Hybrid techniques of pre and assisted processing modify structural, physicochemical and functional characteristics of okra pectin: Controlled-temperature ultrasonic-assisted extraction from preparative dry powders and its field monitoring

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\textbf{ABSTRACT}

Diversiform okra dry powders were prepared and controlled-temperature ultrasonic-assisted extraction (CTUAE) was then utilized to obtain okra pectin (OP) from the preparative powders. During processing of hybrid techniques, 6 types of dry powders were prepared through different drying technologies (hot air drying, HD; freeze-drying, FD) and meshes (60, 80, 120 meshes) at first. Next, the extraction yield, physicochemical and functional characteristics, and molecular structure of OP were analyzed with or without CTUAE technique. Meanwhile, the time–frequency domains of acoustic fields during extraction process of OP were monitored to analyze the effects of ultrasonic fields. Results showed that OP main chains with less cracking by FD than that by HD; the yield, GalA, esterification degree (DE), \(M_w\) and viscosity of OP increased, but its particle size decreased. Water holding capacity (WHC) and oil holding capacity (OHC) of OP by HD were more prominent. Secondly, HD OP had dendritic rigid chains, while FD OP had flexible chains with multiple branches. For HD OP, as meshes of okra dry powders decreased, GalA, viscosity and emulsification ability decreased; while gel strength and thermal stability increased. For FD OP, the reduction of meshes improved thermal stability. Above all, CTUAE technique increased the yield and GalA, and decreased DE, \(M_w\) and particle size of OP. In terms of functional characteristics, the technique also improved gel strength, resilience and viscoelasticity, enhanced emulsifying stability, WHC and thermal stability, and reduced viscosity. Finally, the correlation between functional and structural characteristics of OP was quantified, and some suggestions were made for its application in food areas.

1. Introduction

Pectin is widely used in large demand as a food additive. Nowadays, natural pectin in China is mainly extracted from orange peels and apple residues [1], but the single pectin cannot meet the diversity and multifunctional application of pectin products. Therefore, it is urgent to develop and utilize new pectin with wide source and low price. Okra (\textit{Abelmoschus esculentus} (Linn.) Moench) is comprised of abundant colloidal substances and nutrients. The colloidal substances principally consist of polysaccharides, flavonoids, polyphenols and alkaloids [2]. Specifically, outer pods of okra are rich in water-soluble polysaccharides, including pectin. Okra pectin (OP) is a complex polysaccharide with homogalactururonan (HG) main chains. HG main chains is formed by galacuronic acid (GaLA) linear chains as linked to \(\alpha(1,4)\) bonds, and its primary structure mainly consists of four major structural domains: HG, rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II) and xylogalacturonan (XGA) [3]. Study has shown that the pectin and its chain conformation during extraction process play a fundamental role in the gelation, stability and emulsification of food, medicine and cosmetics [4], which is essential for the commercial application of OP in the food industry.

Fresh okra has high moisture content and is perishable; moreover, its...
large size and weight are also not conducive for pectin extraction. As a result, the drying process of okra is essential. It is well known that effects of drying conditions on properties of porous network of hydrogel are obvious [5]. Hence, different drying methods will affect the properties of OP. Thereinto, hot air drying (HD) with low cost is easy to control through a continuous flow of hot air, and the water evaporation is mainly generated. Freeze drying (FD) is realized on the basis of sublimation dehydration, and can preserve the original morphology, nutrients and other active ingredients of the products [6,7]. At present, there are few studies on effects of different drying methods on physicochemical characteristics and biological activities of OP. Previously, our research group has preliminarily explored effects of ultrasonic pretreatment and different drying methods, that is, HD, pulsatung vacuum drying (PVD) and FD, on structure and functional properties of OP extracted from various dry powders. On the one hand, compared with cold drying method (FD), hot drying method (HD, PVD) has a greater effect on the molecular structure and chain conformation of OP. On the other hand, the effect of ultrasonic pretreatment on pectin structure is not obvious, much less than that of drying methods [8].

Based on the aforementioned research backgrounds, firstly, apparent morphologies of OP molecular chain structure will be supplemented. Meanwhile, the influence of hot drying method (represented by HD) and cold drying method (represented by FD) on the physicochemical, functional and structural characteristics of OP will be deeply explored. Secondly, in view of limitations of ultrasonic pretreatment on pectin structure, ultrasonic-assisted extraction (UAE) will be alternatively utilized to obtain OP in this study. During the pectin extraction process, the acid extraction method is widely used [8]; in recent years, the alkali extraction method with low molecular weight (Mw) has also attracted keen interest, and is beneficial to the application of low sugar products and pectic oligosaccharide production [9]. Compared with acid and alkali extraction, aqueous extraction is green environmental but with low extraction yield [10]. Nowadays, the emerging non-thermal extraction processes, such as microwave-assisted extraction (MAE), high pressure process (HPP), pulsed electric field (PEF), cold plasma (CP) and ultrasound (US), are becoming the focus to improve the pectin extraction efficiency and modify pectin to reduce its Mw [10]. Compared with other non-thermal extraction technologies, UAE is easier and safer in many cases [11]. In addition, when the ultrasound acting on the solution mixes with okra dry powders in the extraction process, acoustic waves generate cavitation bubbles near tissues, and the cell wall may be destroyed as the collapse of bubbles. As a result, the process of the extraction solvent into the cells is accelerated, thereby the cell contents are released [12]. At the same time, the covalent bond between pectin and non-pectin is destroyed so as to improve the purity of pectin [13]. Dry powders with different sizes are obtained by sieving with different meshes, and the dispersion system of dry powders in extraction solution is also different. Dry powders will be affected by acoustic forces and may also scatter acoustic waves. The scattering force of small size can be ignored, so the effect of acoustic field is obvious; while that of large size is considerable, which greatly reduces the effect of acoustic field [14]. Therefore, the UAE process of OP may be affected by ultrasonic forces. However, there is almost no report on the effects of okra powder size on the extraction yield, physicochemical, functional and structural characteristics of OP by UAE. In order to make the research objective clear, the extraction temperature will be controlled to be constant, that is, controlled-temperature ultrasonic-assisted extraction (CTUAE). Based on dry powders with different sizes and mesh sizes, the influence of CTUAE on physicochemical, functional and structural characteristics of OP was studied. The novelty of this research was that the ultrasonic intensity in time and frequency domains was monitored and analyzed in the extraction solution mixed with dry powders, and a theoretical model was hypothesized that okra cell wall was damaged by mechanical force to promote its pectin extraction. Furthermore, effects of two representative drying methods (HD and FD) on apparent morphologies of OP molecular chains were explored. The relationship between physicochemical, structural characteristics (GaIA, DE, Mw, particle size and chain conformation) and functional characteristics (gel, rheological, emulsifying, WHC, OHC and thermal properties) of OP was revealed. Finally, correlation analysis and clustering heat map were used to quantify the relationship, providing theoretical references for the multi-functional application of OP in food industry.

2. Materials and methods

2.1. Materials

Okra was purchased from Fujian Green Ginseng food store and stored at 4°C before the test. Okra with uniform length and size was selected and washed with deionized water to remove contaminants. Before drying, the okra pods were cut into slices with thickness of 1 cm.

2.2. Preparation of okra dry powders by different drying methods and meshes

The preparation of okra dry powders is shown in Fig. 1a. Okra slices were dried by two representative hot and cold drying methods, namely HD (50°C, 24 h) and FD (25°C, 48 h) [6,15] until the moisture content was <10%. HD was carried out in a 1010-3B electric thermostatic blast drying oven (Shanghai Experimental Instrument Co., Ltd., Shanghai, China) and FD was carried out by the Epsilon 2-6D vacuum freeze drying machine (Martin Christ Co., Ltd., Osterode, Germany). Dried okra slices were ground by a MDJ-D4072 dry milling machine (Guangdong Bear Electric Appliance Co., LTD, Guangdong, China). Subsequently, 60 (pore diameter 0.25 mm), 80 (pore diameter 0.18 mm) and 120 meshes (pore diameter 0.125 mm) were used to sieve, and then three different meshes of okra dry powders were obtained: M60 (80 < mesh < 60), M80 (120 < mesh < 80), M120 (mesh < 120). All powders were sealed and stored at ~20°C for further analysis.

