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Extraction solvents influence physicochemical, structural, and functional properties of polysaccharides isolated from okra seeds: A comparative study

[1]Zhang, G., [2]Wei, X., [2]Song, X., [2]Yan, Y., [2]Guo, M. and [2]*Xu, K.

[1]*Suzhou Academy of Agricultural Sciences, Suzhou, 215106, China*
[2]*College of Food Science and Engineering, Shandong Agricultural University, Tai'an, 271018, China*

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Abstract

The present work investigated the physicochemical and functional properties of okra seed polysaccharides extracted by three different solvents, including molecular structure, antioxidant activities, pancreatic lipase, and α-amylase inhibitory activities. Extraction solvents showed remarkable influence on the extraction yield, structural composition, and functional properties of okra seed polysaccharides. Okra seed polysaccharides extracted by hot water, citric acid, and alkali were named as WOSP, COSP and AOSP, respectively. Results showed that arabinose (23.08 - 33.91%) was the main monosaccharide among three kinds of okra seed polysaccharides. The molecular weight of COSP (706.7 kDa) was much higher than that of AOSP (397.6 kDa). COSP had stronger pancreatic lipase and α-amylase inhibitory activities, but weaker antioxidant activities than AOSP. WOSP exhibited higher emulsifying activity (58.77%) and emulsion stability (57.77%) than COSP and AOSP. Thermal treatment significantly improved the emulsifying capacity among three kinds of okra seed polysaccharides, especially the creaming index which increased 190% in the case of COSP stored for 30 d. These results suggested that appropriate solvent could play crucial role in the structure of okra seed polysaccharides and their food potential application.

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Introduction

Plant-derived polysaccharides are natural polymers which possess a wide variety of biological activities, including antioxidation, anti-inflammatory, prebiotic, anti-diabetic, and anti-tumour (Chen *et al*., 2019). Extraction of polysaccharides using different solvents show higher efficiency and lower cost compared to other techniques, including ultrasonic, microwave, and pulsed electric field. Hot water, acid, and alkali are three widely used solvents to extract polysaccharides for various requirements and purposes. On one hand, the use of acid or alkaline can break hydrolysable linkages between cell wall polysaccharides and proteins. On the other hand, it could transform water-insoluble polysaccharides into water-soluble ones in order to increase the extraction yield (Fang *et al*., 2020). Compared to water extraction, acidic extraction produced a higher amount of galacturonic acid (GalA) in blackberry fruit polysaccharides, which increased their glycation end-product inhibitory activity (Dou *et al*., 2021). Similarly, alkali-soluble polysaccharide from purple sweet potato exhibited better anti-inflammation properties than that of water-soluble polysaccharide (Chen *et al*., 2019).

Okra (*Abelmoschus esculentus*) has exhibited various potential health-promoting properties. Okra seedless pods are abundant in water-soluble polysaccharides, mainly comprising pectic polysaccharides, and to a lower extent, xyloglucan and glucuronoxylan (Sengkhamparn *et al*., 2009a; 2009b). Specifically, rhamnogalacturonan-I (RG-I) segments composed of (1,4)-galacturonic acid and (1,2)-rhamnose residues, with disaccharide side chains have been identified as the major pectic polysaccharides in okra (Liu *et al*., 2018). Okra polysaccharides with higher side chains and lower

*Corresponding author.
Email: xukang@sdau.edu.cn

molecular weight could be more beneficial to the intestinal microbial ecology (Wu *et al*., 2022). Xu *et al*. (2020a) noted that okra seedless pods and seeds could improve the nutritional quality and slow down the starch digestion of wheat bread through the delivery of phenolic compounds and dietary fibre. Remarkably, okra seeds possess a much higher content of bound phenolics with better antioxidant activities compared to seedless okra pods. These compounds which play a crucial role in prebiotics could interact with and crosslink cell wall polysaccharides to form a strong network (Araptitsas, 2008; Macagnan *et al*., 2016). However, few related studies on the polysaccharides derived from okra seeds could be found.

In the present work, we hypothesised that differences among extraction solvents would lead to remarkable changes in the chemical structures and biological activities of okra seed polysaccharides as a result of the presence of bound phenolics. Therefore, okra seed polysaccharides were firstly extracted by sequential extraction using hot water, citric acid, and alkali. The composition, microstructure, and physicochemical properties of the extracted polysaccharides, as well as their biological activities, including *in vitro* antioxidant activities and enzyme inhibitory activities, were then investigated. The obtained results would improve our understanding on further exploitation of okra seed polysaccharides as a new functional food ingredient.

Materials and methods

Materials and regents

Okra samples were harvested at the 13th day after blossom from the National Vegetable Research Center of Shandong Agricultural University (Tai'an, Shandong Province, China). Seeds and seedless pods of fresh okra were separated manually. Okra seeds were placed in a food dehydrator (Hamilton Beach, Glen Allen, USA), and air-dried after achieving moisture below 10%. Afterwards, they were ground into flour using a DF-20 grinder (Xinnuo Equipment Co., Ltd., Shanghai, China), and sieved to pass a screen of 250 μm. The collected flour was sealed in vacuum polyethylene bags, and stored at -18°C for further analysis.

