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Characteristics of alkali-extracted peanut polysaccharide-protein complexes and their ability as Pickering emulsifiers

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ABSTRACT

An alkaline isolation method was applied to extract polysaccharide from residues of peanut oil processing while retaining high protein content, in order to enhance the emulsifying ability of these materials. The obtained complexes (PECs) containing protein (13–18%, dry basis) were named as PEC8.0, PEC10.0 and PEC12.0 according to extraction pH values. The protein content of PECs increased with increasing extraction pH value, thereby the hydrophobicity was improved. Additionally, as extraction pH value increased to 10.0, the protein of PECs covalently bonded to polysaccharide and polysaccharide conformation unfolded simultaneously, thus particle size was enlarged. Furthermore, the increasing concentration of PECs further induced the formation of large complex particles. Then, they were used to stabilize the Pickering emulsions with oil fractions (φ) of 0.4–0.7. The emulsions stability especially the gel structure was maintained by the interactions of large particles adsorbed in the interface and those in the continuous phase. Stability analysis indicated the emulsifying capacity of PEC10.0 and PEC12.0 was superior to that of PEC8.0, due to difference of their particle properties. This suggested the promoting effect of alkali in preparation of polysaccharide-protein complex as good Pickering stabilizer.

1. Introduction

The development of oil-in-water (O/W) Pickering emulsions stabilized by food-grade particles has aroused extensive interests in recent years, mainly because of their compatibility with food and some outstanding characteristic, such as extraordinary stability [1–3]. Additionally, the Pickering emulsions would be a healthy and promising approach for creating solid-like textures in the food system of liquid oils. To date, the carbohydrate, protein and the polysaccharide and protein complex are main food-grade particles as effective Pickering stabilizers. Simultaneously, they face the problems of poor hydrophobicity [4], the sensitivity of molecular structure [5], the complex and rigorously controlled fabrication condition [6,7], respectively. Herein, specific procedures are needed to fabricate colloidal particles. Moreover, these stability-influencing properties for solid particles to function as Pickering emulsions stabilizers are primarily related to their particle size, concentration, shape and wettability [8].

Additionally, it is widely recognized that the stabilization of Pickering emulsion is the accumulation of solid particles at the oil-water interface in the form of a densely packed layer. Meanwhile, the adsorption of particles at the interface is considered to be irreversible due to the high desorption energy barrier [8]. These effectively protect the emulsion against coalescence and Ostwald ripening and thereby possess high stability. In addition to the particle layer adsorption around the droplets, some other ordering mechanisms being responsible for the prevention of droplet coalescence may be associated with aggregation of the particles. The aggregated structure held together by inter-particle attractive forces would cause a network of particles adsorbed at the oil-water interface and thereby form the steric particle-based barrier [9]. A noteworthy issue is that inter-particle interactions are highly determined by the particle concentration [10]. At high particle concentrations, the attractive inter-particle force would be strong and most of the particles usually are aggregated. More importantly, the aggregation of particles in the continuous phase could contribute to formation of a gel-like network in the emulsions, as in the chitin nanocrystal, soybean protein and whey protein cases [11–13].

According to the data from USDA, the stocks of dehulled peanut oil were up to 25.8 million pounds in 2019 [14]. The large amounts of residues from peanut oil production inevitably were generated. Up to now, the utilization of by-product mainly focuses on the protein [15,16]. However, after removal of the protein, the development of the rest waste is worth thinking deeply. Recently, the recovery of peanut polysaccharide from by-product of peanut oil gradually arouses extensive interests. Aqueous extraction process (AEP) of peanut oil was an environmentally friendly process method for the recovery of oil from peanut. AEP of peanut oil produced simultaneously an oil-rich cream phase, a protein-rich water phase and a sediment phase [17]. It has

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been previously reported that the polysaccharides from sediment of AEP of peanut oil comprised of the neutral and acidic polysaccharides, of which acidic polysaccharide exhibited large particle aggregates. Meanwhile, this aggregation behavior caused by molecular self-association enhanced with particles concentration increasing [18]. Additionally, alkali-extracted crude polysaccharide could reduce the oil-water interfacial tension, being associated with the residual protein [19]. Therefore, the peanut polysaccharide or its complex with protein were expected to be developed as a novel Pickering stabilizer.

Hence, in present study, the gradient alkali (pH 8.0, pH 10.0 and pH 12.0) was used to extract the complexes of polysaccharide and protein (PECs) from the peanut sediment of AEP, in order to obtain materials with good emulsifying ability. The colloids properties of PECs, including composition, hydrodynamic diameter, microscopic morphology and contact angle were investigated. Meanwhile, the effect of PECs concentration on hydrodynamic diameter and microscopic morphology also was analyzed. These were used to evaluate the potential for stabilizing O/W Pickering emulsions. Furthermore, the PECs at a relatively high concentration were used to prepare the emulsions with various oil volume fractions. The microstructure, droplet size, rheological property and visual appearance of emulsions were examined to assess the stability and explore the mechanism of emulsion stabilization simultaneously. This work would supply theoretical guidelines for exploiting PECs as a novel Pickering stabilizer.

2. Materials and methods

2.1. Materials and reagents

The peanut sediment (Junqi Co., Ltd., Jiangsu, China) from AEP was frozen and stored at −20 °C until its use for PECs extraction. The dry weight of sediment phase (Sp) from AEP of peanut oil was mainly composed of dietary fiber (47.10%) and starch (30.20%), protein (12.50%) and fat (3.40%), according to the previous study [19]. The chemical reagents, including HCl, NaOH, etc., were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The α-amylase was purchased from Novozymes Co., Ltd. (Novo, China). Peanut oil was purchased from Luhua Co., Ltd. (Laiyang, China).

2.2. Extraction

The alkali-extracted PECs were prepared by the procedure described in previous study [19]. The main procedures were following: peanut sediments from AEP dispersed in distilled water were adjusted to pH 8.0, pH 10.0 and pH 12.0, respectively, and then incubated at 121 °C for 40 min by using vertical heating steam pressure sterilizer (Shenan, Shanghai). The resulting slurries were centrifuged and the supernatants were enzymatically hydrolyzed using thermostable α-amylase (5.0 U, per ml of supernatant) at pH 6.00 ± 0.10, 90 ± 2.0 °C for 90 min, in order to hydrolyze the starch in reaction solution. After hydrolyzing, the hydrolysate was desalted by ultrafiltration with a molecular weight cut-off (MWCO) of 10,000 ultrafiltration membrane (Fumei, Xiamen, China), spray-dried and named as PEC8.0, PEC10.0 and PEC12.0 according to the extraction pH value.

2.3. Composition of PECs

Ash was measured according to AOAC standard methods 900.02 [20]. The protein content was determined by the Kjeldahl method with a nitrogen conversion factor of 5.46. Total sugar content was determined using the phenol-sulfuric acid method at 490 nm and D-glucose was used as the standard [21]. Monosaccharide composition was analyzed on an ICS-5000 ion chromatographic system (Dionex, Shanghai) equipped with CarboPac PA20 analytical column (3 × 150 mm) and pulsed amperometric detector. The detailed procedures were described in the previous study [18]. Fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), mannose (Man), fructose (Fru), glucuronic acid (GlcA), and galacturonic acid (GalA) were used as standards.

2.4. Colloidal properties of PECs

2.4.1. Hydrodynamic diameter

The hydrodynamic diameters (d_H) of PECs particles were determined by employing dynamic light scattering (DLS) at a scattering angle of 90° and λ = 640 nm (Nano Brook Omni instrument, Brookhaven Instruments Corporation; Holtsville, NY, U.S.A.). According to literature with a slight modifications [10], to elucidate the pattern of interactive forces involved in formation and maintenance of the particle, PECs powder were dispersed in distilled water and various kinds of perturbative solvents including 3 M urea, 0.5% SDS, and 15 mM DTT or their combinations and then the corresponding d_H were determined, respectively. The pH of all PECs solution used was 6.0 ± 0.5.