2.3. Extraction method of OP

The whole extraction procedure of OP is shown in Fig. 1b. 10 g okra dry powders were washed twice with 75% ethanol (1:20, W/V) at 40°C, stirred at 500 rpm/min for 1 h to remove fat soluble compounds and pigments, and then dried in an oven at 50°C after filtration. The decolorized dry residues were mixed with distilled water (1:40, W/V) to form the extraction solution of OP, and extracted at 60°C for 30 min. In this procedure, CTUAE of OP was utilized (Section 2.4) with magnetic stirring extraction (MSE) at 300 r/min as a control. Subsequently, the supernatant was collected by centrifugation at 10,000 r/min for 10 min, and the residues were extracted again using the same method [16,17]. The total supernatant was collected and free protein was removed by Sevag reagent (chloroform:n-butanol = 4:1). The procedure was repeated until no absorption peak was observed at 280 nm. Afterwards, the OP was dialysed by a dialysis tube (aperture equivalent to 14,000 MWCO) in distilled water at room temperature for 48 h, and the dialysate was changed 3 times a day [17,18]. Finally, the purified OP was obtained after drying, and OP groups were named as: with or without ultrasound (-/U)-drying method (HD/FD)-meshes of okra dry powders (M60/M80/M120) OP.

The yield (%) of OP was calculated by dividing the weight (g) of extracted OP by the weight (g) of dry powder (Eq. (1)) [19]:

\[
\text{Extraction yield} = \frac{w_{\text{extracted OP}}}{w_{\text{dry powder}}} \times 100\%
\]  

(1)

2.4. CTUAE and its acoustic field monitoring in time-frequency domains

During CTUAE of OP (Fig. 1b-A), the beaker containing aforementioned extraction solution was placed in a 40 kHz ultrasonic reactive tank with controlled temperature of 60°C for 30 min and the ultrasonic...
Fig. 1. Research diagrams. (a) Preparation of okra dry powders by different drying methods and meshes, (b) Extraction procedure of OP and CTUAE with its acoustic field monitoring.
power density was 37.5 W/L [19,20]. The specific OP extraction method was shown in Section 2.3. During the CTUAE process, real-time online monitoring system was carried out. More specifically, polyvinylidene fluoride (PVDF) sensor was introduced to collect acoustic signals in time domain. The performance of the PVDF sensor was detailedly described in earlier studies [21-23]. The collected instantaneous pressure of acoustic field was converted into electrical signals through the PVDF sensor, then recorded and stored in the form of voltage signals by MDO3024 oscilloscope (Tektronix, Inc., USA) (Fig. 1b-B2) to study the ultrasonic intensity. Meanwhile, acoustic signals in frequency domain were monitored by a tube hydrophone (Bruel & Kjaer Sound & Vibration Measurement, Denmark) and analyzed using RSA306B Real-time Spectroanalyzer (Tektronix, Inc., USA) (Fig. 1b-B1) to study the acoustic field was converted into electrical signals through the PVDF sensor, then recorded and stored in the form of voltage signals by MDO3024 oscilloscope (Tektronix, Inc., USA) (Fig. 1b-B2). PVDF sensor and hydrophone were located at the same position, that is, on the central vertical line of the beaker and 5–6 cm away from the bottom. The research on time and frequency domains of acoustic field was combined, and effects of CTUAE in different OP extraction solutions were explained from the perspective of acoustics, which was mainly represented through ultrasonic intensity.

2.5. Determination of structural characteristics of OP

2.5.1. Scanning electron microscopy (SEM)

Dried OP was glued on the sample platform by conductive adhesive and completely gold-sputtered under vacuum conditions. SEM images were collected at acceleration voltage of 15.0 kV with the image magnification of 500 × by S-3400 N SEM (Hitachi Ltd, Japan) [18].

2.5.2. Atomic force microscopy (AFM)

OP solution (100 µg/mL) was prepared with pure water, heated and oscillated at 40°C. After dissolving fully, 10 µL of OP solution was dropped on the surface of the freshly peeled mica sheet, and dried naturally overnight at room temperature. Next, the mica sheet was fixed on a round iron sheet with a diameter of 15 mm with double-side adhesive, and the iron sheet was fixed on the sample platform. A Multi-mode8 Nanoscope AFM (Bruker Nano Surfaces Division, Santa Barbara, CA) was used in the semiautomatic tapping mode, and the probe was performed at room temperature of 20°C and a relative humidity of 26–30%. The scanning area was set at 2 µm × 2 µm on the XY plane, and the scanning resolution was 512 × 512 points [24]. AFM images were analyzed offline by using NanoScope Analysis software (Digital Instruments, Santa Barbara, CA, USA).

2.5.3. Fourier-transform infrared spectroscopy (FT-IR)

The structure of OP was analyzed by WQF-510A FT-IR spectroscopy (Beijing Ruili Analytical Instrument Co., Ltd, China). Each OP sample (1.0 mg) was mixed with 100 mg KBr, and pressed into the film. The infrared spectra were recorded in the frequency range of 4000–400 cm \(^{-1}\) at a resolution of 4 cm \(^{-1}\) with an average of 32 scans [20].

Furthermore, the esterification degree (DE) of OP was determined from FT-IR spectra according to a previously reported method [20], DE was calculated from the band areas based on the region of 1700–1750 cm \(^{-1}\) (esterified uronic acids) and 1600–1630 cm \(^{-1}\) (free uronic acids). Afterwards, DE was gained by the equation as follows (Eq. (2)):

\[
\text{DE} = \left( \frac{A_{1740}}{A_{1740} + A_{1620}} \right) \times 100\% 
\]

wherein, \(A_{1740}\) was the peak area of 1700–1750 cm \(^{-1}\), and \(A_{1620}\) was the peak area of 1600–1630 cm \(^{-1}\).

2.6. Determination of physicochemical characteristics of OP

2.6.1. Galacturonic acid content (GalA)

M–hydroxybiphenyl colorimetric method was applied to determine GaA of OP. 3,5-dimethylphenol (≥99%) was dissolved in 5 mg/mL NaOH with a concentration of 1.5 mg/mL as a color reagent. The OP solution (1.0 mL, 200 µg/mL) was poured into a tube, and sulfuric acid/sodium tetraborate (6 mL) was added in an ice bath. Next, the tube was shaken by a vortex mixer, heated in a boiling water bath for 5 min, and cooled in the ice bath. The above-mentioned color reagent was added to the solution, and shaken for 5 min. Finally, the absorbance was measured at 520 nm. By following the same procedure, the GaA standard solution with concentrations of 0–100 µg/mL was prepared, and the UV–vis spectrophotometer (PERSEE, China) was calibrated with deionized water [19].

2.6.2. Molecular weight of OP

Size-exclusion chromatography (SEC) with refractive index (RI) and multichannel laser light-scattering detectors (MALLS) were used to determine the weight-average molecular weight (M\(_w\)), number-average molecular weight (M\(_n\)) and molecular weight distribution (M\(_w)/M\(_n\)). SEC-MALLS system comprised of a LC-20AT isometric pump (Shimadzu, Japan), and a RID-20 RI detector (Shimadzu, Japan) equipped with an OHpak SB-G guard column (Shodex, Japan) and two SEC columns (OHpak SB-806 M HQ and SB-805 HQ, 8 mm × 30 cm, Shodex, Japan) in series at 25°C. OP was dissolved in the eluent (0.5 mg/mL) and filtered through a 0.22 µm microporous membrane before injection. The eluent was mixed by 0.1 M NaCl with 0.02% NaN\(_3\) at a flow rate of 0.5 mL/min, and the injection volume of OP solution (1.0 mg/mL) was 100 µL. The RI increment (dn/dc) was 0.138, and data were analyzed by Astra Version 6.1 software [25].

2.6.3. Particle size of OP

OP solution was diluted to 0.1 mg/mL with deionized water to reduce multiple light scattering effect. Hydromechanical diameters of OP solution were determined by liteSizer 500 particle size analyzer (Anton Paar, Austria) with detection range of 0.3–10 µm. RI of aqueous solution was set to 1.330. Each OP sample was tested in 3 parallel, and all measurements were performed at 25°C [26].

2.7. Determination of functional characteristics of OP

2.7.1. Texture properties of OP gel

A mixture of 15% sucrose, 0.1% xanthan gum, 0.9% carrageenan and 0.1% OP was prepared, heated and stirred at 80°C until completely dissolved [27]. Next, the mixture was sterilized in 85°C water bath for 15 min, poured into the glass container (10 mm × 35 mm) for cooling [28], sealed and refrigerated at 4°C for 18 h. Thus, the OP gel was formed, and the gel without OP was prepared as control. Gel properties were measured by texture analyzer (Stable Micro Systems, UK), TPA mode was selected, and P0.5 probe was used for measurement. The gel was compressed until its height reached 50% of its initial height at a constant speed of 1.0 mm/s. The peak of the compression curve was recorded as gel strength (g) when broken [29].