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), GalA, glucuronic acid (GlcA), and monosaccharides standards [rhamnose (Rha), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), and glucose (Glc)] were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Pancreatic lipase, α-amylase, and *p*-nitrophenyl butyrate (*p*NPB) were purchased from Shanghai Aladdin BioChem Technology Co., Ltd. (Shanghai, China). All other chemicals were at least of analytical grade.

Preparation of okra seed polysaccharides

Okra seed flour was washed with 95% ethanol (1:15, w/v) for 2 h (× three times) under continuous stirring to remove non-cell wall components. After centrifugation (4,500 rpm, 10 min, 20°C), the alcohol-insoluble residue (AIR) was washed with four times the volume of acetone, and centrifuged to remove alcohol-soluble components such as fatty acids, free phenolics, and sugars, and minimise the activity of cell wall degrading enzymes. The AIR pellet was air-dried at ambient temperature overnight.

For the hot water extraction, AIR was extracted with deionised water (1:15, w/v) at 100°C for 1 h under continuous stirring (Xu *et al*., 2020b). The supernatant (S1) was separated from the precipitate by centrifugation at 4,500 rpm for 10 min, and cooled to room temperature. Afterward, the precipitate was divided into two equal parts (A and B) for further extraction using acid or alkali solvent.

For acid solvent extraction, precipitate A was mixed with distilled water (1:15, w/v) and adjusted to pH 2.0 using citric acid solution (4 mol/L), then incubated at 90°C for 2 h under continuous stirring (Roman *et al*., 2021). The acid-extracted supernatant (S2) was obtained after centrifugation at 4,500 rpm for 10 min. For alkali solvent extraction, precipitate B was mixed with 0.05 mol/L sodium hydroxide (1:15, w/v), and incubated at 80°C for 2 h under continuous stirring (Sun *et al*., 2021). The supernatant (S3) was obtained after centrifugation.

Subsequently, S1, S2, and S3 were adjusted to a final ethanol concentration of 70% (v/v), and kept at 4°C overnight. The precipitates were separated by centrifugation at 4,500 rpm for 10 min, and dissolved in deionised water. The ions were removed by dialysing in a dialysis bag of 3.5 kDa against water for 48 h, while changing the water every 4 h. The dialysate was freeze-dried to produce polysaccharides including WOSP, COSP, and AOSP. The yield of okra seed polysaccharides was defined as the mass ratio of polysaccharides and okra seed flour. All extractions were performed in duplicate.

Chemical characterisation

Moisture content was determined by drying 3 g of samples at 100°C for 3 h in a FX101-3 air dryer (Shuli Equipment Co., Ltd., Shanghai, China) following the AACC 44-15.02 method, and ash content was determined by incinerating 3 g of samples at 575°C for 8 h in a SX2 muffle furnace (Yangguang Equipment Co., Ltd., Shanghai, China) following the AACC 08-01.01 method (AACC, 2015). Protein was determined by the Bradford method using bovine serum albumin as standard (Bradford, 1976). Total polyphenol was evaluated by the Folin-Ciocalteu assay using gallic acid as standard (Xu *et al*., 2020a), and molecular weight (Mw) analysis were performed according to Xu *et al*. (2020b). Monosaccharide composition was measured using a polar column (30 m × 0.32 mm, 0.2 μm) (DM-2330, DIKMA, China) in a gas chromatograph (GC-2010, Shimadzu, Japan) equipped with a splitter injection port (split ratio 1:14) and a flame ionisation detector. Molecular weight (Mw) analysis was performed by high-performance size exclusion chromatography (HPSEC) coupled with a refractive index detector (RID-10), and equipped with a TSK guard column (35 × 4.6 mm; Tosoh Co. Ltd., Tokyo, Japan) and a TSK-GEL G4000PWxl column (300 × 7.8 mm; 10 μm).

Spectroscopic analyses

FT-IR spectra were performed from 600 to 4000 cm^{-1} in attenuated total reflection (ATR) mode, with a DTGS detector at a resolution of 4 cm^{-1} using 32 co-added scans (Vertex 70, Bruker, Rheinstetten, Germany). ^{1}H analyses were conducted using a 600 MHz AVANCE III NMR spectrometer (Bruker, Rheinstetten, Germany). Briefly, 100 mg sample was fully dissolved in 2 mL of 99.9% D_2O, and the ^{1}H NMR spectrums were scanned for 256 times.

Microstructure

Polysaccharides were stuck to carbon double sticky tape on a 25 mm diameter specimen holder, and observed under a NeoScope JCM-5000 scanning electron microscope (JEOL, Tokyo, Japan). Photomicrographs were taken in high vacuum mode with an accelerating voltage of 5 kV.