2.4.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out on a Bio-Rad Mini-Protein Tetra Electrophoresis System (Bio-Rad Laboratories, Inc., Hercules, USA) with a 5% stacking gel and 12% separating gel according to literatures with a slight modification [22]. PECs were dispersed in SDS-PAGE sample buffer including SDS and β-mercaptoethanol to a concentration of 8–10 mg/mL. The mixtures were heated in the boiling water for 5 min. After centrifuging at 10,000*g* for 5 min, an amount of 10 μL per sample was loaded onto the prepared gels. After electrophoresis, the gel was cut in half, then being stained for proteins with 0.1% Coomassie Brilliant Blue R-250 Staining Solution and for carbohydrate with 0.5% periodic acid fuchsin, respectively.

2.4.3. Morphology observation

In addition, the transmission electron microscopy (TEM, H-7650, HITACHI, Japan) was employed in observing microstructure of PECs at the concentration of 0.5% and 2.0% (w/w), respectively. 10 μL of prepared PECs aqueous solution (pH = 6.0 ± 0.5) was deposited onto electron microscope copper grids which were first coated with a thin film of carbon and then were dried at 35 °C. Finally, TEM images were taken at an accelerating voltage of 8 kV.

2.4.4. Contact angle measurements

Contact angle of PECs was measured using a contact angle meter equipped with a camera (OCA15EC, Dataphysics Co., Ltd., Germany) with static sessile drop method according to Li et al. [23]. Briefly, PECs powder was compacted into a standard tablet at 10 MPa using an auto press. The resultant tablet was placed on the platform of the OCA15EC system, and then a 2 μL of distilled water was lightly dripped onto the surface of the tablets. After the equilibrium was reached (about 10 s), the droplet shapes of water were recorded and meanwhile the corresponding contact angle was analyzed by the SCA software for both right and left angles of the drop.

2.5. Emulsion preparation

Firstly, PECs particle dispersions at varying concentration values (1.0%, 1.5%, 2.0% and 4.0%, w/w) were applied to prepare the emulsions which were formed at a fixed oil fraction (φ) of 0.6. Secondly, the concentration of PECs was fixed at 4.0% (w/w), and different oil fractions (φ = 0.4, 0.5, 0.6 and 0.7 (v/v), respectively) were used to prepare emulsions. The total volume of all the emulsions was fixed at 20 mL and meanwhile 100 μL of 1.0% (w/w) sodium azide was added to inhibit growth of microorganisms. Briefly, peanut oil was mixed with PECs dispersion using a high-speed homogenizer (T25, IKA Co., Germany) at 8800 rpm for 1 min. These resultant emulsions (fresh) were directly subjected to analysis or stored at room temperature for various periods

of time for emulsion stability evaluation, e.g., 7 or 20 days for coalescence or creaming stability analysis.

2.6. Emulsion characterization

2.6.1. Microstructure observation

The microstructure of PECs-based emulsion was observed by using confocal laser scanning microscopy (CLSM, Leica TCS SP8, Heidelberg, Germany) as previous reports [24]. The diluted emulsion was colored by a hybrid dye solution (1.0 mg/mL Nile red and Nile blue in glycol). The Nile red was used to label peanut oil and Nile blue was to probe the PECs dispersion. The fluorescent dyes were excited by either at 488 nm for Nile red or at 633 nm for Nile blue.

2.6.2. Droplet size

The droplet size of emulsion stabilized by PECs was determined using a laser diffraction instrument (BT-9300S, China) based on a previous method [25]. Emulsions were diluted 6 times with distilled water for determination. The droplet size was reported as the volume weighted average diameter ($d_{4,3}$), which was calculated by Eq. (1).

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

2.6.3. Rheological measurement

Rheological properties of Pickering emulsion were analyzed using a Discovery Hybrid Rheometer-3 (DHR-3) (TA instruments, USA), as previously described with a modification [18]. For steady shear test, a cone-plate geometry of 40 mm diameter, a cone angle of 1°59′57″, and a gap of 56 μm were employed. Shear rate (γ) increased from 0.01 to 300 s^{-1}. Furthermore, a 40 mm diameter parallel geometry and 1000 μm gap were applied in oscillatory measurement. 0.2% strain within the linear viscoelastic region was used. Storage modulus (G′) and loss modulus (G″) over the frequency (f) ranging from 0.01 to 100 Hz were determined.

2.6.4. Centrifugation stability

The centrifugation stability of emulsion was investigated mainly according to the previous method [26]. After the centrifugation at 8000 rpm for 20 min, the emulsions (3 ± 0.02 g) stabilized by PECs were recorded by a camera.

2.7. Statistical analysis and figure plotting

The data was analyzed using analysis of variance (ANOVA) by SPSS software (Version 16.0) and expressed as mean value ± standard deviation (n = 3). The significance of differences between groups were assessed by Duncan's multiple range test using a significance level of $p < 0.05$. The figures were plotted using Origin software 2017 and ChemDraw Ultra 2004.

3. Results and discussion

3.1. Composition analysis

Since the extraction process involves ultrafiltration procedure with a molecular weight cut-off (MWCO) of 10,000, the total sugar content of PECs determined by phenol-sulfuric acid method actually belonged to the polysaccharide content. As a shown in Table 1, the polysaccharide content decreased in the sequence of PEC8.0, PEC10.0 and PEC12.0, which exhibited a little difference compared with the previous study [19]. This mainly was associated with ultrafiltration procedure. The protein content of PECs positively related to extraction pH and simultaneously ranged from 13.87% to 17.76%. On the other hand, the monosaccharide composition among PECs was similar. They primarily consisted of neutral sugar, such as Glc and Ara. The Glc might partly result from the starch hydrolysate of peanut sediment during extraction. Moreover, the PECs was heteropolysaccharide, probably resulting from the degradation of hemicellulose in peanut sediment. Moreover, the GalA comprised a small proportion of PECs, suggesting the existence of acidic polysaccharide.

Table 1
Chemical composition (dry basis), monosaccharide composition of PECs.

Sample	Total sugar %	Protein %	Ash %	Monosaccharide composition molar %					
				Rha	Ara	Gal	Glc	Xyl	GalA
PEC8.0	68.06 ± 2.10^a	13.87 ± 0.25^c	5.23 ± 0.02^b	3.64	32.30	2.59	50.01	5.19	6.34
PEC10.0	64.85 ± 1.68^a	16.45 ± 0.45^b	4.25 ± 0.02^c	4.02	34.21	3.22	49.03	4.04	5.58
PEC12.0	56.75 ± 3.56^b	17.76 ± 0.38^a	6.50 ± 0.56^a	3.40	37.92	4.35	42.35	5.35	6.67

Different superscript letters represent significant difference at $p < 0.05$ level among the same columns.

3.2. Analysis of colloidal properties

3.2.1. Particle size

It is widely recognized that particle size is an important factor in determining the stability of emulsions stabilized by micro- and nanoparticles [27]. As shown in Fig. 1A, at the 0.5% concentration, PEC8.0, PEC10.0, PEC12.0 all showed a nearly mono-modal particle size distribution, with the hydrodynamic diameter of 264.1 ± 5.1 nm, 403.3 ± 7.9 nm and 360.5 ± 13.5 nm. Owing to the polysaccharide comprised a large proportion of PECs, thereby the contribution of particles size of PECs mainly resulted from polysaccharide molecules. Based on this, the relatively large size of PEC10.0 and PEC12.0 may be due to the fact that more alkaline extracted condition promoted unfolding of polysaccharide structure and/or interaction between polysaccharide and protein [28]. However, the particle size of PEC12.0 decreased compared to that of PEC10.0, possibly because of decline in molecular size. According to the literature [19], violent alkali might be likely to degrade polysaccharide into smaller molecular. On the other hand, as the concentration increased to 2.0%, the PECs could self-assemble into large particles (shown in Fig. 1A-1). Meanwhile, the particle distribution of PECs was comparatively polydispersed, with polydispersity index (PDI) ranging from 0.3 to 0.4 shown in Table S1. It was usually accepted that the increase in particle size may be associated with the inter-particle interactions.

