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Research Article

Effects of pH on the Composition and Physical Stability of Peanut Oil Bodies from Aqueous Enzymatic Extraction

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Peanut oil body (POB), which is rich in unsaturated fatty acids and bioactive substances, is widely used in cosmetics, food, and medicine. Compared with synthetic emulsifiers, peanut oil bodies have health advantages as natural emulsions. The physicochemical properties of oil bodies affect their food processing applications. To improve peanut oil body yield, cell-wall-breaking enzymes were screened for aqueous enzymatic extraction. The optimum conditions were as follows: enzymatic hydrolysis time, 2 h; material-to-liquid ratio, 1 : 5 (*m*/*v*); enzyme concentration, 2% (*v*/*m*); and temperature, 50°C. Oil body stability was closely related to pH. With increasing pH, the average particle size and zeta-potential of the oil bodies increased, indicating aggregation, as confirmed by microstructure analysis. At pH 11, exogenous proteins at the oil body interface were eluted, leaving endogenous proteins, which led to a decreased interfacial protein content and oil body aggregation. Therefore, oil body stability decreased under alkaline pH conditions, but no demulsification occurred.

1. Introduction

Recently, oil body, or lipid body, oleosome, has received considerable attention owing to its potential applications in cosmeceuticals, nutraceuticals, and pharmaceuticals [1, 2]. Oil bodies are small (0.2–2 μm) discrete cell organelles composed of triacylglycerols (94.2–98.2%), surrounded by a monolayer of phospholipids (0.6–2%), and embedded with oil body integral proteins (0.3–3%), namely, oleosin (the content is nearly 80–90%), caleosin, and steroleosin [3, 4]. During traditional solvent extraction of oil from oilseeds, oil bodies are destroyed by organic solvents, with the released crude oil requiring to be further refinement [5].

Oil bodies, as organelles for storing oil, are distributed in large quantities in peanut cells [6]. Different methods for extracting oil bodies had been reported. Internal oil bodies are usually obtained using physical crushing to destroy the cell wall. Liu et al. [7] compared the effects of different comminution methods on oil body yield, with wet grinding found to give a higher yield than dry grinding. Wet grinding requires presoaking of the oilseed, but different oil crops types require different presoaking times, such as 72 h for palm kernels [8], 24 h for pecan nut [9], and overnight for safflower seeds [10]. Common extraction methods are aqueous extraction and aqueous enzymatic extraction [11]. Aqueous extraction process involves initially mixing the grinded seeds with water, followed by intensive agitation, which results in the preparation of a dilute oil body extract [12]. Compared with other soaking solvents, oil bodies extracted by water have higher stability and can interact better with the interface composition of oil bodies [13]. The reaction is usually conducted under alkaline conditions, high salt ion concentration and high temperature, which decreases the oil body stability and utilization rate [14, 15]. Although this method has been well researched, the extraction efficiency remains limited. On the other hand, aqueous enzymatic extraction promotes the separation of oil bodies by rupturing polysaccharides, proteins, and other components in the cell wall [16, 17]. The type of enzymes added depends on the composition and structure of the oilseed cell wall. Soybean cell wall is mainly composed of protein, which is released by protease degradation of the

protein oil body [18]. Pectin is the main component of rapeseed cell wall, which can be degraded by pectinase [19]. Peanut cells are mainly composed of cellulose, hemicellulose, and pectin, with cellulose serving as the skeleton of the membrane structure. Therefore, cellulase, hemicellulose, and pectinase can be used to destroy peanut cells and release internal oil bodies [17]. Furthermore, proteases are commonly used to destroy protein in the cell wall but inevitably break down peanut proteins into small molecule peptides, resulting in nutritional and functional loss.

During food processing, the stability of oil bodies must be enhanced to meet environmental changes (pH, temperature, and salt). Stability between oil bodies is mainly due to electrostatic repulsion and steric hindrance, while stability inside the oil body is mainly affected by changes in the interfacial membrane, mainly the protein composition [20, 21]. It is known that the changes in pH can affect the interface charges [22]. Oil bodies are stable at pH higher than 7 but unable in pH range of 3–6 (pH close to the isoelectric point), while electrostatic repulsion is the smallest, and the stability is the lowest. At neutral pH, exogenous proteins, including globulins, albumin, and lipoxygenase, are bound to the oil body surface, which is almost removed under alkaline conditions [23]. At pH 11, only endogenous protein remains on the oil body surface, but whether the oleosin can be eluted by alkaline pH has yet to be determined.

This study aimed to first extract peanut oil bodies and protein using different enzymes and optimize the process. By comparing changes in particle size, zeta-potential, and interface protein composition, changes in oil body stability under alkaline pH will then be analyzed, which will aid the application of peanut oil bodies in the food industry.

2. Material and Methods

2.1. Materials. Newly harvested peanuts (*YuHua-23*) were purchased from Henan Academy of Agricultural Sciences (Zhengzhou, China), which were shelled and stored at 4°C until use. Cellulase, hemicellulose, pectinase, and viscozyme L (main ingredients: cellulase, hemicellulose, and arabinose) were purchased from Novozymes (Beijing, China). Other reagents of analytical grade or higher were purchased from Sigma-Aldrich Trading Co., Ltd.