Zeta potential

Zeta potentials of the polysaccharide solutions (1 mg/mL) were analysed using dynamic light scattering (DLS) on a Malvern Zetasizer, Nano ZS90 (Malvern Instruments Limited, UK) at 25°C.

Emulsifying properties

Emulsifying activity (EA) and emulsion stability (ES) were investigated according to Xu *et al*. (2020b). Creaming index (CI) was defined as the percentage of the height of the emulsion phase and the total height of the emulsion, which was recorded multiple times within 30 d. Microstructures of droplets were observed using an IX73 fluorescence inversion microscope system (Olympus, Tokyo, Japan) at 25°C (Jiang *et al*., 2020).

Antioxidant activities

DPPH radical scavenging activity (DPPH-RSA) and ABTS radical scavenging activity (ABTS-RSA) were determined according to Xu *et al*. (2020b). Vitamin C (Vc) was used as the positive control.

In vitro pancreatic lipase and α-amylase inhibition assay

The *in vitro* pancreatic lipase and α-amylase inhibitory activity were determined following previously reported methods (Guo *et al*., 2018; Wang *et al*., 2018). For the pancreatic lipase inhibition assay, 100 μL of polysaccharide solution (0.25, 0.5, 1.0, 2.5, 5.0, and 10 mg/mL) was mixed with 200 μL of Tris buffer (50 mmol/L, pH 7.4) and 100 μL of pancreatic lipase solution (5 mg/mL), and incubated at 37°C for 10 min. Then, 100 μL of 2 mmol/L *p*NPB was added into the mixture, and incubated at 37°C for 15 min. The absorbance at 405 nm was measured, and the orlistat was used as the positive control. The percentage inhibition was calculated using Eq. 1:

$$\text{Inhibition (\%)} = \left[1 - \frac{\text{A sample} - \text{A sample blank}}{\text{A control}}\right] \times 100\% \quad \text{(Eq. 1)}$$

where, A sample = absorbance of the mixture of sample, buffer, pancreatic lipase, and *p*NPB solution, A sample blank = absorbance of the mixture of sample, buffer, distilled water, and *p*NPB solution and A control = absorbance of the mixture of distilled water, buffer, pancreatic lipase, and *p*NPB solution.

For the α-amylase inhibition assay, 200 μL of polysaccharide solution (0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 mg/mL) mixed with 400 μL of 20 mmol/L

sodium phosphate buffer (pH 6.9) containing α-amylase solution (2 U/mL) were incubated at 37°C for 10 min. Then, 300 μL of starch solution (0.5%) in 20 mmol/L sodium phosphate buffer (pH 6.9) was added as the substrate, and incubated for 15 min. Afterward, 2 mL of DNS reagent (10 mg/mL of 3,5-dinitrosalicylic acid, and 120 mg/mL of sodium potassium tartrate in 0.4 mol/L of sodium hydroxide solution) was added to terminate the reaction, and heated another 15 min at 100°C. After cooling down, the absorbance was measured at 540 nm, and acarbose was used as the positive control. The percentage inhibition was calculated using Eq. 2:

$$\text{Inhibition } (\%) = \left[1 - \frac{\text{A sample} - \text{A sample blank}}{\text{A control}}\right] \times 100\% \quad \text{(Eq. 2)}$$

where, A sample = absorbance of the mixture of sample, starch, α-amylase, and DNS solution, A sample blank = absorbance of the mixture of distilled water, starch, α-amylase, and DNS solution, and A control = absorbance of the mixture of distilled water, buffer, α-amylase, and DNS solution.

Statistical analysis

Each result was obtained from an average of three measurements. Results were expressed as mean ± standard deviation (SD). The data were statistically analysed by SPSS Statistics 22 (SPSS Inc., Chicago, USA), and the significant differences were compared by LSD test on the level of $p < 0.05$.

Results and discussion

Chemical compositions and structural characteristics

The yields of WOSP, COSP, and AOSP were 5.00 ± 0.12, 2.62 ± 0.11, and 5.20 ± 0.04%, respectively. As expected, the decreased yield of COSP was due to the breakage of glycosidic bonds by acid. AOSP displayed the highest extraction yield because the low-concentration alkali solution effectively destroyed the cell walls, which resulted in the change of hemicellulose into a water-soluble polysaccharide (Xiong *et al*., 2022). Neutral sugar, uronic acid, protein, ash, and total phenols exhibited notably different contents (Table 1). The protein content of AOSP was higher than those of WOSP and COSP, which probably because the high hydroxide ion content would lead to the swelling of the okra cell walls, and the disruption of the hydrogen bonds among polysaccharides, proteins, and various phenols (Bai *et al*., 2020). WOSP and COSP showed similar ash contents, but they were slightly higher than AOSP. On the other hand, AOSP showed the highest total polyphenol content.