3.2.2. Inter-particle interactions

It is generally believed that inter-particle interactions play an important role on the particles structure of hydrocolloids [10]. Owing to the fact that the protein comprised a small proportion of PECs, firstly, SDS-PAGE method was used to analyze the linkage between polysaccharide and protein. As shown in Fig. 2A, the stained protein appeared blue, while the stained polysaccharide appeared pink. The Mw of protein components in PECs mainly ranged from 5 to 20 kDa, belonging to the relatively low Mw fraction. Furthermore, the protein bands of PEC10.0 and PEC12.0 also manifested in the region above 20 kDa bands and the stacking gel, suggesting the fact that the protein Mw of PEC10.0 and PEC12.0 significantly increased compared to that of PEC8.0. Simultaneously, the polysaccharide bands also appeared in the corresponding position, which attributed to the fact that the protein component was linked to polysaccharide and thereby increased the Mw of protein [22]. Meanwhile, the polysaccharide linked by protein distributed in above 250 kDa bands, suggesting the large size molecular characteristics. Base on above, it could be concluded that as extraction pH increased to 10.0, partial protein of PECs was linked to large size polysaccharide. This may be explained by the fact that higher amount of protein in

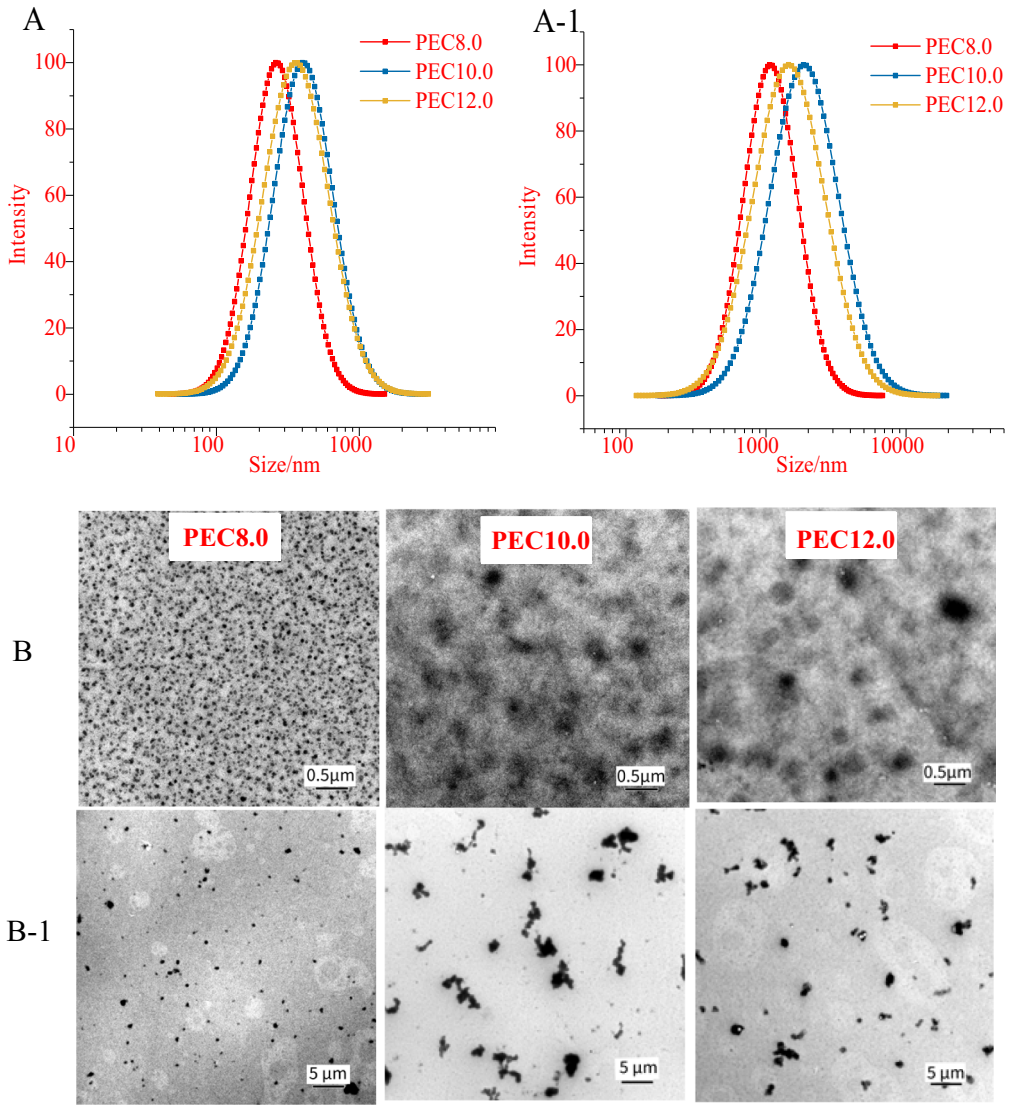

Fig. 1. The d_H distribution of PECs particles at 0.5% (w/w) concentration (A) and 2.0% (w/w) concentration (A-1). TEM image of PECs particles at 0.5% (w/w) concentration (B) and 2.0% (w/w) concentration (B-1).

PEC10.0 and PEC12.0 increased the possibility of reaction between polysaccharide and protein. Additionally, the binding of protein to polysaccharide may further provide basis for the relatively large size of PEC10.0 and PEC12.0 at 0.5% concentration (shown in Fig. 1A).

In addition, various perturbants including DTT, urea and SDS were used to destroy the inter-particles interaction of PECs at a relative high concentration (2.0%). As shown in Fig. 2B, the presence of 3 M urea or 0.5% SDS sharply reduced the d_H of PECs. However, the 15 mM DTT weakly reduced the d_H of PEC10.0 and PEC12.0 and simultaneously had no effect on that of PEC8.0, indicating the fact that hydrogen bonds and hydrophobic interactions were major interactive forces for the aggregation of PECs particles, while disulfide bonding was minor. Owing to the fact that hydrophobic interactions only resulted from the protein, thus, the protein in PECs was involved in the formation of large particles. Additionally, the combination of 3 M urea, 0.5% SDS as well as 15 mM DTT was used to disrupt the particle aggregation. Consequently, the d_H of PEC8.0, PEC10.0 and PEC12.0 were 169.8, 350.7 and 341.6 nm, respectively, which were close to that of PECs at 0.5% concentration. This may suggest that as the concentration of PECs increased, the PECs particles could self-aggregate into large particle by means of hydrogen bonds and hydrophobic interactions. Moreover, polysaccharide and protein together maintained the particle structure of PECs. Additionally, the average d_H of PECs at various perturbants and the corresponding PDI were shown in Table S1.