2.2. Preparation of Peanut Oil Body and Protein. Deionized water (1 : 4, *w*/*v*) was added to skinless peanut seeds (30 g), and this was stored in a refrigerator at 4°C for 18 h. The soaked peanuts were then washed with deionized water three times and then made up a total weight of 150 g with deionized water. The peanuts were then crushed for 120 s with a tissue shredder at 18,000 rpm using a multifunctional food processor (C022E, Joyoung Co., Ltd., Shandong, China). Different enzymes (2%; cellulase, hemicellulose, pectinase, or viscozyme L) were added to the as-obtained peanut pulp, followed by incubation for 2 h at 50°C in a constant temperature bath shaker (THZ-82, Jintan Huafeng Instrument Co., Ltd, Tianjin, China). After enzymatic hydrolysis, the enzyme was inactivated in a boiling water bath for 5 min. After extraction, the peanut pulp was cooled in cold water and centrifuged (DZ267-32C6, Anting Scientific Instrument Factory, Shanghai, China). The upper oil body was collected and repeated three times. Sodium azide (0.2%, *w*/*w*) was then added to prevent microbial growth. The oil body was dried in a vacuum drying oven (DZF-2B, Beijing Yongguang Medical Instrument Co. Ltd, Beijing, China) at 50°C for 10 h to remove water. The oil body yield was calculated using equation (1). The peanut protein in the aqueous phase was alkali-dissolved, acid-precipitated, freeze-dried to a powder (LGJ-25; Beijing Sihuan Scientific Instrument Inc., Beijing, China), and stored in a refrigerator at 4°C for use later. The peanut protein content in the aqueous phase was calculated using the following equation:

$$\text{oil body yield (\%)} = \frac{\text{oil body dry weight}}{\text{peanut weight}} \times 100, \quad (1)$$

$$\text{aqueous phase protein (\%)} = \frac{\text{aqueous phase protein weight}}{\text{peanut protein content}} \times 100. \quad (2)$$

2.3. Particle Size Analysis and Zeta-Potential Measurement. The oil body sample (1 g) to be measured was diluted 20-fold with deionized water, and the evenly dispersed liquid was quickly transferred into the sample pool of the laser particle size analyzer (Baxter BT-9300S, Dandong Baxter Instrument Co., Ltd, Dandong, China) using a plastic straw for particle size determination. The oil body sample was diluted by 10-fold with deionized water, and the zeta-potential was determined using a zeta-potential analyzer (Zetasizer Nano ZSP, Marvin instrument co., Ltd., Marvin, England).

2.4. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE of the oil body protein was conducted using the method of Ying [24] with some modifications. After Kjeldahl determination of the obtained oil body protein content, oil body protein and SDS were fully mixed in a 1 : 1.52 (*w*/*w*) ratio using equal volumes by shaking in a vortex shaker for 2 min. SDS-PAGE was performed on a gel slab comprising 5% stacking gel and 12% separating gel in an SDS-Tris-glycine discontinuous buffer system. The protein was diluted to 2 mg/mL with sample buffer, which was added with SDS (2%, *w*/*w*), thiourea

(2 M), urea (6 m), and DTT (1%, *w*/*w*) based on pH 8.5 Tris-HCl buffer, and a sample (10 μL) was loaded into the sample well. After electrophoresis, the gels were fixed for 1 h, stained for 2 h, and bleached until the background was clear.

2.5. Confocal Laser Scanning Microscopy (CLSM). The extracted peanut oil body was stained with 0.1% (*w*/*v*) Nile Red (soluble in anhydrous ethanol) and 1% (*w*/*v*) Nile Blue (soluble in acetone) for 30 min. After staining, 10 μL of the emulsion was placed on a glass slide, and the oil body distribution and microstructure were observed using a laser confocal microscope (Germany Carl Zeiss co., Ltd., Jean, Germany) under excitation wavelength of 488 nm and 561 nm at 40x magnification.

2.6. Scanning Electron Microscopy (SEM). Scanning electron microscopy was conducted according to the method of Hu et al. [25] with some modifications. The peanut oil body was mixed with n-hexane (1 : 5, *w*/*v*) and stirred at 4°C for 2 h. After centrifugation, the upper layer was removed and repeated for 2~3 times. Methanol/chloroform (2 : 1, *v*/*v*) was added to remove phospholipids, and the upper layer was removed and repeated twice. After drying with nitrogen, the sample obtained is the oil body interface protein. The oil body interface protein was fixed on the sample platform and observed by SEM (JEOL JSM-7500F, Tokyo, Japan).

2.7. Amino Acid Composition Analysis. Amino acid composition was determined using the method reported by Liu et al. [26] with some modifications. Samples (50 mg) were placed in a hydrolysis tube, and hydrochloric acid (6 M) was added, followed by hydrolysis at 110°C for 24 h. The hydrolysate was then dried under vacuum, sodium citrate buffer solution was added, and the amino acid content of the sample was determined using an amino acid analyzer (Sykam S 433D, Germany).

2.8. Proximate Analysis. The oil, protein, moisture, crude fiber, and ash contents were determined using AOAC standard methods.

2.9. Effect of Alkaline pH on Peanut Oil Body. Deionized water (1 : 5, *w*/*v*) was added to the crude oil (5 g), and the pH was adjusted to 6.8 (crude oil body), 8, 9, 10, or 11 with 0.1 M NaOH. The mixture was then subjected to centrifugation (15000 rpm, 15 min, 4°C) using a high-speed refrigerated centrifuge (GL-10000C; Anting Scientific Instrument Factory, Shanghai, China), and the upper oil body was collected. This process was repeated three times, with the as-obtained final oil body samples denoted as pH 6.8-OB, pH 8-OB, pH 9-OB, pH 10-OB, and pH 11-OB.

