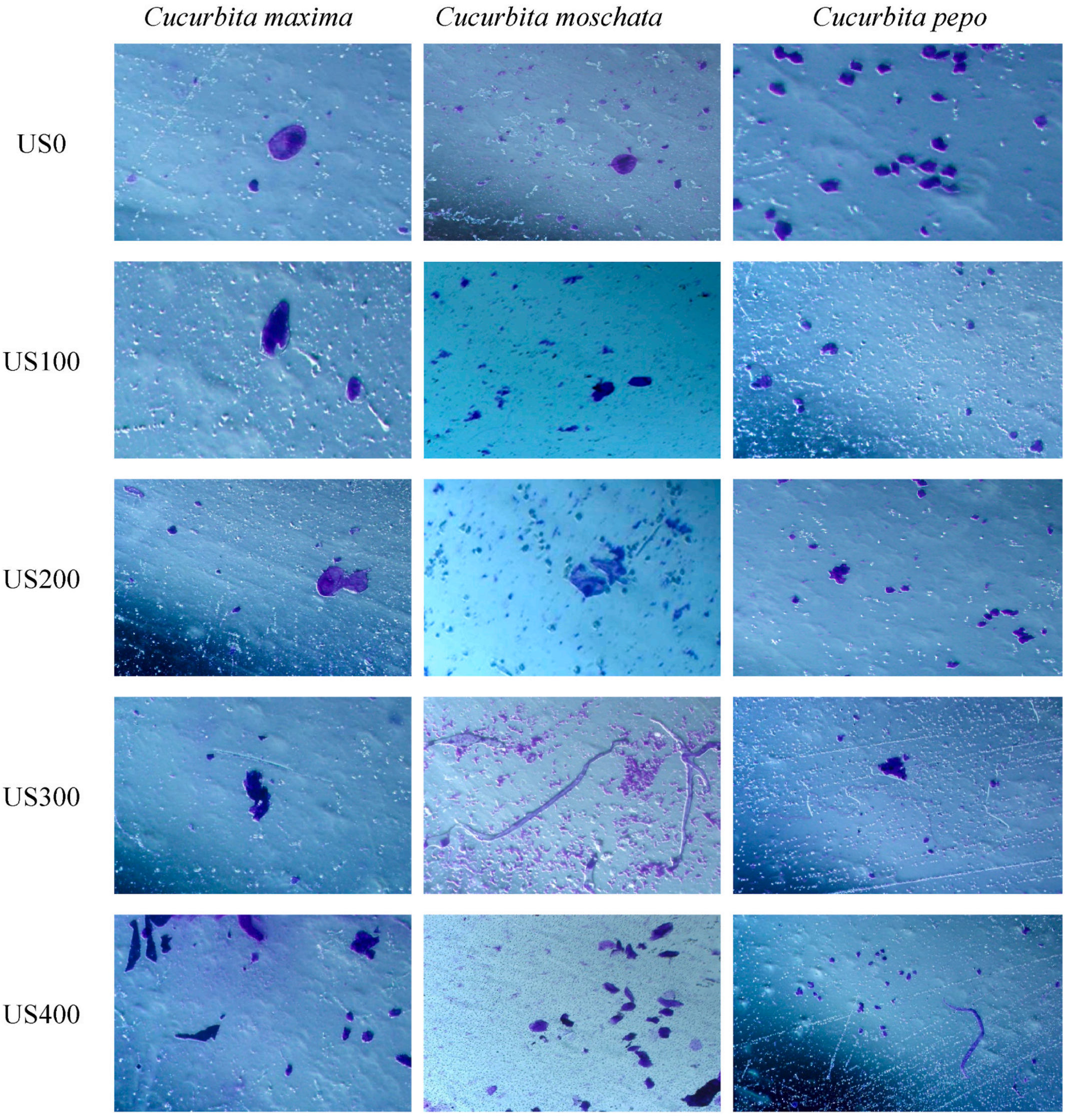


**Fig. 5.** Effect of different ultrasonic power on the microstructure of three types of pumpkin juice
The first line shows the microstructure of *C. maxima* juice after different ultrasonic treatments (0–400 W, 10 min, 20 °C). The second line shows the microstructure of *C. moschata* juice under different ultrasound (0–400 W, 10 min, 20 °C). The third line shows the microstructure of *C. pepo* juice under different ultrasound (0–400 W, 10 min, 20 °C). Microstructure of *C. maxima,C. moschata* and *C. pepo* juice under different power of ultrasound (0–400 W, 10 min, 20 °C) from the first to the fifth rows respectively.