All three polysaccharides were rich in neutral and acidic monosaccharides. The primary monosaccharides were Ara, Gal, and Xyl (over 10%), indicating the presence of xylan-type hemicelluloses. Ara was the most abundant neutral sugar in all three polysaccharides, with the values of 23.08, 26.79, and 33.91% in WOSP, COSP, and AOSP, respectively. The second highest amounts of neutral sugars in WOSP and AOSP were Xyl, accounting for 21.02 and 14.31%; whereas for COSP, Gal (19.65%) was much higher than Xyl (10.42%), indicating the presence of arabinogalactan. The low levels of Rha and Glc indicated the presence of low rhamnogalacturonan or xyloglucan regions. There were notable differences in the uronic acid concentrations among different samples. In all cases, GalA was more abundant than GlcA, and GalA in COSP was much higher than other two samples. Results showed that all polysaccharides belong to heteropolysaccharides, which were composed of various sugar units interconnected by glycosidic bonds, providing structural and bioactive diversity (Muthusamy *et al*., 2021).

The molecular weight distribution indicated that they were heterogeneous due to the presence of multiple asymmetric elution peaks. Both WOSP and AOSP showed similar molecular weight and distributions with three broader peaks of 4954.2 kDa (14.41%), 94.2 kDa (21.13%), and 5.8 kDa (64.46%); and 5132.5 kDa (18.78%), 81.0 kDa (22.11%), and 6.6 kDa (59.11%), respectively. COSP explicitly presented a relatively higher molecular weight, indicating the extraction of larger molecules from the cell walls composed of pectic polysaccharides by the citric acid solution. Generally, higher molecular weight polysaccharides tend to have higher biological activities with more binding affinity (Muthusamy *et al*., 2021). Zeta potentials can reflect the stability of the solution or colloid. All polysaccharides were anionic polysaccharides, which had negative charges due to the presence of uronic acid and phenolic groups. It was noted that COSP and AOSP possessed higher zeta potential values with no remarkable difference, corresponding to the higher contents of uronic acid and total polyphenol.

Table 1. Chemical compositions and structural characteristics of different okra seed polysaccharides.

Parameter	WOSP	COSP	AOSP
Yield (%)	5.00 ± 0.12^{a}	2.62 ± 0.11^{b}	5.20 ± 0.04^{a}
Moisture (%)	13.01 ± 0.33^{b}	8.12 ± 0.27^{c}	14.26 ± 0.42^{a}
Protein (%)	5.10 ± 0.01^{b}	5.16 ± 0.01^{b}	5.88 ± 0.01^{a}
Ash (%)	6.86 ± 0.15^{a}	6.73 ± 0.31^{a}	5.21 ± 0.24^{b}
Total polyphenol (%)	1.06 ± 0.04^{b}	0.82 ± 0.02^{c}	1.34 ± 0.03^{a}
Monosaccharide composition	73.96 ± 2.56^{b}	80.78 ± 1.02^{a}	71.48 ± 3.28^{b}
Rha (%)	3.30 ± 0.11^{b}	4.65 ± 0.20^{a}	4.57 ± 0.14^{a}
Ara (%)	23.08 ± 0.97^{b}	26.79 ± 0.68^{b}	33.91 ± 1.54^{a}
Gal (%)	17.16 ± 0.47^{b}	19.65 ± 1.03^{a}	11.02 ± 0.77^{c}
Glu (%)	2.30 ± 0.11^{b}	4.04 ± 0.18^{a}	1.01 ± 0.09^{c}
Xyl (%)	21.02 ± 1.62^{a}	10.42 ± 0.83^{c}	14.31 ± 1.34^{b}
GalA (%)	4.76 ± 0.12^{b}	14.16 ± 1.33^{a}	4.45 ± 0.25^{b}
GlcA (%)	2.33 ± 0.09^{b}	1.07 ± 0.04^{c}	3.12 ± 0.12^{a}
Molecular weight	454.4 ± 19.7^{b}	706.7 ± 32.8^{a}	397.6 ± 47.1^{c}
Peak 1 (kDa)	4954.2 ± 99.3^{a}	693.3 ± 48.2^{b}	5132.5 ± 121.9^{a}
Area proportion (%)	14.41 ± 0.34^{c}	60.35 ± 1.05^{a}	18.78 ± 0.76^{b}
Peak 2 (kDa)	94.2 ± 5.2^{a}	98.4 ± 7.5^{a}	81.0 ± 6.1^{b}
Area proportion (%)	21.13 ± 1.26^{a}	19.59 ± 1.39^{a}	22.11 ± 1.74^{a}
Peak 3 (kDa)	5.8 ± 0.4^{a}	6.9 ± 0.2^{a}	6.6 ± 0.3^{a}
Area proportion (%)	64.46 ± 2.8^{a}	20.06 ± 2.6^{b}	59.11 ± 3.1^{a}
Zeta potential (mV)	-17.2 ± 0.99^{b}	-28.4 ± 0^{a}	-30.05 ± 1.77^{a}

Different lowercase superscripts in similar columns indicate significant difference ($p < 0.05$). Values of monosaccharide compositions are the sum of Rha, Ara, Gal, Glu, Xyl, GalA, and GlcA.