2.10. Experimental Design and Statistical Analysis. All experiments were repeated at least three times using prepared samples. Average and standard deviations were calculated from three measurements. Data were presented as means ± standard deviation (SD). All data were analyzed by SPSS version 17.0 (SPSS Institute Inc, USA). Images were processed in Origin (version 8.5, OriginLab Institute Inc, USA). Significant difference was analyzed at the $P < 0.05$ level (LSD0.05).

3. Results and Discussion

3.1. Main Components Analysis. The oil and protein contents of skinless peanut were 46.10% and 22.81%, respectively, and were similar to values reported values [27]. Peanut oil contained nearly 80% unsaturated fatty acids, mainly oleic acid (37.00–55.60%) and linoleic acid (25.20–39.70%), and was rich in γ-tocopherol, which was the main source of vitamin E and antioxidants [28]. As shown in Table 1, the oil body was mainly composed of oil (69.54%) and water (26.00%), but the stability was mainly determined by the protein content, which was only 1.78%.

3.2. Effects of Different Cell-Wall-Degrading Enzymes on Oil Body and Aqueous Phase Proteins. As shown in Figure 1, compared with the control, enzyme addition significantly increased the oil body and protein yields. The highest peanut oil body and protein yields extracted by cellulase were 40.5% and 48.55%, respectively. These results closely correlated to the peanut cells structure. In the reported studies, different oil bodies are observed for each oilseed, which was due to the composition of the cell wall of oil seed (mainly proteins, lignin, hemicellulose, and pectic) [29, 30]. Peanut cells are mainly composed of cellulose, hemicellulose, and pectin, with cellulose serving as the skeleton of the membrane structure. After enzymatic hydrolysis, the cellulose breaks down, and the oil bodies and proteins are released. In contrast to the results of Naseri et al. [31] and Liu et al. [32], viscozyme L gave the highest yield owing to the use of different raw material (Naseri used *Eucheuma denticulatum* as the raw material) and different processing methods (Liu used dry grinding).

3.3. Optimization of Aqueous Enzymatic Extraction. According to the results in Section 3.2, cellulase was selected for the subsequent optimization of aqueous enzymatic extraction. Figure 2(a) shows the effect of enzyme concentration on oil body and protein yield. When the enzyme concentration reached 2% (*v*/*w*), the oil body and protein yield reached a maximum of 45.94% and 49.09%. When the enzyme concentration exceeded 2%, the maximum yield was obtained and remained stable; either the substrate was saturated, or the caramelization of soluble sugar limited oil body release. And excess enzyme concentration will produce bitterness and off-flavor [27]. Based on the above results and cost considerations, 2.0% would be more suitable for aqueous enzymatic extraction.

As shown in Figure 2(b), the oil body and protein yield initially increased with increasing temperature, reaching maximum values of 47.74% and 49.21% at 50°C. These results were consistent with the study of Pinelo et al. [33]. That was due to 50°C being a suitable temperature for the

Table 1: Main components of peanut and oil body.

Component	Oil	Protein	Water	Crude fiber	Ash
Peanut (wet %)	46.10 ± 0.41	22.81 ± 0.63	2.34 ± 0.04	4.9 ± 0.5	2.45 ± 0.05
Oil body (wet %)	69.54 ± 0.28	1.78 ± 0.05	26.00 ± 0.10	—	—

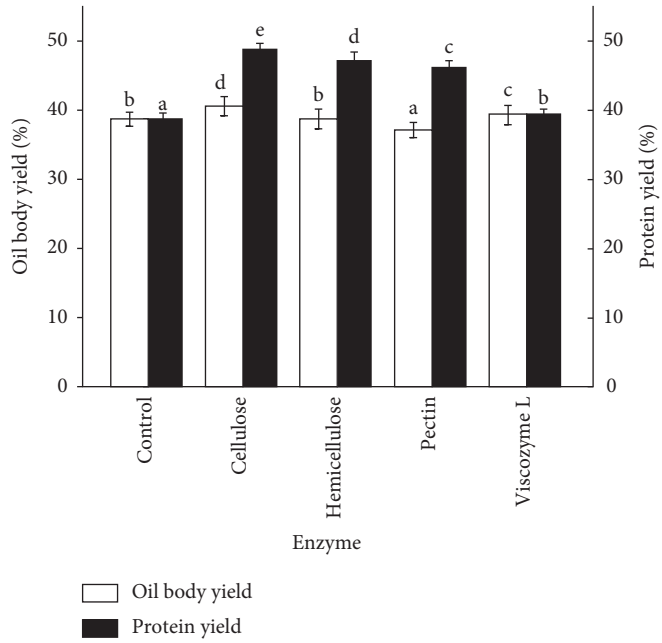

Figure 1: Effects of different cell wall-degrading enzymes on the yields of peanut oil body and aqueous phase protein.

cellulase, with higher temperature causing cellulase inactivation [34]. Accordingly, the appropriate extraction temperature was set to 50°C.