3.2.3. Microscopic morphology

With respect to particle stabilizer, particle shape plays an important role on the performance of its application on emulsion [29]. It is well-known that TEM as a powerful tool is employed to visualize the particle morphology in "near-native" conditions and to investigate the chain conformation of polysaccharide especially at low concentration solution [18,30]. Similarly, the morphology of PECs was observed at different concentration (0.5% and 2.0%). As shown in Fig. 1B, the particle size of PECs was consistent with the result of DLS analysis (Fig. 1A). Additionally, PEC8.0 exhibited uniform and spherical small particle. However, the particles of PEC10.0 and PEC12.0 were comparatively large and slightly associated each other, which might suggest that the conformation of polysaccharide unfolded. As concentration increased to 2.0%, the size of PECs particles significantly increased. It might be associated with the following reasons. On the one hand, owing to the fact neutral sugar comprised a large proportion of PECs, the electrostatic repulsion between polysaccharides or between polysaccharide and protein should be relatively weak. On the other hand, according to the literature [31],

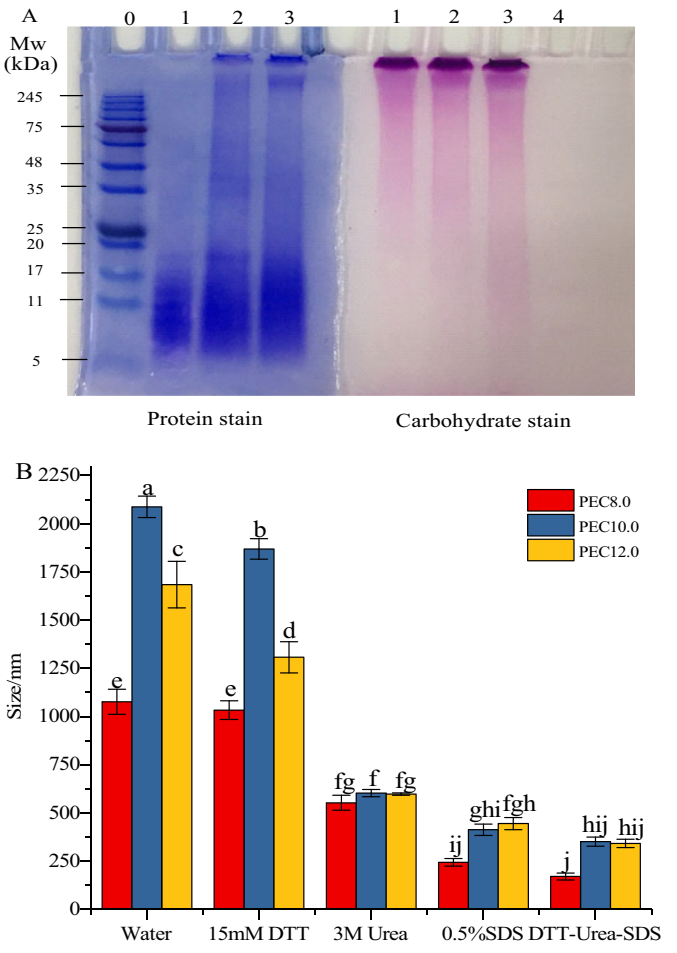

Fig. 2. (A) SDS-PAGE profile of PECs. 0: pre-stain protein marker for monitoring electrophoretic separation; 1: PEC8.0; 2: PEC10.0; 3: PEC12.0; 4: protein marker as negative control for carbohydrate staining. (B) Effects of various perturbative solvents on the d_H of PECs at a concentration of 2.0% (w/w).

once a critical polymer concentration was exceeded, the attractive forces possibly dominated the repulsive forces. Therefore, the increase of PECs concentration could significantly enhance the attractive forces, such as hydrogen bonds, hydrophobic interactions, and thereby induce the aggregations of polysaccharide-polysaccharide, polysaccharide-protein and protein-protein. However, the first two may dominate the contribution to the formation of large particles of PECs, because of low amount and small size of protein. In addition, compared to PEC8.0, PEC10.0 and PEC12.0 exhibited bigger size particles. Meanwhile, the particles of PEC10.0 and PEC12.0 exhibited the extended short chain shape. Base on this, it could be concluded that the particles of PEC10.0 and PEC12.0 possessed higher aspect ratio than that of PEC8.0. This may be explained by the fact that the conformation of polysaccharide unfolded and simultaneously the hydrophobicity of polysaccharide was improved by covalently linked protein, which benefited more polysaccharide or protein to attach and resulted in a higher degree of accumulation. Additionally, the protein content of PEC10.0 and PEC12.0 were higher, possibly inducing self-aggregation of more small molecules protein [10].

3.2.4. Wettability

Additionally, another critical factor, particle wettability, is used to assess the performance of PECs on stabilizing oil-water emulsion [23]. The wettability was evaluated by the contact angle between PECs particles and distilled water presented in Fig. 3. The contact angle of PECs was less than 90°, indicating comparatively hydrophilic feature. This also was consistent with the previous study [19]. Furthermore, the contact angel of PECs increased in the order of PEC8.0, PEC10.0 and PEC12.0, which was in line with tendency to the amount of protein in PECs. It indicated that the improvement of wettability benefited from the amount of protein in PECs. Herein, it can be concluded that the dissolution of protein could improve the hydrophobicity of PECs during extraction.

3.3. Determination of particle concentration for preparing stable emulsions

The results of colloidal properties indicated that as concentration increased to 2.0%, the PECs molecules could self-aggregate into the large particles with a size ranging from 1 μm to 2 μm, which indicated that the increase of particles concentration could obviously enhance the inter-particles attractive forces. Furthermore, as stated by researchers [32], the inter-particles attraction could induce the gelation of oil droplets and thereby maintain the stability of emulsion. Thus, the PECs dispersions at various concentration were applied to stabilize the fixed oil volume ($\varphi = 0.6$) of emulsions. The stability of these emulsions was investigated by centrifugation and shown in Fig. S1. The results indicated that the phenomenon of oil releasing gradually decreased with the particles concentration increasing. As concentration increased to 4.0%, this phenomenon disappeared, suggesting the emulsion was relatively stable. Furthermore, the further increase on concentration brought about the difficulty in dissolution of PECs. Consequently, the 4.0% concentration was used to prepare the stable emulsion and further assess the differences of the performance as emulsion stabilizers and emulsifying capacity among PECs.

3.4. Characteristics of emulsion stabilized by PECs

3.4.1. Microstructure

The CLSM imaging could provide an intuitive way to illustrate the interfacial structure which is vital to the performance of the emulsions [33]. As shown in Fig. 4A, the oil phase was in the interior of the droplet, while the PECs particles adsorbed on the boundary of droplets, belonging to the typical O/W Pickering emulsion. Since the PECs could self-aggregated into large size particles at relatively high concentration, the adsorption layer at the oil/water interface should be relatively thick, effectively avoiding the oil droplet coalescence and thereby enhancing emulsion stability. Furthermore, there were many PECs particles dispersed in the continue phase. With respect to φ of 0.4 and 0.5, all emulsions stabilized by PECs exhibited comparatively uniform droplet size, implying their stable property. As φ increased to 0.6 and further, the droplets size of emulsions stabilized by PEC8.0 significantly increased and consequently these emulsions were unstable. This may associated with the fact that oil-water interface of emulsions could not be covered well by PEC8.0 particles [34]. However, the emulsions stabilized by PEC10.0 and PEC12.0 exhibited slight increase in droplet size and still

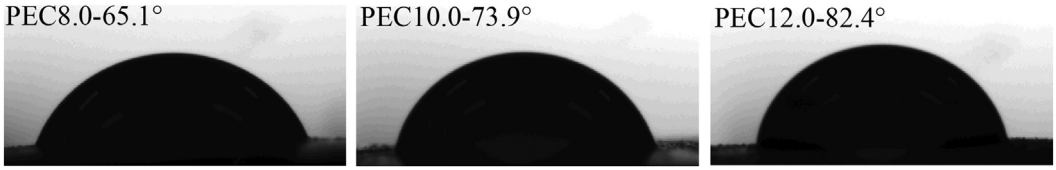

Fig. 3. Contact angles between PECs and distilled water.