FTIR and NMR analyses

Based on Figure 1A, all polysaccharides exhibited intense broad bands between 3200 and 3600 cm^{-1}, which was due to the stretching vibration of O-H groups of the inter- and intra-molecular hydrogen bonding of the polysaccharide backbone (Roman *et al.*, 2021). However, there were some distinctions in the peak intensities and positions for several characteristic bands. The weak absorption peaks at 3000 - 2800 cm^{-1}, which corresponded to C-H stretching bands of the -CH or -CH_2 groups of polysaccharides, were less intense for WOSP compared to the other two samples. The absorption peak around 1740 cm^{-1}, which corresponded to the stretching vibration of C=O in the carboxyl groups (-COOH), was relatively more intense in COSP (larger peak area than WOSP and AOSP). Consistent with Table 1, these results suggested that acid extraction were more likely to produce higher uronic acids contents. The absorption peak at 1640 cm^{-1} represented the O-H bending vibration of adsorbed water (Wang *et al.*, 2022). The difference in peak intensities suggested that AOSP contained more

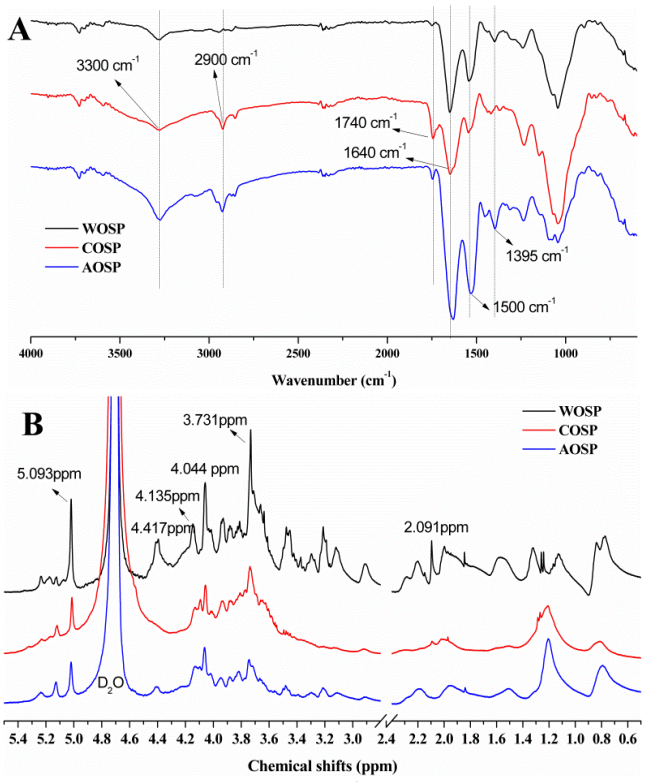

Figure 1. (A) FT-IR and **(B)** ^{1}H NMR spectra of okra seed polysaccharides extracted by three solvents.

moisture. Besides, the peak at 1740 and 1640 cm^{-1} were the typical signals of esterified and free carboxyl groups in polysaccharides (Roman *et al*., 2021), which could provide information to calculate the degree of esterification (*i.e.* the relative proportion of the area of the peak at 1740 cm^{-1} to the sum of the 1740 and 1620 cm^{-1} peaks). The peak intensities at about 1500 cm^{-1} were characteristic of stretching vibrations of the aromatic rings such as lignin, which is the most abundant renewable natural phenolic polymer, and is one of the main components of the secondary cell walls in plants. AOSP showed the highest peak intensity at 1500 cm^{-1} in AOSP, followed by WOSP and COSP, which is in agreement with the total polyphenol contents reported in Table 1. A signal at approximately 1395 cm^{-1} was observed for WOSP and AOSP, which might be ascribable to the non-symmetrical and symmetrical CH_3 bending.

As shown in Figure 1B, the polysaccharides extracted by different solvents contained the α-galactosyl and rhamnosyl residues. The three spectra displayed an intense signal at 4.723 ppm due to the presence of D_2O medium. The 1,2-linked-rhamnosal units and t-α-rhamnosyl residues could only be observed in WOSP. Moreover, the significant signals at 2.091, belonging to an O-acetyl group, suggested the presence of O-acetylated GalA in WOSP. Another novelty of α-galactose substitution at O-4 of the backbone rhamnose residue (RG-I) was seen in WOSP and AOSP. However, no signal at about 4.417 was found in COSP, which implied RG-I containing α-galactose substitution was not present under acidic conditions. Similarly, the anomeric signals of GalA residues among all samples, with a proton chemical shift of 3.731, suggested that non-substituted GalA existed and belonged to HG.