2.7.2. Rheological properties of OP

Rheological properties of OP were measured on a Discovery Hybrid Rheometer-1 (TA, USA) equipped with a parallel steel plate (40 mm diameter, 1.0 mm gap). OP samples were prepared in deionized water at a concentration of 6 mg/mL. The steady shear viscosity was measured within the shear rate range of 0.1–10 s\(^{-1}\) at 25°C. The storage modulus (G’) and loss modulus (G”) were determined by oscillation frequency mode at the range of 0.1 to 100 rad/s with a constant strain of 10% at 25°C [7].

2.7.3. Emulsifying properties of OP

The emulsifying activity (EA) and emulsifying stability (ES) of OP were investigated. 4 mL of OP solution (1.0%, w/v) were homogenized with 4 mL of refined sunflower oil in a 10 mL centrifuge tube at room temperature by a high-speed shear homogenizer at 10,000 rpm for 1
min. Next, the mixture was centrifuged at 1300 g for 5 min, total volume of system (V\textsubscript{1}) and volume of emulsifying layer (V\textsubscript{2}) were measured. Thereafter, the emulsion was heated at 80 °C for 30 min, cooled down to ambient temperature, and centrifuged at 1300 g for 5 min. The volume of remaining emulsifying layer (V\textsubscript{3}) was measured. EA and ES were calculated as follows [6]:

\[
EA\% = \frac{V_1}{V_{10}} \times 100\% \tag{3}
\]

\[
ES\% = \frac{V_2}{V_{20}} \times 100\% \tag{4}
\]

2.7.4. WHC and OHC of OP

The centrifuge tube and 0.10 g OP were weighed together as m\textsubscript{1}, 10 mL of distilled water or refined sunflower oil was mixed with OP (dry weight m\textsubscript{0}) in the centrifuge tube, soaked at ambient temperature for 1 h, and stirred every 10 min. The mixture was centrifuged at 1300 g for 10 min, the supernatant was obtained, and superfluous water and oil were removed by filter paper standing for 5 min before weighing. Next, the centrifuge tube and the remaining residues were recorded as m\textsubscript{2}, WHC and OHC were calculated and expressed on a dry basis as grams of water/oil bound with per gram of OP (Eq. (5)) [6]:

\[
\text{WHC/OHC (g/g)} = \frac{m_2 - m_1}{m_0} \tag{5}
\]

2.7.5. Thermal stability of OP

OP samples were dried to constant weight at 40 °C and 6 mbar in a desiccator before the test, then performed by Thermogravimetric (TG) analysis using the following conditions: linear heating rate of 10°C/min from 30 to 600°C, dynamic inert nitrogen atmosphere of 75 mL/min, 70 μL crucible (70μLSDT-Q/TA6.5) without lid, and sample weight of 5–10 mg [30].

2.8. Statistical analysis

All the experiments were conducted in three replicates and the mean ± standard deviation (SD) was used in the analysis by using Excel Version 2010 (Microsoft Corp., USA) and OriginPro Software Version 8.0 (OriginLab Corp., MA, USA). p < 0.05 indicated statistically significant differences. The correlation coefficient and agglomerative hierarchical clustering was done using R package.

3. Results and discussion

3.1. Study on CTUAE process of OP

3.1.1. Extraction yield of OP

The moisture content of HD and FD dry powders was (9.78 ± 0.78)% and (8.52 ± 0.18)%, respectively. The extraction yield of OP is shown in Table 1. Results showed that the extraction yield ranged from (10.21 ± 0.38) to (15.54 ± 1.18)%. Most of all, CTUAE can significantly improve the extraction yield by 1.09–17.81%; especially for U-HD OP, compared with HD OP, the increase rate reached 11.46–17.81%. Hence, it was shown that CTUAE can improve the traditional pectin extraction method and promote its further development. It was observed in earlier studies the pectin also can be extracted from pulp (200 W, 35 min) and pomelo peel (50 W, 30 min) by ultrasonic technique, the extraction yield significantly improved and the extraction time was shortened [31,32]. The advantage of pectin extraction assisted by ultrasonic technique was attributed to micro jet generated as the collapse of ultrasonic bubbles decomposed the middle layer structure of cells and promoted the pectin dissolution [33].

Secondly, based on different drying methods to obtain okra dry powders, it was found that the extraction yield of FD OP was higher than that of HD OP. Similar results were also obtained that it was related to drying methods [34]. HD usually causes pore collapse due to high capillary pressure, and the resulting material shows high density and low porosity [35]; while the high density of the tissue leads to the difficulty of pectin extraction. To the opposite, FD can better maintain the initial polymeric structure of the hydrogel [36], which facilitates the mass transfer and the dissolution of OP.

Finally, effects of meshes of okra dry powders on extraction yield of OP were compared. As for HD, the smaller the mesh of okra dry powders, the higher the extraction yield of OP. Some studies have proved that there was a close relationship between the mesh and the extraction yield [37,38]. This is because the smaller the meshes of dry powders, the greater the exposure of pectin chains. Therefore, water penetration is

| Groups | Extraction yield (%) | GalA (%) | Degrees of esterification (DE %) | Hydrodynamic diameter (nm) | Weight-average molecular weight M\textsubscript{w} (×10\textsuperscript{3}Da) | Number-average molecular weight M\textsubscript{n} (×10\textsuperscript{3}Da) | Polydispersity Pd (M\textsubscript{w}/M\textsubscript{n}) |
|--------|----------------------|----------|-------------------------------|---------------------------|---------------------------------|---------------------------------|-----------------------------|
| HD-M60 | 10.21 ± 0.38\textsuperscript{a} | 56.32 ± 0.83\textsuperscript{a} | 39.54 ± 0.99\textsuperscript{e} | 3029.33 ± 170.35\textsuperscript{f} | 6.48 ± 0.84% | 6.26 ± 0.80% | 1.04 ± 1.16% |
| HD-M80 | 10.33 ± 0.31\textsuperscript{a} | 57.02 ± 0.90\textsuperscript{a} | 39.68 ± 1.18\textsuperscript{e} | 8851.67 ± 535.58\textsuperscript{d} | 6.57 ± 0.68% | 6.39 ± 0.51% | 1.03 ± 0.85% |
| HD-M120 | 11.21 ± 0.51\textsuperscript{d} | 54.74 ± 0.98\textsuperscript{b} | 37.57 ± 0.73\textsuperscript{d} | 5576.00 ± 349.87\textsuperscript{b} | 6.28 ± 0.69% | 6.14 ± 0.54% | 1.02 ± 0.87% |
| FD-M60 | 12.63 ± 0.38\textsuperscript{d} | 61.85 ± 0.88\textsuperscript{b} | 45.04 ± 0.90\textsuperscript{d} | 4783.33 ± 415.77\textsuperscript{d} | 6.63 ± 0.67% | 6.47 ± 0.45% | 1.03 ± 0.80% |
| FD-M80 | 14.63 ± 0.45\textsuperscript{b} | 59.04 ± 0.83\textsuperscript{d} | 44.13 ± 0.13\textsuperscript{d} | 3524.33 ± 399.85\textsuperscript{d} | 7.30 ± 0.60% | 7.00 ± 0.47% | 1.04 ± 0.80% |
| FD-M120 | 14.29 ± 0.44\textsuperscript{b} | 59.97 ± 0.61\textsuperscript{e} | 42.06 ± 1.06\textsuperscript{d} | 1263.57 ± 354.38\textsuperscript{c} | 7.03 ± 0.64% | 6.80 ± 0.52% | 1.03 ± 0.82% |
| U-HD-M60 | 11.38 ± 0.25\textsuperscript{a} | 59.62 ± 0.66\textsuperscript{d} | 38.10 ± 0.73\textsuperscript{ef} | 1279.53 ± 200.37\textsuperscript{c} | 6.35 ± 0.75% | 6.05 ± 0.46% | 1.05 ± 0.80% |
| U-HD-M80 | 12.17 ± 0.52\textsuperscript{d} | 60.44 ± 0.58\textsuperscript{cd} | 38.55 ± 0.81\textsuperscript{ef} | 3202.67 ± 516.65\textsuperscript{d} | 6.21 ± 0.51% | 6.11 ± 0.44% | 1.02 ± 0.85% |
| U-HD-M120 | 12.63 ± 0.50\textsuperscript{d} | 59.22 ± 0.61\textsuperscript{f} | 37.29 ± 1.14\textsuperscript{d} | 3183.33 ± 293.15\textsuperscript{d} | 5.62 ± 1.00% | 5.50 ± 0.89% | 1.02 ± 1.34% |
| U-FD-M60 | 13.83 ± 0.44\textsuperscript{d} | 62.37 ± 0.88\textsuperscript{d} | 42.05 ± 1.28\textsuperscript{ef} | 3647.00 ± 806.54\textsuperscript{d} | 6.49 ± 0.73% | 6.30 ± 0.50% | 1.03 ± 0.89% |
| U-FD-M80 | 14.79 ± 0.31\textsuperscript{b} | 63.39 ± 0.61\textsuperscript{d} | 42.32 ± 1.37\textsuperscript{b} | 3032.67 ± 122.15\textsuperscript{d} | 6.40 ± 0.65% | 6.17 ± 0.39% | 1.04 ± 0.76% |
| U-FD-M120 | 15.54 ± 0.38\textsuperscript{a} | 61.49 ± 0.77\textsuperscript{bc} | 40.53 ± 0.63\textsuperscript{cd} | 810.90 ± 133.45\textsuperscript{c} | 5.95 ± 0.52% | 5.75 ± 0.38% | 1.03 ± 0.69% |
enhanced during the extraction process, and the water extraction capacity of cell wall polysaccharides is improved [39]. However, for FD, when the meshes of dry powders decreased from M80 to M120, the extraction yield of OP decreased from 14.63 ± 0.45% to 14.29 ± 0.44%. The decreased yield of small meshes may be related to the accumulation of small particles which blocked the flow of solvent [40]. However, the extraction yield increased from 14.79 ± 0.31% to 15.54 ± 0.38% through CTUAE technique, when the meshes decreased from M80 to M120. It can be established that accumulation of small particles was ameliorated by CTUAE technique.