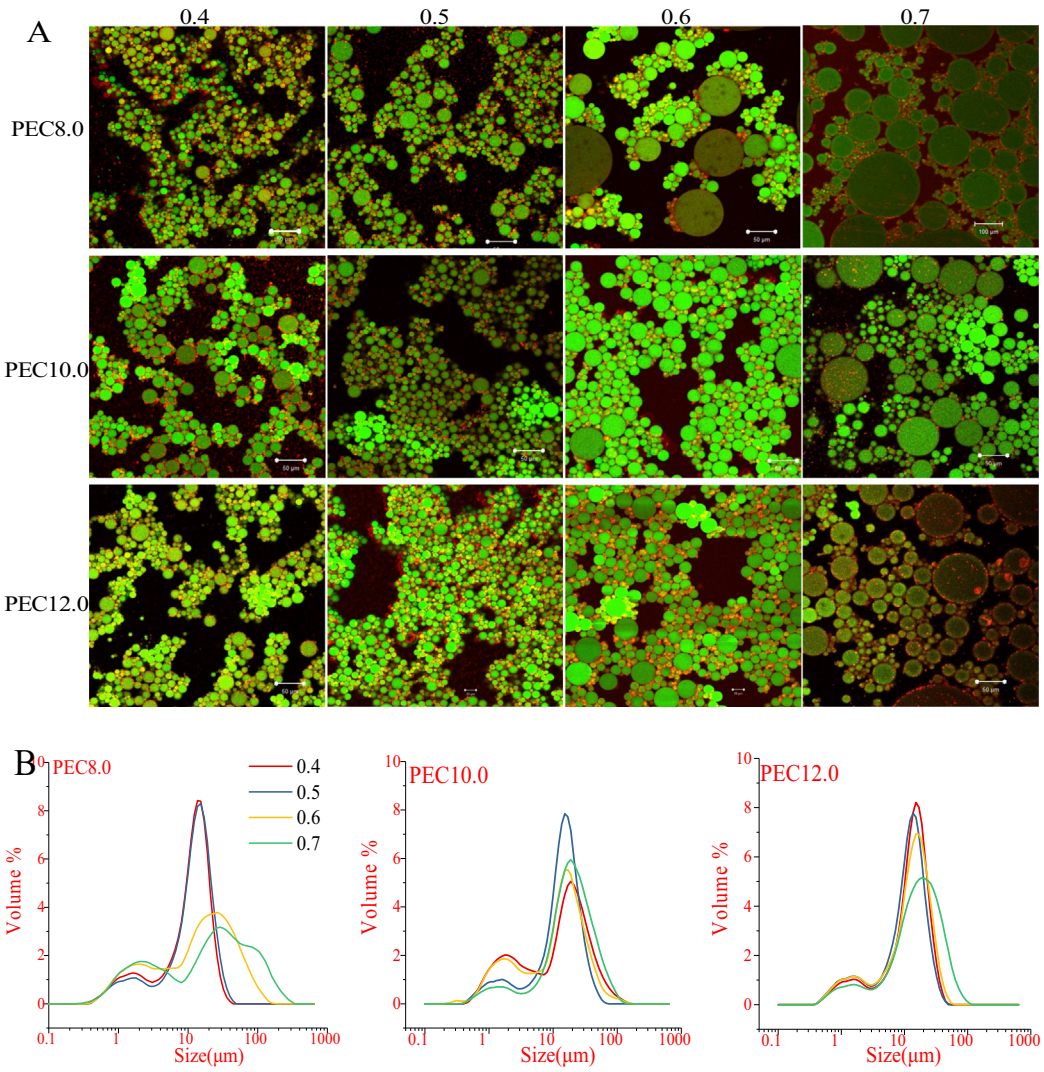

Fig. 4. (A) CLSM images of emulsions stabilized by PECs at φ = 0.4, 0.5, 0.6 and 0.7 with a magnification of 250 times. The oil phase was stained with Nile red (green) and PECs particles was stained with Nile blue (red); (B) The $d_{4,3}$ distribution of the fresh prepared emulsions.

homogenous, suggesting that the oil droplets had been successfully wrapped. The difference of emulsifying capacity primarily was due to their diversity in wettability. Moreover, the flocculation behavior was observed in all emulsions. This may be ascribed to the fact the adsorption and anchoring of particles between two or more oil droplets simultaneously happened [6]. Meanwhile, the particles adsorbing surface of oil droplets probably were associated with each other by the virtue of the inter-particles attractive forces. Additionally, the flocculation of emulsion droplet could cause the formation of gel-like network [10].

3.4.2. Droplet size

The size distribution of emulsions stabilized by PECs was shown in Fig. 4B. All emulsions presented multimodal distributions. While the droplet size ranging from 0.5 to 5.0 μm might be related to the unabsorbed PECs particles (also shown in Fig. 4A). Additionally, the droplets size of emulsions stabilized by PECs at various φ exhibited relatively homogeneous except for that of emulsions stabilized by PEC8.0 at φ = 0.6–0.7, suggesting that PECs particles could adsorb the surface of oil droplets and further effectively avoided the coalescence of oil droplets. The $d_{4,3}$ of the homogeneous emulsions stabilized by PECs gradually expanded (shown in Table S2) in the sequence of PEC8.0, PEC12.0 and PEC10.0, which was consistent with the tendency to the d_H of PECs. It indicated that the droplet size of emulsion depended on the original particles size. However, the droplet size of emulsions stabilized by PEC8.0 at φ = 0.6–0.7 obviously increased, probably resulting from the coalescence of oil droplets (shown in Fig. 4A). This indicated that the emulsions stabilized by PEC8.0 were unstable under comparatively high internal phase condition.

3.4.3. Rheological properties

The rheological properties of emulsions during storage were presented in Fig. 5. As shown in Fig. 5A, the G″ and G′ of the freshly prepared emulsions (storage for 0 d) were almost dependent on the frequency. Interestingly, when storage for 7 d and further prolonging, the G′ and G″ of all emulsion significantly increased. Furthermore, the G′ was higher than G″ and simultaneously they were parallel to the frequency, suggesting cross-link gel structure characteristic [10]. The formation of gel structure may be attributed to on the one hand the flocculation behavior of emulsion droplets (shown in Fig. 4A) [10], on the other hand the molecular association or aggregation of PECs particles in continuous phase [18,35]. Moreover, the gel structure was further strengthened with storage time increasing. This may imply that inter-particles interaction in continuous phase played a more important role on strengthen of gel structure, compared to that of inter-droplets, owing to the fact that droplets flocculation had been formed at the fresh emulsion. As stated by other researchers [13], when high