Morphology analysis

As shown in Figure $2A_2$, WOSP presented a small irregular sheet with a rough surface at 500× magnification, while COSP and AOSP showed a state of massive lumpy and thick bulk (Figures $2B_2$ and $2C_2$). Despite the apparent morphological differences, they were all sporadically distributed as long thin fibrous sticks. At 10,000× magnification, WOSP displayed irregular protrusions on the surface, and possessed small amounts of cavities with varying sizes (Figure $2A_3$). By contrast, COSP and AOSP showed more homogenous protrusions, and had intact cell structures. But the elliptic structure of AOSP was much smoother and bigger than COSP (Figures $2B_3$ and $2C_3$).

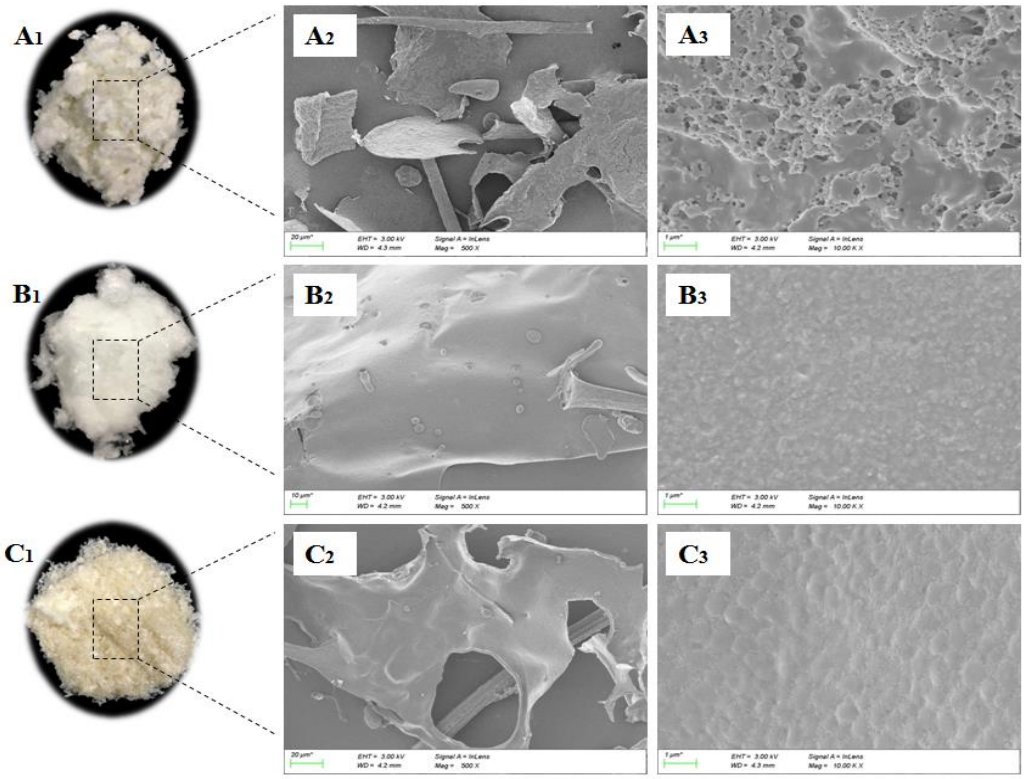

Figure 2. Surface morphological images of **(A)** WOSP, **(B)** COSP, and **(C)** AOSP. The subscripts 1, 2, 3 refer to the original, 500×, and 1,000× magnification, respectively.

Emulsifying properties

WOSP displayed the highest EA of 58.77% and ES of 57.77%, followed by COSP and AOSP (Figure 3A). The protein attached to the polysaccharides was a key factor affecting the emulsifying properties. For ES, it was in line with EA in all cases. Although AOSP showed slightly higher protein content than other samples (Table 1), WOSP exhibited better ES, which was probably due to its smaller particle size. WOSP acted as a holding space to position the oil droplets on the surface of polysaccharides, thereby preventing the coalescence of oil droplets, and improving emulsion stability (Huang *et al*., 2020).

Optical micrographs of emulsions during storage for 30 days are shown in Figure 3C. All fresh emulsions were homogeneous, which indicated that the repulsive electrostatic interactions among the droplets in the emulsion were sufficient to counteract the attractive interactions, and prevent further flocculation (Tavasoli *et al*., 2022). However, with the extension of storage time, the oil droplets started to aggregate, and formed a cream layer on the top. As seen in Figure 3B, a negative correlation between CI and storage time was observed. WOSP and AOSP displayed remarkably higher CI than COSP, indicating a greater extent of droplet flocculation and coalescence in COSP (Figure 3C). This phenomenon