3.1.2. Acoustic field signal monitoring in OP extraction solution

Fig. 2 shows the ultrasonic intensity signals monitored in OP extraction solution at the final extraction stage (15–30 min). It should be noted that during this stage, a large amount of OP was dissolved, and the viscous pectin system dissipated the ultrasonic signals. Using the aforementioned acoustic field signal monitoring system (Section 2.4), the voltage peak of ultrasonic signals in time domain and the power of ultrasonic signals in frequency domain can be collected online during CTUAE process. The voltage peak is a characteristic value of acoustic field in time domain monitored by PVDF. The stronger the acoustic field, the more obvious the cavitation and mechanical effects, and the greater the pressure of the micro-jet generated as bubbles collapse. When the micro-jet acts on PVDF, the larger the deformation of PVDF, the greater the electrical energy converted from mechanical energy, so the greater the peak voltage [21]. The power value is the intensity of ultrasonic signals in frequency domain processed by the spectroanalyzer, which is a negative value and also a characteristic value of acoustic field. Ultrasonic waves propagate in the medium accompanied by the energy propagation, the stronger the acoustic field is, the more obvious the radiated signals are, and so the power peak is greater. Meanwhile, the transmission medium obtained the corresponding acoustic energy and its morphology was changed due to the ultrasonic disturbance [41].

Fig. 2a shows voltage peaks monitored in time domain. Water is Newtonian fluid with a small viscosity of 2.98 × 10⁻³ Pa·s, which is easy to induce ultrasonic cavitation and obtain a strong acoustic field; and the strongest voltage peak is 0.0095 V. The viscosity (measured in Section 3.4.2) of HD OP and FD OP extraction solution was much greater than that of water, thus the propagation ultrasonic waves was obstructed in OP extraction solution at the final stage of CTUAE process. Therefore, the ultrasonic voltage peak detected at the same level became significantly smaller because of dissipation by the viscous OP solution. More importantly, it was found that voltage peaks were obviously different in all OP extraction solution, because the signal intensity of acoustic field was different. Voltage peaks (0.0033, 0.0028, 0.0026 V) in HD OP extraction solution were higher than that (0.0019, 0.0018, 0.0012 V) in FD OP extraction solution, but the extraction yield of HD OP was lower than that of FD OP. Nevertheless, the increment of extraction yield (12.67–17.81%) of HD OP was significantly higher than that (1.09–11.71%) of FD OP owing to CTUAE technique (Table 1). Thus, it was further indicated that CTUAE technique was beneficial to the traditional pectin extraction method. In addition, the smaller the mesh of okra dry powders, the weaker the monitored ultrasonic signal intensity, namely, M60 > M80 > M120; but the higher the OP extraction yield, namely, U-HD-M60 < U-HD-M80 < U-HD-M120, U-FD-M60 < U-FD-M80 < U-FD-M120.

In conclusion, here it was found that the weaker the monitored ultrasonic signal intensity, the higher the OP extraction yield, which seemed to be contrary to our previous research results [10]. However, all the results were not contradictory, and the key point was that ultrasonic waves propagated in different medium. The ultrasonic pre-treatment was utilized in previous research and acoustic field was monitored in water medium. Water is Newtonian fluid, and its viscosity does not change with the increase of ultrasonic shear strain rate. As a result, when the intensity of acoustic field was strong, the time–frequency signal intensity was also strong [10]. Comparatively
speaking, when ultrasonic waves propagated in the OP extraction solution, as CTUAE processed, more viscous OP was generated in the extraction solution. As a result, the propagation of ultrasonic waves was obstructed at the final stage of CTUAE process, and most of ultrasonic energies were consumed by the viscous OP system, so the collected time–frequency domain signal intensity became weak. Therefore, it can be concluded that in the OP extraction system in this research, the weaker the ultrasonic signal intensity at the final stage of CTUAE process, the more the OP extraction yield. In short, OP extraction process was promoted by CTUAE technique, while ultrasonic signal intensity was closely related to the properties of the extraction solution and the dissolution rate of OP. The smaller the mesh of okra dry powders, the faster the dissolution rate of OP; and it was easier to form the viscous solution, which brought a significant barrier effect on the propagation of ultrasonic waves. Ultrasonic signals were obtained after ultrasonic waves propagated in the OP extraction solution and then acted on sensors. The more viscous the pectin system, the more dissipated the ultrasonic waves caused, which was corresponding to results of OP extraction yield (Table 1).

The trend of ultrasonic signals monitored in frequency domain in Fig. 2b-c is consistent with that in time domain in Fig. 2a. The voltage peak value in time domain was low, and the maximum power value in frequency domain was also low (the dark blue part in Fig. 2b-c), which indicated that both of the two monitoring technologies can be used to quantify the intensity of the acoustic field. Besides, the amount of power peaks of ultrasonic signals monitored in HD OP extraction solution was more than that in FD OP extraction solution. This was related to the dense structure of HD okra dry powders, the structure was relatively complex, and the corresponding vibration spectrum was wider with more power peaks. However, dense structure led to relatively less soluble pectin, eventually the extraction yield of HD OP was lower than that of FD OP, and U-FD OP had the highest extraction yield with the help of CTUAE technique.

3.1.3. Morphologies of okra dry powder and its residue

Fig. 3a shows the macro-morphologies of okra dry powders by different drying methods and meshes. Before extraction, the structure of HD okra dry powders was dense, while that of FD okra dry powders was loose and relatively small (Fig. 3b). Furthermore, as the decrease of mesh, the uniformity of dry powders was greatly improved. Cell walls of M120 group were seriously disintegrated, and the natural regular structure was almost eliminated. In addition, the increased specific
surface area of fine powders undoubtedly reduced mass transfer barriers to release pectin, and accelerated pectin dissolution [39].

OP obtained after CTUAE was wet, and dried by FD in order to keep it integrated, as shown in Fig. 3c. It was an aerogel with sponge texture, and uneven morphologies on the surface of OP were mainly caused by ice crystals formed by pre-freezing (-20°C) before FD. U-HD-M60 and U-FD-M120 OP had smaller and more regular ice crystals. As the freezing speed was same, the ice crystal formation was related to the uniformity of pectin distribution due to aggregated behaviors of OP. It was highly correlated with particle size of OP, that is, hydrodynamic diameter in Table 1, and the particle size of U-HD-M60 and U-FD-M120 OP was significantly smaller than the other groups. In terms of different drying methods, ice crystals of HD OP had straight and clear edges, while that of FD OP had irregular shapes (see Red circles in Fig. 3c) when wet OP was dried by FD. In fact, the formation of ice crystals was also related to morphologies of pectin chains (Fig. 4). Furthermore, the extraction of OP was significantly related to the destroying degree of okra dry powders, so the microstructure of dry powder residues after extraction was observed in Fig. 3d. It was found that the destroying degree was in connection with exposed tissue vessels of powder residues after extraction. Residues of HD powders maintained the blocky structure; although some parts of vessels were damaged, other parts owned the original skeleton. Residues of FD powders had the loose layer structure, and vessels were almost destroyed. As the mesh of dry powders became smaller, the destroying degree of vessels was intensified in Fig. 3d: cracked ring structure (yellow circles 1) → split into two pieces (yellow circles 2) → separated filaments (yellow circles 3). Ultimately, intensified destroying degree of okra dry powders correlated with increasing extraction yield (Table 1).