contents, and increased porosity and intracellular space after 10 min of ultrasonic treatment of orange carrot composite juice at 60 °C, 40 kHz. A possible explanation for this might be that the structural integrity of the cells induced by the ultrasound treatment decreased with increasing power. These microstructural changes were attributed to the ultrasound-induced sponge effect. The sponge effect creates microscopic pore channels in the juice sample tissue, which may reduce the diffusion boundary layer and increase the convective mass transfer in the juice sample [19]. The structural changes observed in the US100 and US200 treated pumpkin juice samples may be attributed to the cavitation effect of ultrasound, which generates a large number of tiny bubbles that travel through the sound field, leading to microflows that collide with the cells and cause minor damage to the cells [52]. US300 and US400 caused structural damage to the pumpkin juice, probably because of the large amount of energy released during ultrasound, which caused large shear forces on the tissue cell surface, resulting in cell wall breakage, tearing, structural distortion, and loss of content. This result is similar to that reported by Gao et al. [17] who treated tomatoes with cold ultrasound (87.52 W/cm$^2$, 10 °C, 10 min). In addition, microstructures showed that high-intensity sonication produced a more disorganised structure and the appearance of small irregular pieces. These results indicated that the microstructure of the pumpkin juice after ultrasound treatment changed significantly compared to the control.

### 3.9. *Sensory evaluation of three types of pumpkin juice after ultrasonic treatment*

Sensory evaluation provides valid and reliable product information. Fig. 6 shows the sensory evaluation of *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* juices after treatment with different ultrasound intensities. Increasing ultrasound intensity resulted in little significant change in the sensory evaluation of the different groups. Fig. 6(A) shows the sensory evaluation of *C. maxima* juice subjected to ultrasonic treatments of differing intensities. The overall acceptability of the freshly squeezed *C. maxima* juice was 7.39 and was slightly higher under US400 treatment at 7.5. The color was higher and acidity was lowest, with a slight increase in turbidity after ultrasonic treatment, which is consistent with the particle size results. In Fig. 6(B), *C. moschata* juice had a higher overall consumer acceptance, with an average of 8.22, better taste, and lower acidity. Color, sweetness, and flavor maintained better quality after treatment and did not change significantly compared with the untreated group. In the case of *C. pepo* juice (Fig. 6 (C)), the overall acceptability was the lowest among the three (6.66) with large variation in taste, probably because of the reduction of particles in the juice after ultrasound treatment, a slight increase in turbidity, and the precipitation of cell contents, which resulted in a richer taste. Among the three types of pumpkin juice, *C. moschata* juice had the lowest acidity, the highest sweetness, the best taste, and the best overall consumer acceptance. *C. pepo* juice had the lowest turbidity among the three types of pumpkin juice, with a clearer solution and brighter color, making it more attractive to consumers. In addition, ultrasonic treatment had no significant effect on the sensory evaluation, and the quality of the treated juice was close to that of fresh pumpkin juice, indicating that ultrasonic treatment could better preserve the nutritional value of pumpkin juice and that appropriate ultrasonic treatment is beneficial for consumer acceptance. This is in agreement with the results reported by Anjaly et al. [53] for apple juice and Esatbeyoglu et al. [54] for the juice of *Aronia melanocarpa*. Vollmer et al. [55] found that people still tend to buy hot-pasteurized juices and that consumers may not know much about non-thermally processed fruit and vegetable juices, but Lopes et al. [56] found that freshly prepared juices and orange juice treated with ultrasound (pulsed method, 20 kHz, 100 W, 40 °C/15 min) had better color and longer shelf life and showed better sensory characteristics. The data reported in this study indicate that ultrasonic pretreatment can maintain the nutrients of pumpkin juice to the maximum extent, and it can better retain the taste and flavour of pumpkin juice. Therefore ultrasound is a suitable non-thermal processing method for pumpkin juice and is worth promoting in the field of pumpkin juice processing.