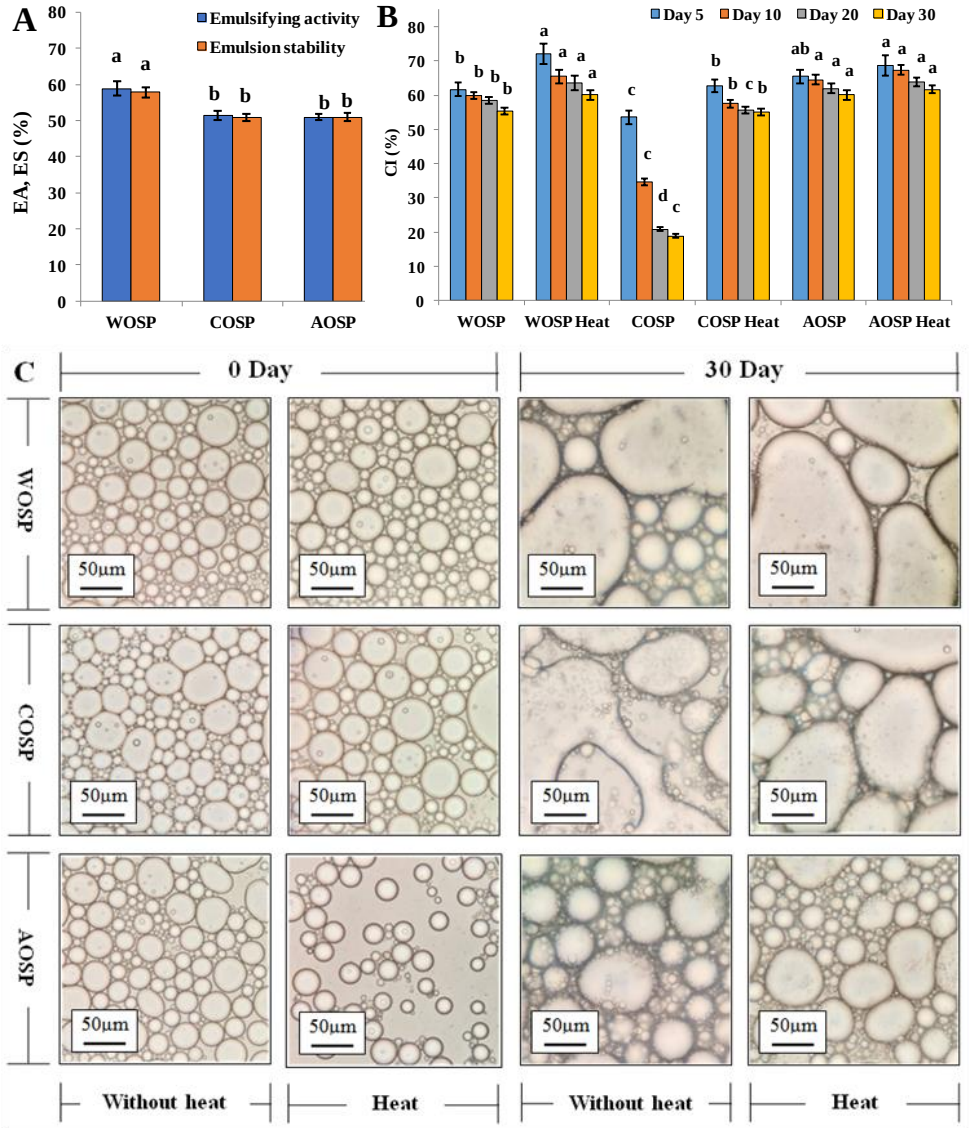

Figure 3. (A) Emulsifying activity (EA) and emulsion stability (ES) of okra seed polysaccharides; **(B)** creaming index (CI) of emulsions prepared at room temperature / with heat treatment within 30 days; **(C)** optical micrographs of the oil-in-water emulsions stabilised by okra seed polysaccharides. Different lowercase letters among treatments in Figure 3A, and among all treatments of the same storage time in Figure 3B indicate significant difference ($p < 0.05$).

was due to higher molecular weight of COSP (in Table 1), which resulted in higher viscosity in the continuous phase, and increased the aggregation rate of droplets (Liu *et al*., 2019). The droplet size distributions had a negative relationship with the relative stability, where smaller sizes created increased creaming stability (Jiang *et al*., 2020). Interestingly, the CI of all samples significantly increased with heat treatment (Figure 3B). This phenomenon was likely driven by protein molecules attached to the polysaccharides gradually unfolded with the increasing temperature, enhancing the adsorption on the droplet interface due to the exposure of hydrophobic residues.