3.2. Structural characteristics of OP

3.2.1. Sem

Fig. 4 shows SEM of OP morphologies. HD OP chains were thick and rigid, while FD OP chains were thin and flexible. It was further found that with the increment of dry powder meshes, the proportion of thick pectin chains got larger, while that of thin pectin chains decreased. Furthermore, aggregation behaviors of OP were found after CTUAE (red circles in Fig. 4). That is because branch chains of OP were reduced after CTUAE, chains got more flexible, and the steric hindrance was weakened, resulting in high mobility of OP molecules [42].
charged polymer chains, hydrogen bonds, or van der Waals forces [45]. During the process of CTUAE, the network structure formed by intra-
molecular or intermolecular entanglement of OP chains became disor-
dered and partially fractured, and then obvious dissociation occurred. Meanwhile, nodes forming network structures were also damaged, and 
then some single fragments were generated because of high ultrasonic 
intensity. Whereupon, glycosidic bonds and neutral sugar side chains of 
OP molecules were broken, thus the reduction of OP molecular weight in 
Table 1 and the shortening of chain length were induced. Recent studies 
found that high-intensity (104.7 W/cm²) ultrasonic treatment signifi-
cantly reduced molecular weight and intrinsic viscosity, and confor-
national transitions from semi-flexible chains to flexible chains or even 
flexible coils became more prominent [4].

In view of different drying methods, chains of HD OP were thick and 
sparse, with a dendritic multi-branch structure. [46] a similar structure of 
HD pectin was also observed. By comparison, chains of FD OP were 
thin and branched with a high density per unit area, and had slender 
filamentous entangled structures. A similar structure of FD pectin was 
also observed [26]. On the side, chains of HD OP were rigid, while that 
of FD OP belonged to a slightly curled flexible branch chain which was in 
accordance with SEM of OP in Fig. 4.

The peak width of OP was calculated by NanoScope Analysis soft-
ware, and the chain width of OP is shown in Fig. 5b. It was reported that 
the peak width can be used to represent the chain width of pectin 
molecules [47]. Due to the broadening effect of the probe, the peak 
width measured here was larger than the actual chain width of OP 
molecules, and linear single fragments were randomly selected for 
measurement. The chain width of HD OP was between 334 and 677 nm. 
By contrast, that of U-HD OP between 96 and 160 nm was reduced by 
57.35%-75.7%. The mesh of okra dry powders was proportional to the 
chain width. The larger the mesh of dry powders, the thicker the chains 
that connected pectins, and the fewer the branches. Conversely, the 
smaller the mesh of dry powders, the thinner and denser the chains, and 
the more the branches. The chain width of FD OP was between 81 and 
289 nm; while the mesh and CTUAE had no significant effect on its chain 
width, because nano-morphologies of OP were also closely related to its 
molecular weight and structure.

Fig. 5 shows the mechanism of OP by CTUAE with different drying 
methods and meshes. It can be concluded from SEM (Fig. 4) and AFM 
(Fig. 5) that HD OP had dendritic rigid chains, while FD OP had flexible 
chains with multiple branches. When okra was crushed and sieved after 
drying in different ways, the cell wall was damaged by the strong me-
chanical force, and then the exposure of OP in the middle layer of the 
cell wall was promoted. The smaller the meshes of okra dry powders, the 
more damaged the cell wall, such as vessels in the residue tissue of dry 
powders (Fig. 3). As a consequence, the penetration of water was suf-
ficient, which facilitated the dissolution of OP and improved its 
oxtraction yield (Table 1). Most noteworthy, the collapse of cavitation 
bubbles during CTUAE process generated micro jet to shear OP chains 
randomly; OP was degraded and broken into smaller molecules, and 
diffused from the middle layer of the cell wall. In the process of CTUAE, 
the smaller the mesh of okra dry powders, the more damaged the cell 
wall, and the greater the degradation effect based on ultrasound, such as 
the decrease of molecular weight (Table 1) and viscosity (Fig. 10a). Compared with HD OP, CTUAE technique was easier to degrade FD OP.

3.2.2. FT-IR

From Fig. 7, FT-IR spectra of OP obtained by different methods are 
similar, and structures of OP prepared from powders by different drying 
methods and meshes are similar between traditional MSE and CTUAE in 
aqueous solution. The band at 3420 cm⁻¹ was induced due to the 
stretching vibration of hydroxyl group (O–H), and mainly came from 
intermolecular and intramolecular hydrogen bonding of GalA main 
chains. Bands in the range of 3000–2800 cm⁻¹ were attributed to the 
C–H absorption, including stretching vibrations of CH, CH₂ and CH₃ 
[48]. The absorption band at 1740 cm⁻¹ corresponded to C = O

Fig. 5. AFM of OP (a), and width distribution of main chains (b).
stretching vibration of methyl esterified groups, and the intense band at 1620 cm\(^{-1}\) was the C=O asymmetric stretching of –COO\(^-\), indicating the existence of uronic acids [49]. The bands at 1420 cm\(^{-1}\) and 1380 cm\(^{-1}\) were attributed to bending vibration of C–H or O–H [50]. Moreover, two absorption peaks at 1620 and 1420 cm\(^{-1}\) were attributed to the asymmetric and symmetric stretching of –COO\(^-\), and it demonstrated that OP was acidic polysaccharide [51]. The band at 1247 cm\(^{-1}\) was the asymmetric C-O-C stretching vibration with the presence of –OCH\(_3\). Typical bands of protein at 1651 and 1555 cm\(^{-1}\) were not detected, which indicated that the amount of protein in OP was very low [49]. The fingerprint region (1200–800 cm\(^{-1}\)) of OP spectra can reflect some changes of monosaccharide components, as previously described by [52]. Intense bands at 1150 and 1070 cm\(^{-1}\) were assigned to stretching vibrations of glycosidic bonds (C-O) and pyranoid rings (C-C) due to high content of GalA. Furthermore, the correction of the relative ratio of the peak area at 1740 cm\(^{-1}\) to the sum of peak areas at 1740 and 1620 cm\(^{-1}\) provided information about DE (Table 1). Results showed that the DE of OP ranged from 37.29% to 45.04% but<50%, which belonged to low methoxyl pectin. The OP also had similar structure [6]. In comparison among all extracted OP, the average DE of FD OP (43.74%) was 10.99% higher than that of HD OP (38.93%). It was also showed that DE of FD pectin was higher [7]. Here, FD OP was more flexible than HD OP because of soft and curved main chain rather than less branches, while chains of HD were straight. In addition, DE of OP after CTUAE was reduced by 0.75–6.65%. The decrease of DE may be attributed to the hydrolysis of ester groups in pectin chains by ultrasonic cavitation, or the reaction of ester groups with ionized groups generated through acoustic decomposition, resulting in the formation of free –COO\(^-\), and GalA content increased [53]. Moreover, the decrease of DE weakened the steric hindrance of OP molecular chains to a certain extent, so that chains after CTUAE became more flexible and curly, which corresponded to Figs. 4-5. Among all the OP extracted from dry powders by different meshes, DE of M120 OP was significantly reduced than that of other meshes, indicating that more pectin chain fractured due to the acting area increment of dry powders with decrement of meshes. Studies showed that DE of polysaccharides affected functional properties, such as emulsifying, gel properties and viscosity [20], and low DE may contribute to the relatively high antioxidant activity of natural polysaccharides [54].

3.3. Physicochemical characteristics of OP

3.3.1. GalA

GalA content (Table 1) can be used as an indicator of OP content, because GalA mainly comes from the HGA and RG-1 of OP main chains [55]. As shown in Table 1, firstly, it is interesting that GalA was affected by drying methods. It can be gained that GalA of FD OP (59.04–61.85%) was higher than that of HD OP (54.74–57.02%). This may be that degradation of the OP main chains was prevented due to the low oxygen concentration and temperature during FD [34], and similar result was reported by [6]. It was indicated that FD preserved the OP structure better than HD.