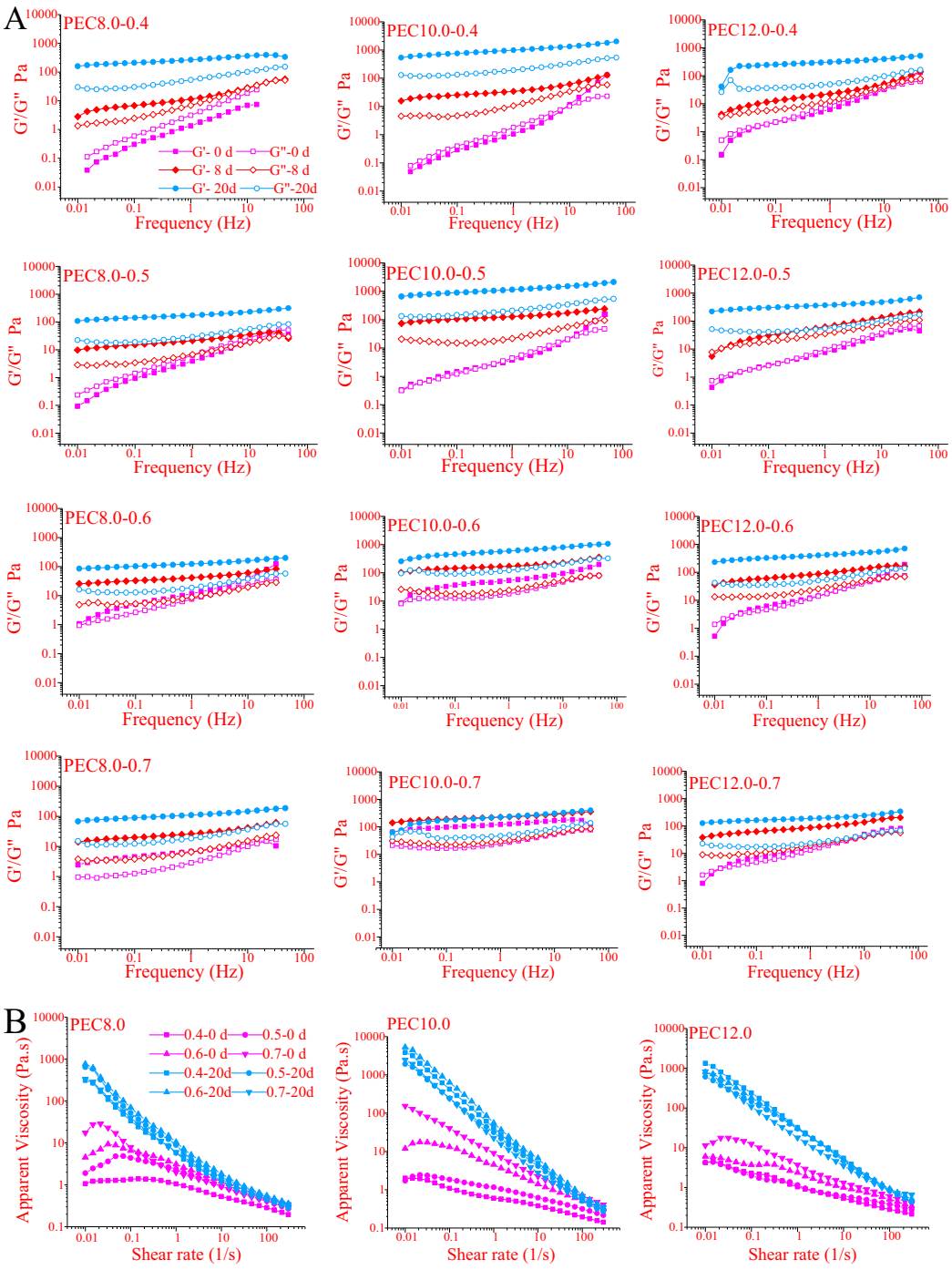

Fig. 5. Rheological properties of emulsions prepared by PECs during storage. (A) Oscillatory frequency sweep curves storage for 0, 7 and 20 d; (B) steady shear flow curves storage for 0 and 20 d.

concentration of particle stabilizer was used to prepare emulsion, the association or aggregation of particles in the continuous phase was more favorable to induce the formation of a gel-like network in the emulsions. In addition, it was observed that the augmentation on φ shorten the time required to form the strong gel structure, probably owing to the fact that the raise of relative density of emulsion droplet increased the chances of interaction between PECs particles. However, after forming stable structure, the G′ of emulsion deceased with the oil fraction increasing, indicating the decline in gel strength. This might be associated with the interaction between PECs particles in whole emulsion systems. The increase of oil fraction brought about decrease of the amount of PECs particles. This further reduced the association between particles and thereby weakens the gel strength. Furthermore, after storage for 20 days, under the same φ, the G′ of emulsions stabilized by PECs increased in the order of PEC8.0, PEC12.0 and PEC10.0. This was consistent with the sequence of particles size of PECs, which may indicate the large size particles would be beneficial for the formation of strong gel structure in emulsions. Similar conclusion also was obtained in the literature [29].

On the other hand, the relationship of apparent viscosity and shear rate for emulsions during storage was shown in Fig. 5B. As expected, all emulsions exhibited shear-thinning behavior and while the apparent viscosity progressively decreased as the shear rate increased from 0.01 to 300 s^{-1}. With respect to the freshly prepared emulsions, the apparent

viscosity increased with φ increasing. However, the extension of storage time weakened this difference. Additionally, the apparent viscosity of the PEC-stabilized emulsions increased in the order of PEC8.0, PEC12.0 and PEC10.0, keeping in line with the tendency to gel strength of their emulsion [26].

3.4.4. Visual appearances and emulsion stability

The typical appearance of PECs stabilized emulsions was presented in Fig. 6A. After storage for 20 d, all emulsions exhibited gel behavior by inverted observation, which was consistent with the results of rheological properties. However, the creaming stability displayed significant differences. The emulsions stabilized by PEC8.0 possessed the best stability against creaming. However, the emulsion stabilized by PEC10.0 at $\varphi = 0.4$ and the PEC12.0 stabilized emulsions at any φ developed into two layers, a creamed layer (top) and a turbid serum layer (bottom). This implied that the emulsions stabilized by PEC10.0 was creaming stability superior to that of PEC12.0, which mainly was ascribed to the fact that the relatively high viscosity feature limited the droplets movement and thereby improved the creaming stability. Additionally, the turbid serum probably was associated with the redundant particles in the emulsions [36]. The creaming behavior of emulsions stabilized by PEC8.0 disappeared could be explained by the reason that all PEC8.0 particles participated in the process of stabilizing emulsion.

On the one hand, the stability of emulsion also was investigated by centrifugation shown in Fig. 6B. As a result, the emulsions prepared by PEC8.0 at the φ of 0.6 and 0.7 were unstable with the presence of top oil layer. Nevertheless, all emulsions stabilized by PEC10.0 and PEC12.0 were still stable, which was contrary to the result of creaming stability analysis. Furthermore, Fig. 6A-2 displayed that after storage for 20 d, when the emulsions were remove from the glass tubes, the emulsions stabilized by PEC8.0 at the φ of 0.6 and 0.7 exhibited oil self-releasing behavior. This may result from the combination effect of thin adsorption, large droplet size and weak gel structure. However, other emulsions still were self-supporting gel. Compared to the creaming stability, coalescence stability is more important index to evaluate the emulsion performance. Consequently, it could be concluded that the emulsions prepared by PEC10.0 and PEC12.0 were stability superior to that of PEC8.0, especially at high oil phase system. Meanwhile, the emulsion stabilized by PECs provided a route for converting liquid oils into solid-like fats.

4. Conclusions

The PEC were obtained from peanut sediment of AEP. Polysaccharide content in PECs ranged from 68% to 56% and protein content was 13%–18%. These protein improved hydrophobicity of PECs. The binding of protein to polysaccharide and unfolding of polysaccharide conformation promoted the expansion of particle size in PEC10.0 and PEC12.0. Furthermore, the increase of concentration enhanced the attractive forces between particles and significantly increased size of PECs. Consequently, PEC10.0 and PEC12.0 possessed bigger particle size and higher aspect ratio than that of PEC8.0. Additionally, 4.0% PECs were used to stabilize emulsions at $\varphi = 0.4–0.7$. The interactions of particles adsorbed in the interface together with those in the continuous phase maintained emulsion stability and strong gel structure. However, the emulsions prepared by PEC8.0 at $\varphi = 0.6–0.7$ were unstable. PEC10.0 and PEC12.0 could stabilize these emulsions, which might provide possibility for fabrication of high internal phase emulsion basing on PEC10.0 and PEC12.0. Additionally, there results may guide for the highly-added-value utilization of residues from oil processing.