Antioxidant and enzyme inhibitory activities

Based on Figures 4A and 4B, DPPH-RSA and ABTS-RSA increased dose-dependently with the increase in the concentration from 0 to 4 and 2.5 mg/mL, respectively. For the absolute values of DPPH-RSA and ABTS-RSA, AOSP showed the highest scavenging activities, followed by WOSP and COSP. This phenomenon might be related to the increased molecular weight. As previous study reported, polysaccharides with lower molecular weight contributed to the increased radical-scavenging ability (Yan *et al*., 2019). In addition, okra seeds are rich in oligomeric catechins (2.5 mg/g) and flavonol derivatives (3.4 mg/g) (Araptitsas, 2008). As seen in Table 1, AOSP possessed significantly higher total polyphenol than the other two samples. Correspondingly, the antioxidant activity of AOSP was also higher due to the high content of total polyphenol interacting with polysaccharides (Xu *et al*., 2020b).

As seen in Figures 4C and 4D, pancreatic lipase and α-amylase inhibitory activities increased with the increase in the concentration from 0 to 10 mg/mL. COSP and AOSP had a higher inhibitory effect on pancreatic lipase activities than WOSP in the range of 0.25 to 10.0 mg/mL. The inhibitory rate of AOSP was significantly higher than COSP up to the concentration of 2.5 mg/mL. But COSP exhibited the highest inhibitory rate (88.43%) at the concentration of 10 mg/mL, followed by AOSP (65.42%) and WOSP (53.93%). Likewise, COSP exhibited the highest α-amylase inhibitory rate (92.62%), followed by AOSP (85.50%) and WOSP (76.06%) at the concentration of 10 mg/mL. The stronger inhibitory activities are probably due to its higher molecular weight (Guo *et al*., 2018). In addition, combined polyphenol with polysaccharide was another factor that demonstrated excellent inhibitory activities in a dose-dependent manner (Sienień *et al*., 2021). The higher inhibitory activities of AOSP might be partially attributed to the higher polyphenol content (Table 1).

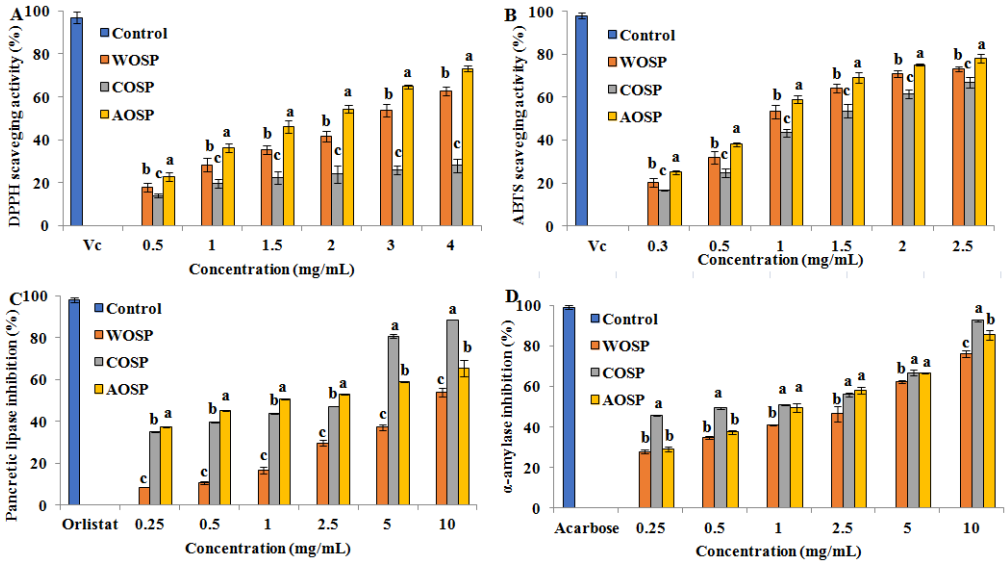

Figure 4. Antioxidant activities, *in vitro* pancreatic lipase, and α-amylase inhibitory activities of polysaccharides. **(A)** DPPH radical scavenging activity, **(B)** ABTS radical scavenging activity, **(C)** pancreatic lipase inhibitory activity, and **(D)** α-amylase inhibitory activity. Different lowercase letters among all treatments of same concentration indicate significant difference ($p < 0.05$). The positive control in Figures 4A, 4B, 4C, and 4D were given at the concentration of 0.25 mg/mL.

Conclusion

The present work provided unique information about okra seed polysaccharides extracted with different solvents, including extraction yield, molecular weight, particle size, surface morphology, emulsifying properties, antioxidant activities, and enzyme inhibitory activities. WOSP with an O-acetylated group and lower zeta potential exhibited better emulsifying activity than the other two samples. COSP with higher molecular weight and uronic acid displayed stronger pancreatic lipase and α-amylase inhibitory activities. However, AOSP with lower molecular weight but higher bound phenolics showed stronger DPPH-RSA and ABTS-RSA. The O-acetyl group on the sugar ring significantly influenced the EA and ES, and molecular weight played a crucial role in the enzyme inhibitory and antioxidant activity. To sum up, okra seed polysaccharides extracted by different solvents could potentially be used as new source of natural and functional ingredients in the food industry due to their inherent benefits.

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