Secondly, owing to CTUAE technique, GalA of OP increased significantly. The pectin was composed of linear structure and side chains, while the degradation induced by ultrasound mainly occurred in side chains rather than main chains [31]. Therefore, the increase of GalA could be attributed to the breakage of OP side chains, and the increase in GalA-rich HG main chains [56]. It was also concluded that ultrasound...
caused the rupture of pectin side chains, so the proportion of GalA in sweet potato pectin got higher [53]. However, the degradation of OP occurred during CTUAE, and the degradation of HD OP (6.07%) was more obvious than that of FD OP (3.54%). That is because ultrasonic effect was more apparent for HD OP extraction (Fig. 2a), and ultrasonic waves caused structural change that was related to polysaccharide properties (monosaccharide composition, chain length, chain conformation, molecular weight and glycosidic bonds) and structure (linear, branched or cross-linked). HD OP tended to form dendritic chains with rod shape, while FD OP formed flexible chains with multiple-branch curly conformations (Fig. 5a). As a result, HD OP with linear rod-shaped conformations was more easily destroyed than FD OP with curly conformations in the process of CTUAE. More mechanical forces were produced by ultrasonic waves in the same spatial direction along long distances of linear chains in comparison with polysaccharides with curly conformations [11].

Finally, meshes of okra dry powders also affected GalA content. For GalA of HD OP, HD-M60 > HD-M80 > HD-M120, because a moderate increase of material fragmentation improved the OP extraction yield. The decrease of HD-M120 may be due to the weakening of pectin HG main chain width and rigidity, and OP was easier to be damaged by heat and ultrasound during the extraction process. As HD OP was obtained by CTUAE, U-HD-M60, U-HD-M80 and U-HD-M120 all increased by 5.86, 6.00 and 6.36%, respectively. Relatively speaking, GalA of FD OP was generally higher than that of HD OP, and it increased further during CTUAE, that is, U-FD-M60, U-FD-M80 and U-FD-M120 increased by 0.84, 7.37 and 2.53%, respectively. In general, the effect of CTUAE on HD OP was higher than FD OP, and it was further indicated that CTUAE technique was an effective method to improve traditional pectin extraction, which was consistent with the results above (Sections 3.1.1 and 3.1.2).

### 3.3.2. Molecular weight

Molecular weight distribution of polysaccharides plays an important role in structural, functional and biological characteristics. Elution of the pectin detected by light scattering (LS) and refraction (RI) rates can provide information about the molar mass of the pectin [20]. The elution curve of OP molecular weight is shown in Fig. 8 and corresponding estimated values of its molecular properties are given in Table 1. The RI signal is proportional to the polymer concentration, while the LS signal depends on the molecular weight, particle size and concentration of OP molecules eluted by the chromatographic column [57]. LS can provide information about particle size, molar mass, and concentration of the polymer, because the polarized light intensity of molecules is proportional to its hydrodynamic volume [58]. One peak of LS and RI was observed simultaneously by detectors at the elution time from 25 to 35 min, indicating the presence of high molar mass polymer. The peaks of elution results coincide with each other and are close to normal distribution, indicating that OP homogeneity was relatively high within the determined molar mass range. \( M_w / M_n \) in Table 1 ranges from 1.02 to 1.05. It was further indicated that OP was relatively homogenous [25]. The elution time of larger molecules with higher mass was the fastest because their retention time was shorter; while that of molecules with smaller mass was at the end of the process [58], so only peaks with overlapping areas and the largest components were discussed. Table 1 shows \( M_w = 5.62 \times 10^5 \text{ Da} \) and \( M_n = 5.50 \times 10^5 \text{ Da} \), while the LS signal depends on the molecule weight, particle size and concentration of OP molecules eluted by the chromatographic column [57].

The molecular weight distribution of OP was significantly affected by drying and CTUAE techniques. It was concluded that \( M_w \) of FD OP was higher than that of HD OP. Due to the removal of the hydration layer of some polysaccharides during HD, the structure of polysaccharides was destroyed, and then high-temperature thermal degradation resulted in a significant decrease of \( M_w \) [59]. Moreover, \( M_w \) of OP through CTUAE was reduced by 2.05–15.41%, indicating that ultrasonic waves can properly degrade the pectin. The research showed that the pectin degradation may become more serious when the intensity of ultrasonic treatment was higher, which was also observed by Ref. [43]. Ultrasonic waves degraded the pectin mainly through chemical action (increasing the transformation of free radicals) and mechanical action (breaking down polysaccharide clusters). During the ultrasonic degradation of pectin chains, the secondary bonds between the molecules of the pectin chains were firstly destroyed, and then the higher order structures of the pectin chains were destroyed, resulting in the loosening of the macromolecular structure and the breaking of the glycosidic bonds. Therefore, the macromolecular pectin depolymerized during CTUAE process, the molecular weight decreased, which may lead OP easier digested by human body.

In terms of meshes of okra dry powders, \( M_w \) of M80 OP was the highest, while that of U-M60 OP became the highest after using CTUAE. In addition, \( M_w \) of M60, M80 and M120 OP through CTUAE were all
reduced by 2.03–2.05%, 5.42–12.37% and 10.43–15.41%, respectively. It was shown that the degradation of OP got stronger with finer meshes of dry powders, which might be related to the contact area between ultrasound and OP. Hu et al. [12] found that $M_w$ increased with the decrease of peel particle size and the distribution of pectin became more uniform. Here, the milling and ultrasonic process may generate higher shear force to break the okra tissue cells, so the cellulose network was broken under the high-strength mechanical action and OP was cut into smaller fragments. OP suffered mechanical degradation, resulting in a decrease in $M_w$. Overall, mechanical force can break down the cell wall of plants, releasing strong cell wall-bound pectin components, or through mechanical degradation to obtain a more uniformly distributed products. Moreover, $M_w$ of HD OP and FD OP were reduced by 2.03–10.43% and 2.05–15.41% in the process of CTUAE, respectively, so it was indicated that ultrasound was easier to degrade FD OP than HD OP. It was reviewed effects of ultrasound on $M_w$ of the two common pectin products (citrus and apple pectin), and also concluded that $M_w$ was all significantly reduced because of ultrasound [60]. Due to the strong mechanical force and sonochemical reaction provided by ultrasonic treatment, the destruction of glycoside bonds was generated, as shown in Fig. 6.

### 3.3.3. Particle size of OP

The influence of CTUAE technique on particle size of OP is given in Table 1. Compared with OP extracted by common MSE (1264–8852 nm), the average particle size of OP (811–3647 nm) significantly decreased by 13.95–63.82% after 30 min of CTUAE, so it can be said that ultrasound caused a significant reduction of OP particle size. Meanwhile, Section 3.3.2 also showed that ultrasonic treatment reduced $M_w$ of OP by 2.05–15.41%. It was inferred that the reduction of OP particle size was notably correlated with that of $M_w$. It was verified that ultrasonic treatment was an effective method to reduce hydrodynamic diameter and $M_w$ of the pectin [26]. It was reported that polysaccharides of peach juice were degraded by ultrasonic treatment with 6.67 W/mL for 15 min, and then their uniformity was enhanced but particle size was reduced significantly [61]. It was also gained that $M_w$ of pectin in orange juice decreased through ultrasonic treatment with 0.42 W/mL for 2 min, and particle size was reduced by 50% [62]. The significant reduction of particle size of OP was in relation to the microstructure destruction of okra tissue caused by cavitation during CTUAE (Figs. 3 and 6). Cell walls of okra were ruptured because of ultrasound waves and the pectin was cut into smaller pieces [63]. In the process of CTUAE, the wide-spread rupture of molecular chains led to the degradation of OP, the reduction of $M_w$, the decrement of pectin molecule size and the enhancement of uniformity, so the reduction of particle size in OP system was observed.

For OP extracted by MSE without ultrasound, the average particle size of HD OP was 82.39% larger than that of FD OP. Nevertheless, HD OP was just 2.34% larger than that of FD OP after CTUAE. On the one hand, the particle size of HD OP was larger than that of FD OP, corresponding to the effect of drying methods on pectin structure mentioned in Section 3.1.3. Pectin is a naturally amphiphilic material that the auto-aggregation and intermolecular aggregation appear normally when dispersed in water [64]. Here, particle sizes of HD and FD OP decreased by 42.91–63.82% and 13.95–35.82% after CTUAE, respectively; and it was deduced that the aggregation of FD OP was easier than that of HD OP. Because the side chains can support the rigidity of pectin molecules, and limits the form of aggregation, entanglement and connection areas [44], ultrasonic waves reduced side chains of FD OP and made it easier to aggregate. It can also be seen from SEM and AFM (Figs. 4-5) that FD OP by CTUAE experienced obvious aggregation behavior. On the other hand, the influence of ultrasound on HD OP was significantly greater than that of FD OP, which was consistent with aforementioned results of extraction yields, GalA and $M_w$, and it also can be said that CTUAE had advantages of improving traditional pectin extraction methods.