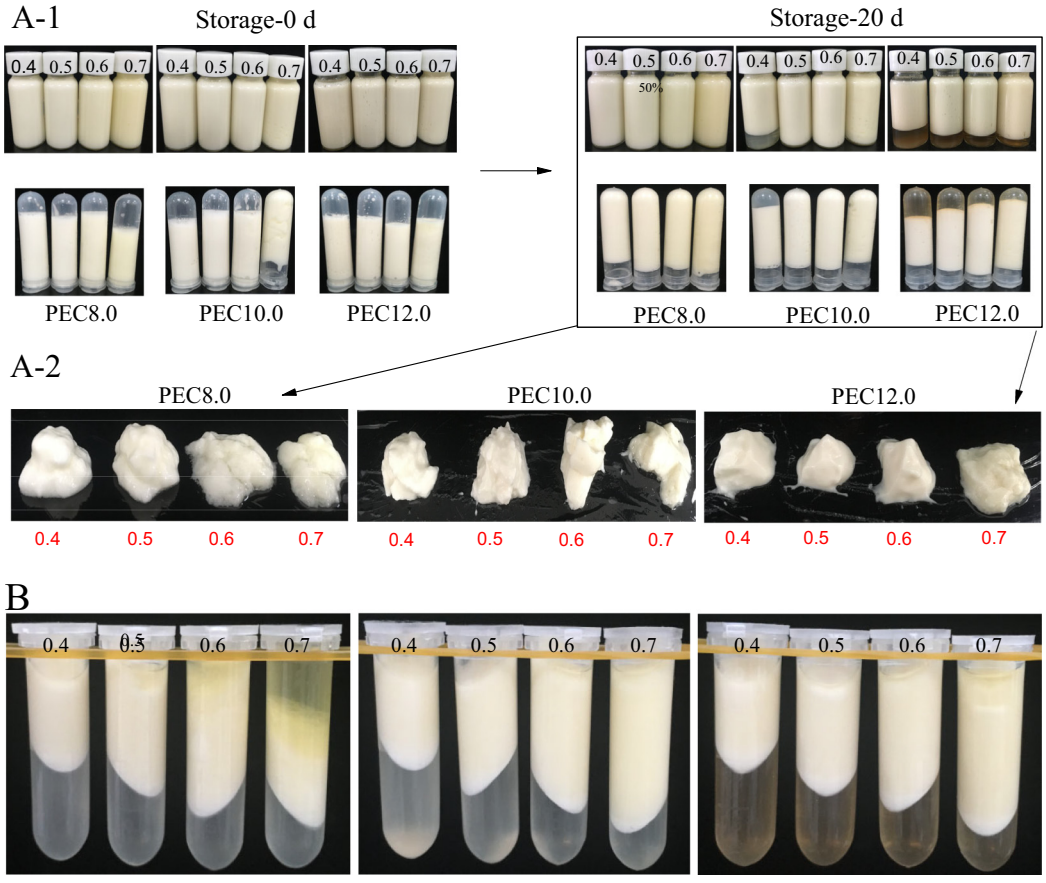

Fig. 6. (A) Visual appearance of emulsions prepared by PECs at $\varphi = 0.4, 0.5, 0.6$, and 0.7 (storage for 0 and 20 d, respectively); (A-1) Photograph of the emulsions stored for 20 d and then placed on a black desktop; (B) Visual appearance of the freshly prepared emulsions after centrifugation.

CRediT authorship contribution statement

Jianfen Ye: Data curation, Writing - original draft, Writing - review & editing. **Xiao Hua:** Conceptualization, Formal analysis. **Qiyan Zhao:** Data curation, Investigation. **Ziyi Dong:** Software, Methodology. **Zhuoyuan Li:** Software, Methodology. **Wenbin Zhang:** Funding acquisition, Resources. **Ruijin Yang:** Funding acquisition, Resources, Supervision.

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Declaration of competing interest

There is no conflict of interest regarding the publication of this article.

Appendix A. Supplementary data

Figure visual appearance of the emulsions stabilized by various PECs concentration after centrifugation (Fig.S1); Table with The hydrodynamic diameters (d_H, nm) of PECs particles (2.0%, w/w) at different solvents (Table S1) and the $d_{4,3}$ (μm) of fresh emulsion stabilized by PECs particles at different oil fractions (Table S2). Supplementary data to this article can be found online at doi:https://doi.org/10.1016/j.ijbiomac.2020.06.245.