Figure 2(c) shows that the oil body and protein yields first increased and then decreased with an decreasing material-to-liquid ratio, reaching the maximum value of 45.28% at 1 : 5. These results were consistent with the mass transfer principle. When the material-to-liquid ratio was relatively high, cellulase was unable to fully contact with peanut cells to effectively conduct enzymolysis, which was attributed to the fact that the mixture was too viscous and difficult to homogenize [35]. However, a low material-to-liquid ratio decreased the enzyme and substrate concentrations, decreasing the opportunity for collision between enzyme and substrate at a molecular level [36].

Figure 2(d) shows that the oil body and protein yields initially increased with time, reaching a maximum value 45.14% and 49.09% after 2 h. With further increasing time, the yield remained stable, which can be explained due to the enzymatic limit and the reverse inhibition of the reaction product [35]. Therefore, the optimal enzymatic hydrolysis time was set as 2 h.

3.4. Effect of pH on Oil Body Stability

3.4.1. Effect of pH on Oil Body Particle Size. The stability of oil bodies against droplet coalescence is crucial as it determines their application in food products. To investigate the effect of pH on the extracted peanut oil bodies, the average particle size was measured. The average particle size of peanut oil bodies directly reflected the dispersibility of droplets in the oil body, as shown in Figure 3. At initial pH 6.8, the average particle size was 2.16 μm, which was higher than reported by Sukhotu (0.99 μm), possibly due to the differences in the extraction method and peanut variety [28]. As the pH increased from 6.8 to 11, the average particle size gradually increased. A previous study showed that aggregation occurred to some extent even under neutral conditions [37]. Under alkaline conditions (pH increased to 11), the oil bodies underwent alkaline washing, causing exogenous proteins to be eluted, and the interface protein content to decrease. Adjacent oil body droplets fused and aggregated into large droplets, and the oil bodies stability decreased.

3.4.2. Effect of pH on Oil Body Zeta-Potential. Zeta-potential measurements are usually used to determine the colloidal interactions, which elucidate the emulsion stability [38]. Changes in the zeta-potential can reflect the chargeability of particles in the oil body and the strength of electric repulsion between the oil bodies. At the isoelectric point, the zeta-potential of the oil body was close to 0, and the hydrophobicity of the particles was strongest due to the decrease in electrostatic repulsion between particles and the action of van der Waals forces [39, 40]. As shown in Figure 4, the zeta-potential increased from—9.7 mV to–3.8 mV as the pH increased from 6.8 to 11. According to the oil body