In addition, for HD OP, M80 OP had the largest particle size. However, for FD OP, the larger the meshes of okra dry powders, the larger the particle size and $M_w$ of OP. Previous studies have found that particle size was highly positively correlated with $M_w$ of pectin [65].

### 3.4. Functional characteristics of OP

#### 3.4.1. Texture properties

Gel properties are important indicators of pectin functional characteristics, and hardness and cohesiveness are important indicators of gel properties. Hardness is related to the gel strength, and cohesiveness refers to the internal adhesion of the gel, which reflects the compactness of gel structure and the ability to resist external damage [27]. Due to hydrogen bonding between pectin molecules, the pectin can form gels under acidic and high sugar concentration conditions [66]. Fig. 9 shows gel properties of different OP. Compared with control group, the hardness, gumminess, chewiness and resilience of the gel samples were significantly increased after OP added. Meanwhile, it can be seen that the gel strength related to hardness of OP was good. Adhesiveness, springiness and cohesiveness were decreased. Due to OP, the gel samples became harder and firmer, rather than sticking and bouncy. As shown in Fig. 9a, trends of gel strength, gumminess and chewiness were the same, while CTUAE obviously improved the three indicators. The gel strength is decided by the degree of pectin polymerization to a great extent, and each molecule must have at least two connection regions to form a continuous network [67]. CTUAE technique promoted the exposure of pectin connection sites and made the crosslinking of pectin denser, as can be seen from AFM (Fig. 5). Similarly, a significant increment was found in gel strength of κ-carrageenan under short-term ultrasonic treatment [68].

For HD OP, meshes of dry powders were inversely proportional to gel strength. The smaller the meshes of dry powders, the more chains were connected between pectin with denser entanglement, and the gel strength increased, which can be proved by AFM (Fig. 5). For FD-M120 OP, linking sites of original chains were dense enough, and pectin chains were fractured by CTUAE technique in a large area with the destruction of binding sites. Fig. 9b shows the adhesiveness of OP. CTUAE technique had no extremely significant effect on the adhesiveness of OP with different meshes, but significantly reduced that of FD OP. Fig. 9c shows that there is also no significant difference among the springiness of OP with different meshes, but can increase the resilience of OP. Overall, the resilience of HD OP was higher than that of FD OP. Fig. 9d shows that there is no significant effect on the cohesiveness of OP by different drying methods. However, the cohesiveness of M120 was reduced, which may be related to the strength of pectin chains. It has been reported that gel properties of pectin are also affected by internal properties such as DE, GalA, $M_w$ and neutral sugar composition [69].

#### 3.4.2. Rheological properties

Fig. 10a shows rheological relationship between shear rate and viscosity of OP. Each sample showed a typical shear thinning behavior, the viscosity decreased sharply as the shear rate from 1 to 50 1/s and then the Newtonian platform was observed at high shear rate from 50 to 100 1/s. This may be due to the entanglement of chains is restrained a little at low shear rates, and longer polysaccharide chains were further untangled at high shear rates [70]. The viscosity at low shear rate represented the shear strength of OP network formed in the solution, and showed the initial viscosity. Among all the groups, OP without ultrasonic-assisted treatment showed higher viscosity compared with OP through CTUAE. It was reported that fractured chains and untangling chain structure of the pectin were induced by ultrasonic waves [71]. This was also consistent with AFM (Fig. 5) that the regular reticular structure of OP was damaged.

The average viscosity of FD OP at 50 1/s was 9.35% higher than that of HD OP (Fig. 10a-c). This is because as the density of the polysaccharide chains increase with the increment of polysaccharide molecules per unit region, the chain segment density increased; hence
intermolecular hydrogen bonds and van der Waals force increased, resulting in the resistance to flow of the pectin solution [72].

For the viscosity of HD OP (Fig. 10c), HD-M60 > U-HD-M60 > HD-M80 > HD-M120 > U-HD-M80 > U-HD-M120. It was indicated that the viscosity of HD OP was positively correlated with meshes of okra dry powders; moreover, CTUAE technique can reduce that of OP which corresponded to molecular weight. It was also showed that the viscosity was mainly determined by molecular weight of pectin [34]. For the viscosity of FD OP, FD-M60 > FD-M120 > FD-M80 > U-FD-M80 > U-FD-M60 > U-FD-M120. It was clearly found that the viscosity of OP was reduced after CTUAE; while the influence of meshes on the viscosity of FD OP was irregular and not significant. It was reported that the decrease of polysaccharide viscosity was also related to the rupture of glycosidic bonds and the weakening of the microstructural network during ultrasonic-assisted extraction [73].

In addition, the pectin with more ester groups is presumed to have a higher viscosity, because methoxyl groups can limit the mobility of pectin molecules and enhance the anti-shear ability of the pectin solution [74]. Higher DE values lead to more hydrophobic interactions with the increment of the viscosity [16], and it may be related to GalA [39], side chains, molecular weight and polydispersity [26].

Fig. 10d-g shows storage modulus $G'$ and loss modulus $G''$ of OP with angular frequency. Polysaccharide is a viscoelastic material with both solid and fluid behaviors. $G'$ represents the elastic part and $G''$ represents the viscous one. When $G'' > G'$, the polysaccharide solution shows fluid behaviors and is considered as colloid properties. When $G' > G''$, the polysaccharide solution exhibits solid behaviors and is considered gel properties. $G'$ and $G''$ of all samples increased with angular frequency, indicating that all samples showed angular frequency dependence [75]. As shown in Fig. 10d-e, for HD OP, $G'$ was always greater than $G''$, and HD OP was elastic and always in a gel state. Fig. 10f shows FD OP without ultrasonic-assisted treatment, and the intersection point of $G'$ and $G''$ well indicated the viscoelastic behavior of the material, that is, the beginning of viscous behavior or near solution state. The smaller the angular frequency of the intersection point, the smaller the elastic contribution [17]. The intersection points of FD-M60, FD-M80 and FD-M120 OP were 76.5, 95.6 and 90.3 rad/s, respectively, indicating that FD-M80 had better elasticity. These phenomena may be attributed to enhanced untangling and rearrangement of polysaccharide chains during long oscillations, and intersection points between polysaccharides were changed [16]. The study [16] has shown that $G'$ and $G''$ of acidic-extracted polysaccharides were larger than that of alkaline-extracted ones with positive correlation with molecular weight, and the viscoelasticity of okra polysaccharides had molecular weight dependence. In Fig. 10g, there is no intersection between $G'$ and $G''$, and the elastic behavior of FD OP increased after CTUAE. In conclusion, $G'$ and $G''$ of OP could be affected by different drying methods and meshes through CTUAE.

3.4.3. Emulsifying properties

OP can reduce the interfacial tension between oil and water phase, and can be used to prepare emulsion effectively. EA (10.42–77.92%) and ES (33.22–87.35%) of OP molecular aqueous solution were significantly different (Fig. 11a); while all the OP had quite good emulsifying properties, and can be comparable to the reported research of [20].

CTUAE technique reduced EA of HD-M60 and HD-M80 OP, but increased HD-M120 OP. On the contrary, CTUAE technique increased EA of FD-M60 OP, but decreased EA of FD-M80 and FD-M120 OP, and the regular conclusions of OP EA with different drying methods and meshes could not be found temporarily. The emulsifying capacity of OP is usually related to the chemical structure of main chains of biological polymerization, macromolecular properties of pectin chains (molecular weight, branching degree of side chains, hydrodynamic volume), functional units (such as proteins and ferulic acid) and the control of intra-chain and interchain interactions. Evaluating the respective advantages of these structural parameters for emulsification and their contribution to emulsifying properties of OP can be a good approach to improve the emulsifying properties of OP.
Fig. 10. Viscosity and viscoelasticity of OP.
to pectin production capacity is still under development [76].