References

[1] E. Dickinson, Stabilising emulsion-based colloidal structures with mixed food ingredients, J. Sci. Food Agric. 93 (2013) 710–721, https://doi.org/10.1002/jsfa.6013.
[2] M. Kargar, K. Fayazmanesh, M. Alavi, F. Spyropoulos, I.T. Norton, Investigation into the potential ability of Pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions, J. Colloid Interface Sci. 366 (2012) 209–215, https://doi.org/10.1016/j.jcis.2011.09.073.
[3] J. Xiao, Y. Li, Q. Huang, Recent advances on food-grade particles stabilized Pickering emulsions: fabrication, characterization and research trends, Trends Food Sci. Technol. 55 (2016) 48–60, https://doi.org/10.1016/j.tifs.2016.05.010.
[4] Q.-H. Chen, J. Zheng, Y.-T. Xu, S.-W. Yin, F. Liu, C.-H. Tang, Surface modification improves fabrication of Pickering high internal phase emulsions stabilized by cellulose nanocrystals, Food Hydrocoll. 75 (2018) 125–130, https://doi.org/10.1016/j.foodhyd.2017.09.005.
[5] C. Linke, S. Drusch, Pickering emulsions in foods-opportunities and limitations, Crit. Rev. Food Sci. Nutr. 58 (2018) 1971–1985, https://doi.org/10.1080/10408398.2017.1290578.
[6] M.-F. Li, Z.-Y. He, G.-Y. Li, Q.-Z. Zeng, D.-X. Su, J.-L. Zhang, Q. Wang, Y. Yuan, S. He, The formation and characterization of antioxidant Pickering emulsions: effect of the interactions between gliadin and chitosan, Food Hydrocoll. 90 (2019) 482–489, https://doi.org/10.1016/j.foodhyd.2018.12.052.
[7] T. Zeng, Z.L. Wu, J.Y. Zhu, S.W. Yin, C.H. Tang, L.Y. Wu, X.Q. Yang, Development of antioxidant Pickering high internal phase emulsions (HIPEs) stabilized by protein/polysaccharide hybrid particles as potential alternative for PHOs, Food Chem. 231 (2017) 122–130, https://doi.org/10.1016/j.foodchem.2017.03.116.
[8] I. Tavernier, W. Wijaya, P. Van der Meeren, K. Dewettinck, A.R. Patel, Food-grade particles for emulsion stabilization, Trends Food Sci. Technol. 50 (2016) 159–174, https://doi.org/10.1016/j.tifs.2016.01.023.
[9] F. Gautier, M. Destribats, R. Perrier-Cornet, J.-F. Dechézelles, J. Giermanska, V. Héroguez, S. Ravaine, F. Leal-Calderon, V. Schmitt, Pickering emulsions with stimulable particles: from highly-to weakly-covered interfaces, Phys. Chem. Chem. Phys. 9 (2007) 6455–6462, https://doi.org/10.1039/B710226G.
[10] F. Liu, C.H. Tang, Emulsifying properties of soy protein nanoparticles: influence of the protein concentration and/or emulsification process, J. Agric. Food Chem. 62 (2014) 2644–2654, https://doi.org/10.1021/jf405348k.
[11] F. Liu, C.-H. Tang, Cold, gel-like whey protein emulsions by microfluidisation emulsification: rheological properties and microstructures, Food Chem. 127 (2011) 1641–1647, https://doi.org/10.1016/j.foodchem.2011.02.031.
[12] C.-H. Tang, F. Liu, Cold, gel-like soy protein emulsions by microfluidization: emulsion characteristics, rheological and microstructural properties, and gelling mechanism, Food Hydrocoll. 30 (2013) 61–72, https://doi.org/10.1016/j.foodhyd.2012.05.008.
[13] M.V. Tzoumaki, T. Moschakis, V. Kiosseoglou, C.G. Biliaderis, Oil-in-water emulsions stabilized by chitin nanocrystal particles, Food Hydrocoll. 25 (2011) 1521–1529, https://doi.org/10.1016/j.foodhyd.2011.02.008.
[14] USDA-NASS, Peanut stocks and processing, Latest Releases from https://downloads.usda.library.cornell.edu/usda-esmis/files/02870v87z/bk128k43k/4q77g072x/pnst0419.pdf 2019.
[15] N. Reddy, L. Chen, Y. Yang, Thermoplastic films from peanut proteins extracted from peanut meal, Ind. Crop. Prod. 43 (2013) 159–164, https://doi.org/10.1016/j.indcrop.2012.06.051.
[16] A. Sanchez-Resendiz, S. Rodriguez-Barrientos, J. Rodriguez-Rodriguez, B. Barba-Davila, S.O. Serna-Saldivar, C. Chuck-Hernandez, Phosphoesterification of soybean and peanut proteins with sodium trimetaphosphate (STMP): changes in structure to improve functionality for food applications, Food Chem. 260 (2018) 299–305, https://doi.org/10.1016/j.foodchem.2018.04.009.
[17] P. Li, W. Zhang, X. Han, J. Liu, Y. Liu, M.A.A. Gasmalla, R. Yang, Demulsification of oil-rich emulsion and characterization of protein hydrolysates from peanut cream emulsion of aqueous extraction processing, J. Food Eng. 204 (2017) 64–72, https://doi.org/10.1016/j.jfoodeng.2017.02.009.
[18] J. Ye, X. Hua, Q. Zhao, W. Zhao, G. Chu, W. Zhang, R. Yang, Chain conformation and rheological properties of an acid-extracted polysaccharide from peanut sediment of aqueous extraction process, Carbohydr. Polym. 228 (2020), 115410. https://doi.org/10.1016/j.carbpol.2019.115410.
[19] J. Ye, X. Hua, M. Wang, W. Zhang, R. Yang, Effect of extraction pH on the yield and physicochemical properties of polysaccharides extracts from peanut sediment of aqueous extraction process, Lwt 106 (2019) 137–144, https://doi.org/10.1016/j.lwt.2019.02.049.
[20] Association of Official Analytical Chemists, Official Methods of Analysis of AOAC *International*, 17th ed, Method 900.02, , AOAC, Gaithersburg, MD, USA, 2000.
[21] M. Dubois, K.A. Gilles, J.K. Hamilton, P.t. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356, https://doi.org/10.1021/ac60111a017.
[22] I. Kutzli, M. Gibis, S.K. Baier, J. Weiss, Formation of whey protein isolate (WPI)-maltodextrin conjugates in fibers produced by needleless electrospinning, J. Agric. Food Chem. 66 (2018) 10283–10291, https://doi.org/10.1021/acs.jafc.8b02104.
[23] S. Li, C. Li, Y. Yang, X. He, B. Zhang, X. Fu, C.P. Tan, Q. Huang, Starch granules as Pickering emulsifiers: role of octenylsuccinylation and particle size, Food Chem. 283 (2019) 437–444, https://doi.org/10.1016/j.foodchem.2019.01.020.
[24] L.J. Wang, Y.Q. Hu, S.W. Yin, X.Q. Yang, F.R. Lai, S.Q. Wang, Fabrication and characterization of antioxidant Pickering emulsions stabilized by zein/chitosan complex particles (ZCPs), J. Agric. Food Chem. 63 (2015) 2514–2524, https://doi.org/10.1021/jf505227a.
[25] Y. Cai, X. Deng, T. Liu, M. Zhao, Q. Zhao, S. Chen, Effect of xanthan gum on walnut protein/xanthan gum mixtures, interfacial adsorption, and emulsion properties, Food Hydrocoll. 79 (2018) 391–398, https://doi.org/10.1016/j.foodhyd.2018.01.006.
[26] C. Yan, D.J. McClements, Y. Zhu, L. Zou, W. Zhou, W. Liu, Fabrication of OSA starch/chitosan polysaccharide-based high internal phase emulsion via altering interfacial behaviors, J. Agric. Food Chem. 67 (2019) 10937–10946, https://doi.org/10.1021/acs.jafc.9b04009.
[27] R. Aveyard, B.P. Binks, J.H. Clint, Emulsions stabilised solely by colloidal particles, Adv. Colloid Interf. Sci. 100 (2003) 503–546, https://doi.org/10.1016/S0001-8686(02)00069-6.
[28] Y. Yi, M.-W. Zhang, S.-T. Liao, R.-F. Zhang, Y.-Y. Deng, Z.-C. Wei, B. Yang, Effects of alkali dissociation on the molecular conformation and immunomodulatory activity of longan pulp polysaccharide (LPI), Carbohydr. Polym. 87 (2012) 1311–1317, https://doi.org/10.1016/j.carbpol.2011.09.014.
[29] B. Madivala, S. Vandebril, J. Fransaer, J. Vermant, Exploiting particle shape in solid stabilized emulsions, Soft Matter 5 (2009) 1717, https://doi.org/10.1039/b816680c.
[30] Z. Huang, Y. Huang, X. Li, L. Zhang, Molecular mass and chain conformations of Rhizoma panacis japonici polysaccharides, Carbohydr. Polym. 78 (2009) 596–601, https://doi.org/10.1016/j.carbpol.2009.05.027.
[31] O.K. Simo, Y. Mao, T. Tokle, E.A. Decker, D.J. McClements, Novel strategies for fabricating reduced fat foods: heteroaggregation of lipid droplets with polysaccharides, Food Res. Int. 48 (2012) 337–345, https://doi.org/10.1016/j.foodres.2012.04.018.
[32] Y. Cao, R. Mezzenga, Design principles of food gels, Nat. Food 1 (2020) 106–118, https://doi.org/10.1038/s43016-019-0009-x.
[33] K.P. Drapala, D.M. Mulvihill, J.A. O'Mahony, A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems, Food Struct. 16 (2018) 27–42, https://doi.org/10.1016/j.foostr.2018.01.004.
[34] Y. Jiang, C. Zhang, J. Yuan, Y. Wu, F. Li, D. Li, Q. Huang, Effects of pectin polydispersity on zein/pectin composite nanoparticles (ZAPs) as high internal-phase Pickering emulsion stabilizers, Carbohydr. Polym. 219 (2019) 77–86, https://doi.org/10.1016/j.carbpol.2019.05.025.
[35] F.Z. Zhou, X.N. Huang, Z.L. Wu, S.W. Yin, J.H. Zhu, C.H. Tang, X.Q. Yang, Fabrication of zein/pectin hybrid particle-stabilized Pickering high internal phase emulsions with robust and ordered Interface architecture, J. Agric. Food Chem. 66 (2018) 11113–11123, https://doi.org/10.1021/acs.jafc.8b03714.
[36] P. Chivero, S. Gohtani, H. Yoshii, A. Nakamura, Assessment of soy soluble polysaccharide, gum arabic and OSA-starch as emulsifiers for mayonnaise-like emulsions, LWT Food Sci. Technol. 69 (2016) 59–66, https://doi.org/10.1016/j.lwt.2015.12.064.