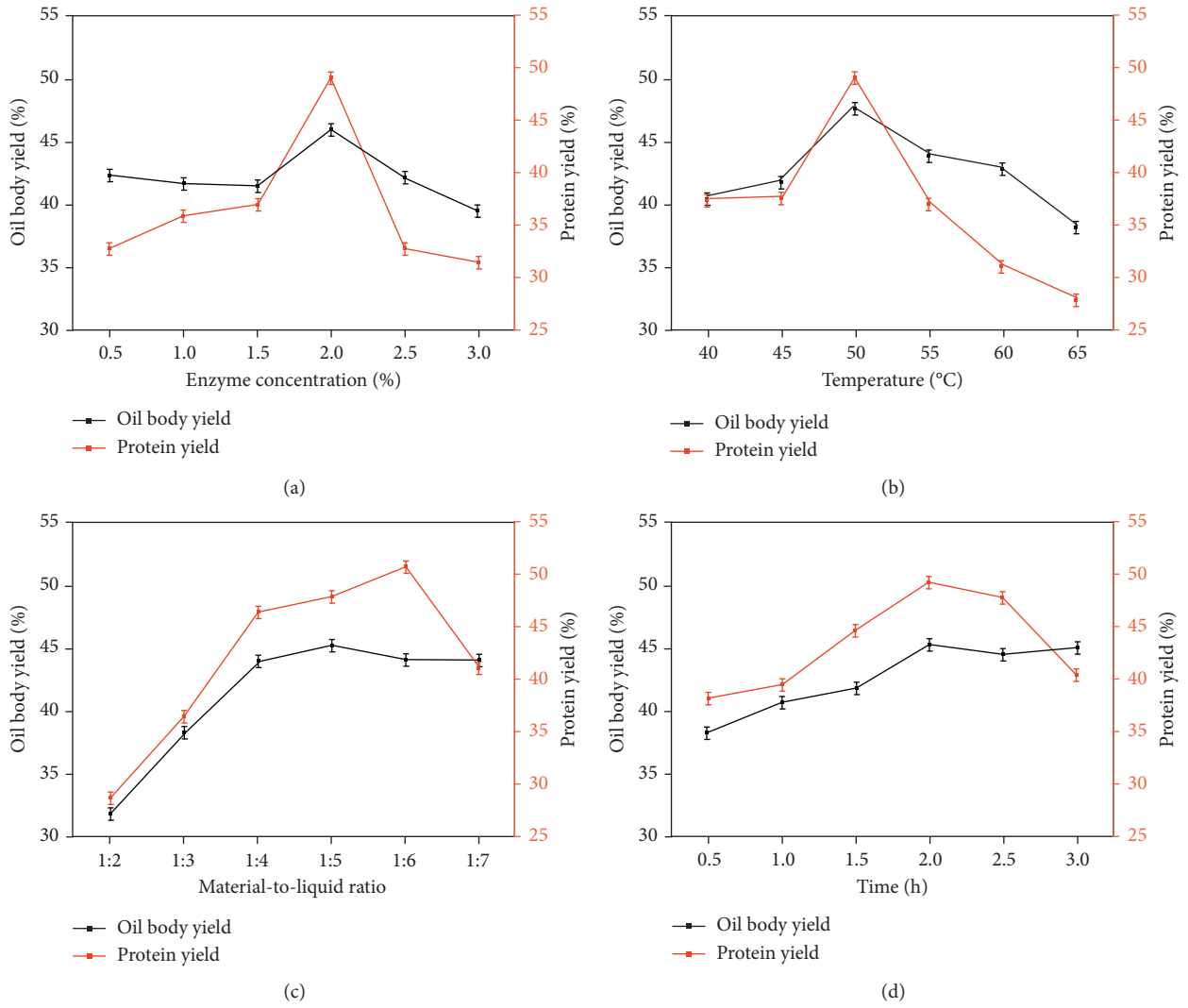

Figure 2: Effects of different parameters on the yields of oil body and protein extracted by cellulase: (a) enzyme concentration, (b) temperature, (c) material-to-liquid ratio, and (d) time.

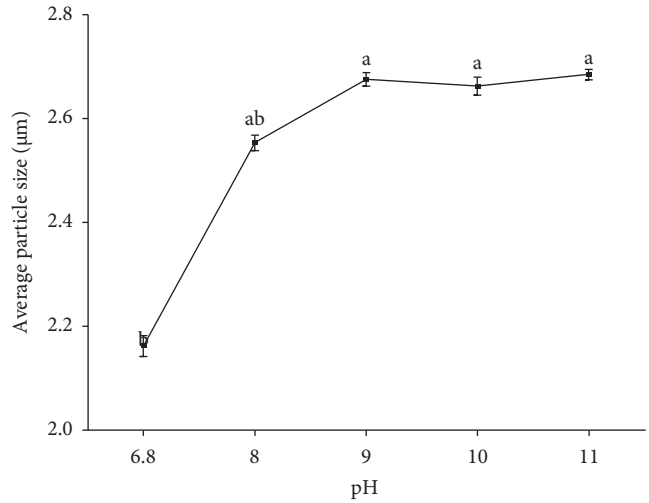

Figure 3: Effect of different pH values on the average particle size of peanut oil body (pH range of 6.8–11).

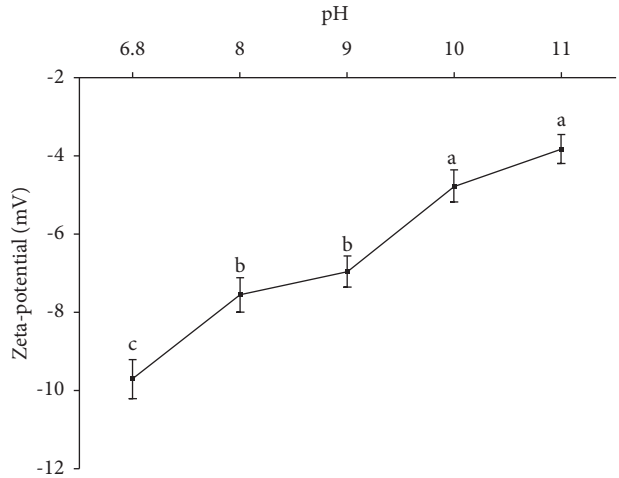

Figure 4: Effect of different pH values on the zeta-potential of peanut oil body (pH range of 6.8–11).

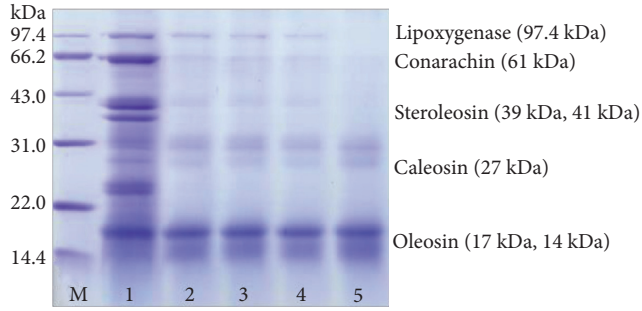

Figure 5: Effects of different pH treatments on the composition of interfacial proteins in peanut oil body (M, 1, 2, 3, 4, and 5 represent marker, pH 6.8-OB, pH 8-OB, pH 9-OB, pH 10-OB, and pH 11-OB, respectively).

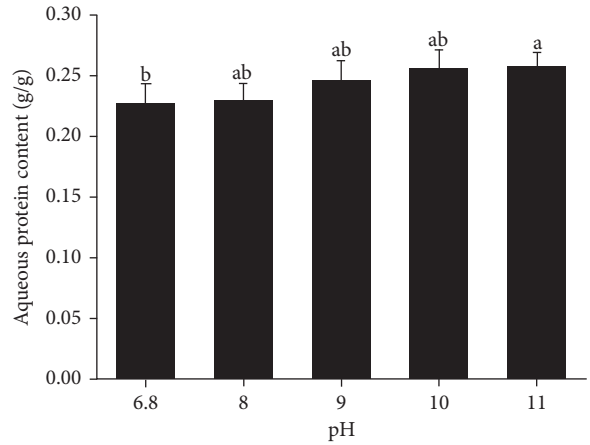

Figure 6: Effect of pH treatment of oil bodies on the aqueous phase protein content.

model reported by Tzen and Huang [41], the negatively charged residue of the oil body protein at the oil body interface was exposed on the outside, resulting in the oil body being negatively charged and providing steric hindrance and electrostatic repulsion [42].