Compared with FD OP, HD OP showed better ES potential (Fig. 11b), [77] showed that dry heat treatment could be used as a simple method to improve the emulsifying properties of the pectin. More usefully, ES of OP after CTUAE became higher, especially for FD OP. On the one hand, ultrasonic waves can promote the formation of denser interface layers of pectin emulsion [26]. On the other hand, the emulsifying ability of the pectin depends on its RG-I, molecular weight and other structural characteristics [78], and ultrasonic waves can enrich RG-I [79]. Side chains of neutral sugar in RG-I contribute to long-term ES and provide effective spatial stability due to the formation of thick interface layers, thus preventing emulsion separation [80]. It was shown that ultrasound improved ES of a salad dressing containing acacia gum to a greater extent compared with MSE, because more stable oil-in-water emulsion was generated by the reduced particle size of acacia gum after ultrasonic treatment [81]. In general, OP after CTUAE showed stronger ES.

3.4.4. WHC and OHC

WHC and OHC of OP ranged from 4.15 to 9.64 g/g and 2.71 to 5.25 g/g, respectively. Results showed that all OP had fairly good WHC and OHC, which was comparable to the reported research of [82]. WHC and OHC represent the amount of water and oil held and absorbed by pectin samples, respectively. Both WHC and OHC are important factors affecting food texture through interactions between other components in food processing [6].

In Fig. 11c-d, CTUAE technique significantly increases WHC of M60 and M80 OP, but decreases WHC of M120 OP. [83] showed that ultrasound could induce cavitation in pectin structure and improve water permeability and absorption rate. The non-covalent intramolecular and intermolecular bonds are destroyed, so the hydration is more easily generated between exposed hydrophilic polysaccharide groups and water molecules [83]. Due to CTUAE, cavitation and shear forces damaged the pectin structure (Section 3.2), thus the level of structural completeness was reduced and its WHC was affected. For the decrease in WHC of M120, its structure was more easily damaged [11].

The average WHC and OHC of HD OP (8.30 g/g and 4.67 g/g) were higher than that of FD OP (6.21 g/g and 3.57 g/g). M80 OP had the highest WHC in HD groups, while M60 OP had the highest WHC in FD groups, and CTUAE can improve all the aforementioned WHC. High WHC of the pectin suggests that it can effectively overcome dehydration shrinkage problems, such as yogurt [84]. However, an opposite trend was observed for OHC (circled in red in Fig. 11c-d) in comparison with WHC. In general, HD OP exhibited higher OHC due to the exposure of more hydrophobic residues during heating, which might increase the physical entrapment of oil [85]. Total charge density and hydrophilicity are the most important factors that may affect OHC of the pectin [84]. Various factors also may influence WHC, such as the concentration of free hydroxyl groups in the structure, calcium content, particle size and porosity of the pectin, ionic strength and ionic form in solution, pH and temperature [86].

3.4.5. Thermogravimetric

TG is used to evaluate effects of different treatments on the thermal stability of OP, as shown in Fig. 12. TG curves of all OP were similar and there were two thermal degradation regions in temperature region of 35-600°C. The temperature region of thermal degradation at the first stage was below 200°C, the evaporation of free and bound water of OP was generated with a weight loss of about 20%, and it was also reported in the study of [87]. The second stage of thermal degradation from 200 to 400°C was attributed to pyrolytic decomposition of OP chains with a
rapid and huge weight loss of 60%, including primary and secondary decarboxylation, bonds or functional groups cracking, and chain breaking. For complex reactions in the gas phase, such as the oxidation of volatile organic compounds to water, carbon dioxide, and carbon monoxide, firstly, bonds formed by neutral sugars thermally degraded, due to the most unstable property. Subsequently, polygalacturonic acid units hydrolyzed, and then glycoside bonds formed by uronic acids degraded at higher temperatures [87].

In Fig. 12, weight loss curves show that the average weight loss of FD OP (66.60%) is greater than that of HD OP (64.92%), but the average endurance temperature of HD OP in the first derivative curve is higher, so it is difficult to judge which drying method can bring better thermal stability for OP. In addition, the average weight loss of HD OP (64.92%) was greater than that of U-HD OP (62.00%). The average weight loss of FD OP (66.60%) was also greater than that of U-FD OP (61.82%). All this can demonstrate that CTUAE technique enhanced the thermal stability of OP. It was also found that polysaccharides treated by ultrasound had less weight loss than control [88]. The effective improvement of thermal stability after ultrasonic treatment may be related to molecular weight and microstructure of the pectin [56]. Interestingly, based on the first derivative curves, with the decrease of meshes of okra dry particles, the peak area became smaller and red-shifted to a higher temperature with higher thermal stability of OP. In the food processing industry, high thermal stability of the pectin indicates its potential application in hot processing [89].

3.5. Correlation coefficient and agglomerative hierarchical clustering (AHC) of OP

Correlation coefficient and AHC have been used to examine relationships between variables in Fig. 13. It has been reported that several inherently variable properties of the pectin, including $M_\text{w}$, DE and spatial conformation, can greatly affect their functional characteristics, and the exploration of main structure and spatial conformation of different pectin will provide a better understanding of functional characteristics; while the evaluation of specific contributions of single structure parameter to functional characteristics is still under development [90]. The correlation coefficient between structural, physico-chemical and functional characteristics of OP is shown in Fig. 13a. GalA of OP was positively correlated with gel strength, but negatively correlated with particle size and OHC. It was also showed that GalA of citrus increased with the decrease of particle size [26]. DE of OP was negatively correlated with OHC and ES. $M_\text{w}$ of OP showed a significant and high positive correlation with viscosity and EA. [91] showed the correlation between high $M_\text{w}$ of pectin and high emulsifying properties. Cui et al. [92] and Dranca et al. [93] also proposed that high viscosity of pectin might be related to high $M_\text{w}$ of pectin and high emulsifying properties. Cui et al. [92] and Dranca et al. [93] also proposed that high viscosity of pectin might be related to high $M_\text{w}$ of pectin and high emulsifying properties. The relationship between structural and functional characteristics can be identified by correlation analysis, as the selection of one parameter leads to that of another correlated parameter. However, only the correlation analysis cannot give a clear illustration of inter-relationships with the other traits, as only two traits are taken into account at a time while the connection with other traits are neglected [94]. Therefore, AHC was performed to reveal inter-relationships between different traits in a complete data set.

As shown in Fig. 13b, clusters are formed through correlation coefficients between various functional characteristics. It was easy to find parameters related to specific function, and help pectin selectors determine the optimal linear combination of functional parameters. It is known that the pectin can be used as emulsifier, thickener, stabilizer and gelling agent, but different functional characteristics of the pectin will be considered according to different product needs. The heat map (Fig. 13b) visualized the strength of various functional characteristics of OP, and can help pectin selectors quickly find suitable OP. Based on the similarity of all functional indicators, AHC was performed to help select optimum groups. For example, the pectin is used as a thickener in fruit drinks, soft drinks, sauces and sorbet at low concentration, which is
related to rheological properties, and HD-M60 OP was a better choice. The pectin also can be used as gelling agents for the jelly-like consistency of jellies and jams, which is related to their gel properties, and U-FD-M80 OP was a better choice. [95] reported that the pectin alone could improve ES of cod liver oil, which focused on emulsifying properties, while non-significant correlations are in white. (b) Heat map of AHC of functional indicators of OP. The color temperature scheme indicates the level of corresponding variables, ranging from the minimum (blue) to the maximum (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

Okra pods are rich in water-soluble polysaccharides, including pectin. The extraction yield, GaA, DE, Mw, particle size, gel, rheological and emulsifying properties, WHC, OHC and thermal stability of OP were significantly affected by different preparations. The molecular chain of HD OP was rigid dendritic, while that of FD OP was flexible with tangled multiple branches. The yield, GaA, DE and Mw, viscosity of FD OP increased, particle size decreased, and the reduction of meshes improved thermal stability. WHC and OHC of HD OP were more prominent, as meshes of dry powders decreased, GaA, viscosity and emulsification ability decreased, while gel strength and thermal stability increased. CTUAE increased the yield and GaA, and decreased DE, Mw and particle size of OP. The technique also improved gel strength, resilience and viscoelasticity, enhanced ES, WHC and thermal stability, and reduced viscosity. Ultrasonic effects on HD OP were more significant than that on FD OP. Finally, the correlation between functional and structural characteristics of OP was quantified.

It was provided unique information about most suitable production techniques of OP for different types of food applications, and linked these effects with basic understanding on the molecular scale.

CRediT authorship contribution statement

Lei Zhang: Data curation, Writing – original draft, Funding acquisition. Yang Hu: Data curation, Writing – original draft. Xue Wang: Data curation, Writing – original draft. Ao Zhang: Data curation, Writing – original draft. Olugbenga Abiola Fakayode: Writing – review & editing. Haile Ma: Supervision. Cunshan Zhou: Supervision.

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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