## 4. Conclusion

In this study, the effect of ultrasonic treatment on microbiological, physicochemical properties, and sensory quality of three

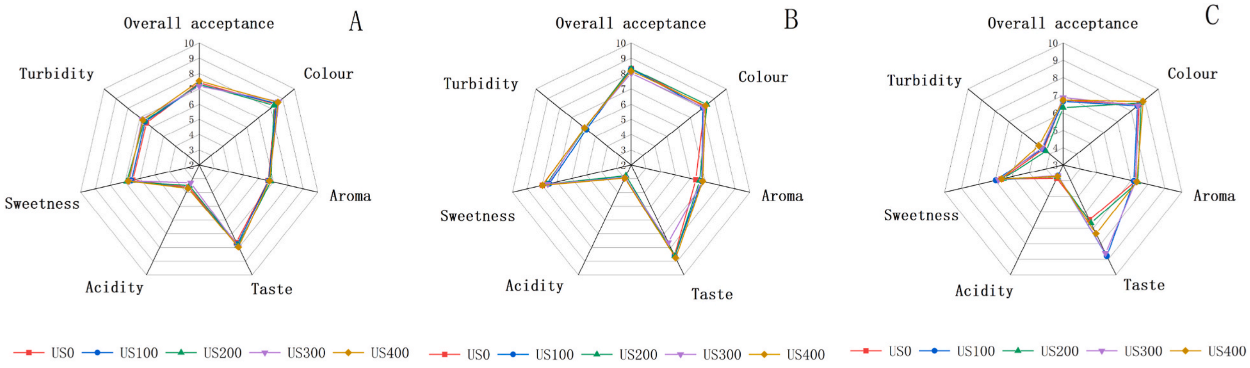


**Fig. 6.** Sensory evaluation of three pumpkin juices, *Cucurbita maxima*, *Cucurbita moschata* and *Cucurbita pepo* juice, treated with different ultrasonic power (Fig. A–C)Figure A: Sensory evaluation of *C. maxima* juice treated with different power of ultrasound (0–400 W, 10 min, 20 °C). Figure B: Sensory evaluation of *C. moschata* juice treated with different power of ultrasound (0–400 W, 10 min, 20 °C). Figure C: Sensory evaluation of *C. pepo* juice treated with different power of ultrasound (0–400 W, 10 min, 20 °C).

pumpkin juices was investigated. The results revealed that different ultrasonic treatments sterilized the pumpkin juice better than conventional pasteurization, with the time factor having the greatest effect on the sterilization rate. The ultrasonic treatments successfully retained the physicochemical properties of the three pumpkin juices (color, carotenoids, sugar content, soluble solids, and organic acid content). The antioxidant properties were also significantly enhanced by 9.09%, 10.25%, and 16.9%, respectively. However, not all the treatment conditions were effective in inhibiting the activities of PPO and POD. The microscopic analysis revealed different degrees of homogenization of pumpkin juice related to the intensity of ultrasonic applied. Sensory evaluation showed that the quality of the ultrasonically treated pumpkin juice was closer to that of fresh pumpkin juice. In summary, based on the above experimental data we can conclude that *C. moschata* juice is the most promising juice in terms of microbiological effects, carotenoid content, antioxidant capacity and sensory evaluation by consumers.

Ultrasonic is an alternative to pasteurization as it is a non-thermal process that can effectively sterilize pumpkin juice while retaining the quality of the juice. Ultrasonic enhances the physical and chemical properties of the pumpkin juice and ensures that the taste quality of the juice is maintained without affecting its nutritional value. The benefits of using ultrasonic include reduced energy loss, simplicity of operation, and virtually no emissions during the process. However, the effect of ultrasonic treatment during storage and its influence on the aroma substances needs to be further investigated. In conclusion, in terms of natural bioactivities in pumpkin juice, ultrasound has better retention of carotenoid content and antioxidant capacity, and it is better than thermal treatment in sterilising the microorganisms of pumpkin juice. This provides more useful information for pumpkin juice flavour enhancement techniques and non-thermal processing methods. In summary, we can make the conclusion that ultrasonic treatment is a promising method to enhance the functional properties of fruit juice.

## Data availability

No data was used for the research described in the article.

### *Declaration of adherence to ethical and professional practices for sensory analysis*

Consumers were recruited from students and staff of Jiangxi Science and Technology Normal University. Written informed consent was obtained from all participants before the experiment was conducted. Participant consent was ensured by all participants signing and returning their consent forms to the research team prior to participation in the study. The sensory analysis experiments in this study strictly followed the ethical and professional practices outlined in the IFST (Institute of Food Science and Technology, UK) Ethical and Professional Practice Guidelines for Sensory Analysis of Food. Authors confirmed that the rights and privacy of all participants were protected during the execution of the research.

## CRediT authorship contribution statement

**Manjun Zhang:** Writing – original draft, Data curation. **Chunli Zhou:** Writing – review & editing, Funding acquisition, Conceptualization. **Long Ma:** Supervision, Software. **Wei Su:** Methodology. **Jian Jiang:** Data curation. **Xueyan Hu:** Project administration.

## Declaration of competing interest

**All authors declare that there were no conflicts of interest with respect to 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.heliyon.2024.e27927.

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