3.4.3. Interfacial Protein Composition of Peanut Oil Body under Different pH. According to the SDS-PAGE results (Figure 5), in addition to endogenous proteins (oleosin, caleosin, and steroleosin), exogenous proteins, such as lipoxygenase and conarachin, were present in line 1 (pH 6.8-OB).

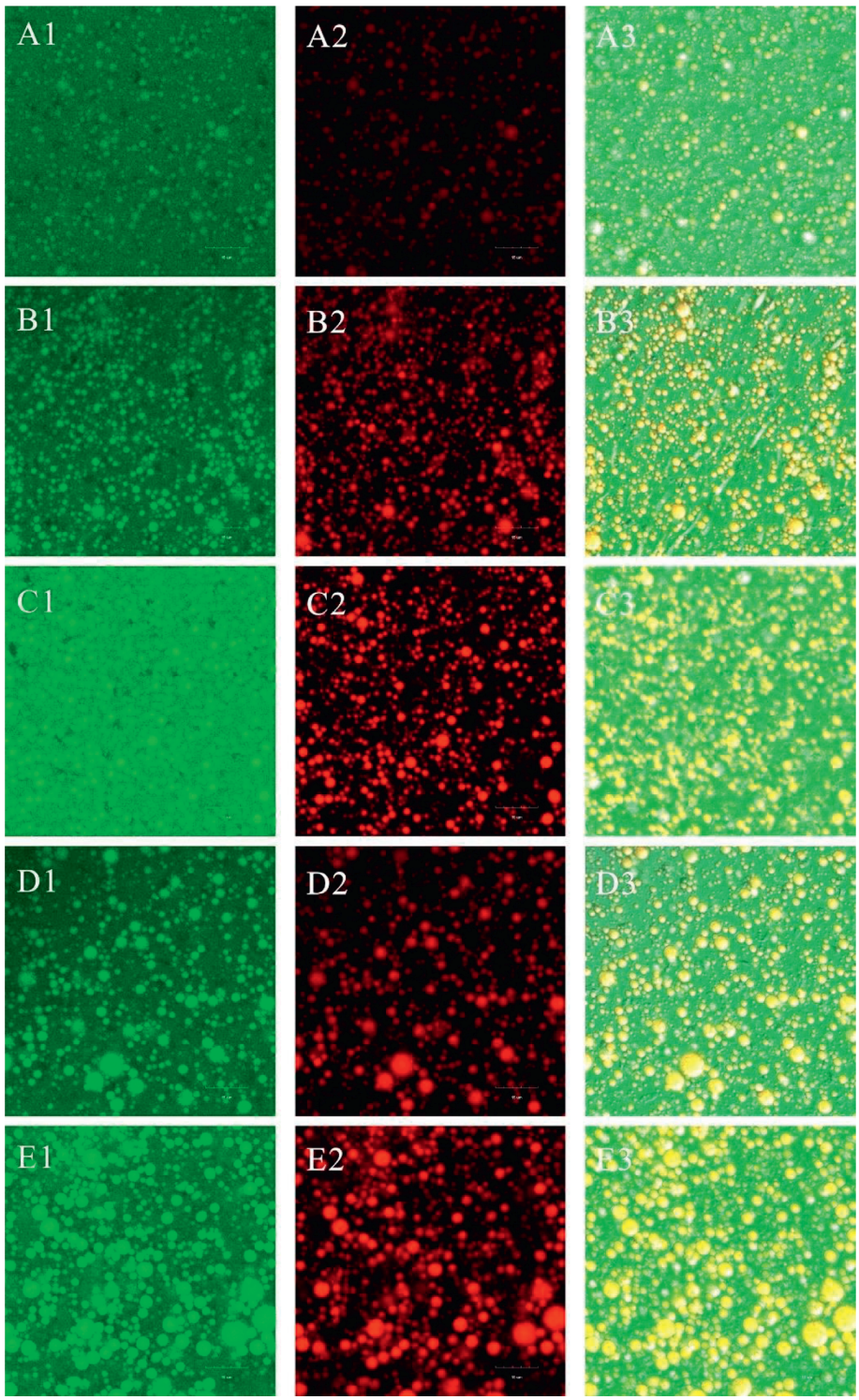

Figure 7: CLSM images of the peanut oil body microstructure under different pH conditions. (a–e) pH 6.8, 8, 9, 10, and 11, respectively; 1 denotes protein stained green, 2 denotes oil stained red, and 3 denotes oil bodies with oil and protein). According to the previous experimental methods, oil body suspensions were mixed with Nile Red (excitation 561 nm, emission 588 nm) to stain neutral lipids and FITC (excitation 488 nm, emission 517 nm) to stain proteins. In the CLSM results, the oil located in the core of the oil body was labeled with Nile Red to emit red fluorescence, and the protein at the oil body interface was labeled with FITC to emit green fluorescence.

By comparing soybean crude oil body and refined oil body, Ishii et al. [43] also found that the electrophoresis band of the crude oil body was significantly more than that of the refined oil body, indicating that the crude oil interface membrane not only contained endogenous protein, but was also rich in soybean storage proteins and some other low molecular-weight

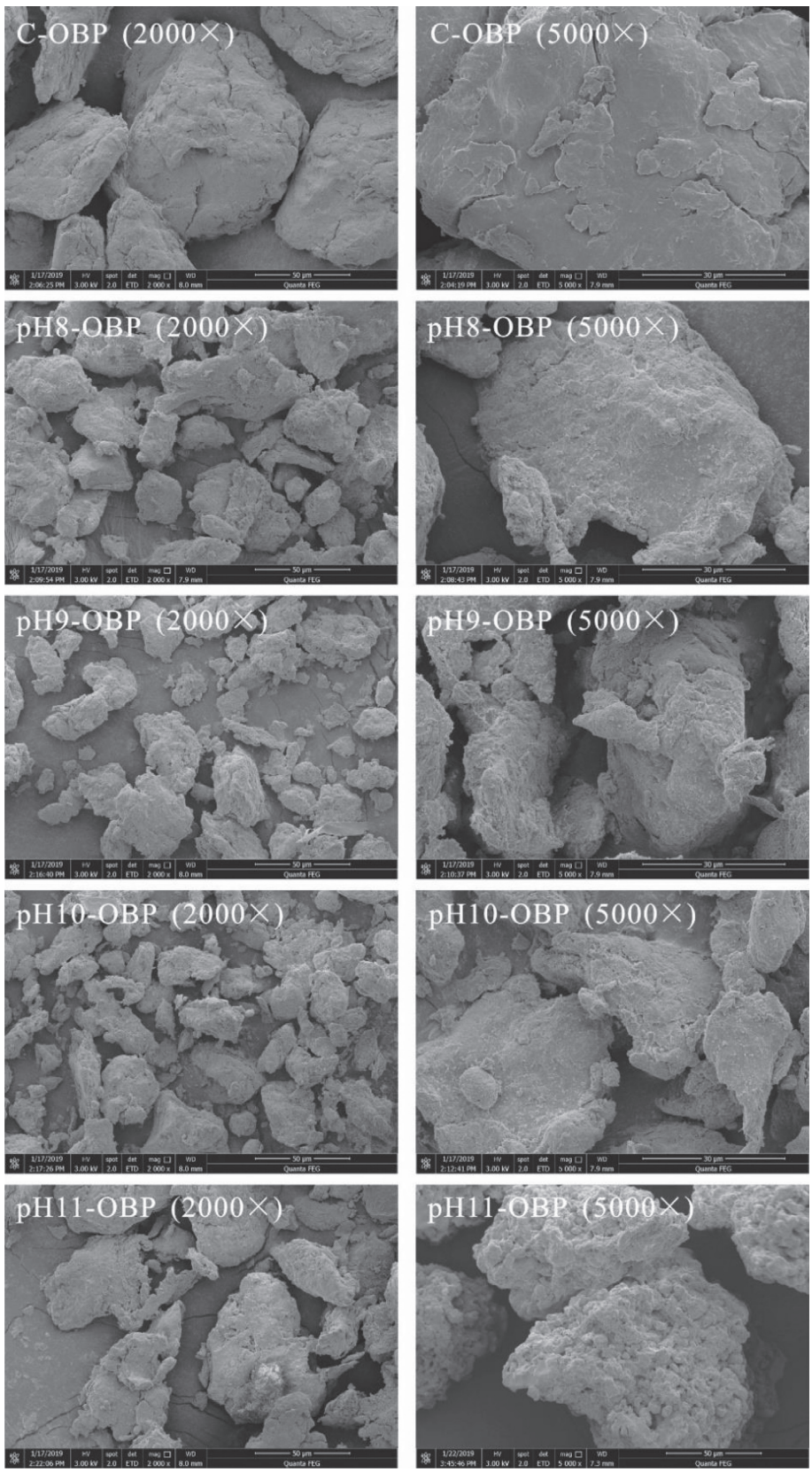

Figure 8: Scanning electron microscopy (SEM) images of oil body interface proteins under different pH conditions (magnification of 2,000x and 5,000x).

Table 2: Amino acid composition of oil body interface protein under different pH conditions.

Amino acid	pH 6.8	pH 8	pH 9	pH 10	pH 11
Asp	0.670 ± 0.02	0.645 ± 0.01	0.676 ± 0.03	0.725 ± 0.03	0.593 ± 0.01
Thr	0.727 ± 0.01	0.732 ± 0.05	0.787 ± 0.03	0.867 ± 0.04	0.740 ± 0.03
Ser	0.580 ± 0.03	0.586 ± 0.02	0.623 ± 0.02	0.683 ± 0.03	0.581 ± 0.02
Glu	0.795 ± 0.02	0.756 ± 0.04	0.807 ± 0.04	0.865 ± 0.04	0.704 ± 0.04
Gly	0.720 ± 0.01	0.712 ± 0.03	0.767 ± 0.03	0.846 ± 0.03	0.731 ± 0.03
Ala	0.756 ± 0.01	0.738 ± 0.02	0.817 ± 0.04	0.892 ± 0.02	0.764 ± 0.03
Cys	0.057 ± 0.01	0.045 ± 0.02	0.049 ± 0.02	0.053 ± 0.02	0.042 ± 0.02
Val	0.550 ± 0.04	0.536 ± 0.01	0.570 ± 0.02	0.620 ± 0.03	0.535 ± 0.02
Met	0.107 ± 0.01	0.128 ± 0.01	0.105 ± 0.01	0.102 ± 0.02	0.092 ± 0.01
Ile	0.529 ± 0.02	0.542 ± 0.02	0.562 ± 0.03	0.608 ± 0.02	0.521 ± 0.03
Leu	0.917 ± 0.03	0.917 ± 0.04	0.986 ± 0.03	1.068 ± 0.04	0.913 ± 0.02
Tyr	0.567 ± 0.02	0.591 ± 0.02	0.601 ± 0.04	0.656 ± 0.02	0.556 ± 0.03
Phe	0.463 ± 0.02	0.479 ± 0.02	0.481 ± 0.02	0.512 ± 0.03	0.435 ± 0.02
His	0.252 ± 0.01	0.164 ± 0.02	0.183 ± 0.01	0.188 ± 0.02	0.152 ± 0.02
Lys	0.451 ± 0.01	0.442 ± 0.02	0.469 ± 0.01	0.504 ± 0.04	0.407 ± 0.02
Arg	0.589 ± 0.03	0.564 ± 0.03	0.617 ± 0.03	0.599 ± 0.02	0.528 ± 0.02
TAA	14.100 ± 0.04	14.400 ± 0.04	14.600 ± 0.05	14.200 ± 0.04	14.600 ± 0.05
EAA	3.995 ± 0.03	3.941 ± 0.02	4.142 ± 0.03	4.467 ± 0.02	3.796 ± 0.02
E/T (%)	28.34 ± 0.02	27.37 ± 0.02	28.37 ± 0.02	31.46 ± 0.03	26.00 ± 0.02
HAA	3.322 ± 0.02	3.341 ± 0.02	3.520 ± 0.02	3.802 ± 0.02	3.261 ± 0.02

Note: TAA, EAA, E/T, and HAA represent the total amino acid, essential amino acid, EAA/TAA, and hydrophobic amino acid contents, respectively.

proteins. Exogenous proteins, derived from protein bodies, interact with oil body interface proteins during the extraction process to form a second interface membrane that stabilizes the oil bodies [12]. With increasing pH, the exogenous protein content decreased gradually, indicating that the oil body interface binding was relatively loose. At pH 11, lipoxygenase and conarachin had completely disappeared, and most of steroleosin had disappeared, leaving only oleosin and caleosin. This result was consistent with Cao et al. [21], and the oleosin band became denser with increasing pH, while exogenous protein content decreased significantly. Furthermore, as shown in Figure 6, the increase in aqueous protein content after pH washing also indicated that exogenous proteins were shed. With increasing pH, the interfacial protein content, negative charge of the oil body interface, and oil body stability decreased, while the oil droplets accumulated, and particle size increased.

3.4.4. Microstructure of Peanut Oil Body. As shown in Figure 7, oils (stained red) were surrounded by an extra protein layer (stained green). The protein layer contained endogenous proteins (oleosin, caleosin, and steroleosin) and exogenous proteins (conarachin, lipoxygenase, and other), which might have been extracted by aqueous enzymatic extraction [44, 45]. The oil bodies had a smaller particle size and uniform dispersion at pH 6.8. With increasing pH, the particle size gradually increased, forming large droplets in a polydisperse state. At pH 11, a large amount of oil body was observed to aggregate to form large particle sizes, which still existed in the form of droplets. The results showed that an alkaline pH affected the oil body stability, but with a limited influence, while demulsification was not achieved.

The microstructures of the oil body interface proteins were observed using SEM at two different magnifications (2000x and 5000x). As shown in Figure 8, the surface of the crude oil body interface protein (C-OBP) was relatively flat and smooth, and the endogenous and exogenous proteins were tightly bound. With increasing pH, the interface proteins became rough and porous, which was most obvious at pH 11. This phenomenon might be due to the solubility of the endogenous proteins decreasing, and exogenous protein falling off the oil body interface to make the interface rough. From the analysis of the amino acid composition of oil body interfacial protein (Table 2), the hydrophobic amino acid content of the interface protein initially increased and then decreased. Hydrophobic amino acids (Asp, Thr, Ser, Glu, Gly, Cys, Tyr, His, Lys, and Arg) maintained the protein tertiary structure [46]. When the hydrophobic amino acid content decreased, the hydrophobic area of the oil body interface decreased, and oil body aggregation increased.

4. Conclusion

By screening wall-breaking enzymes, cellulase was selected to extract peanut oil bodies and protein. The optimal extraction conditions, obtained by single factor experiments, were as follows: enzymatic hydrolysis time 2 h; material-to-liquid ratio 1 : 5; enzyme concentration 2%; temperature 50°C. The obtained oil body and protein yields were 47.8% and 49.2%, respectively. Oil body stability changed with changing pH. With increasing pH from 6.8 to 11, the particle size and zeta-potential increased, indicating that the oil bodies had aggregated. SDS-PAGE showed that endogenous proteins were completely eluted at pH11, while endogenous proteins were mostly retained, and the hydrophobic amino acid content of the oil body protein was lowest, resulting in increased oil body particle size and decreased stability. Microstructure analysis showed that this change in oil body

stability was due to change in the interface protein composition.

Data Availability

All the data that support the findings of the this study are displayed in the manuscript.

Additional Points

Highlight. Cellulase hydrolyzed peanut cell wall was better than other polysaccharide enzymes, releasing oil bodies and protein. The effect of pH on of oil body stability was evaluated from the particle size, zeta-potential, and interfacial protein composition. With increasing pH, exogenous protein was gradually eluted, leaving only the endogenous protein (mainly oleosin).

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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