Effects of Pretreatment on the Yield of Peanut Oil and Protein Extracted by Aqueous Enzymatic Extraction and the Characteristics of the Emulsion
Chen Liu, Fu-sheng Chen*, Rui-hao Niu, and Yu-hang Gao

College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, CHINA

Abstract: Effects of comminution on peanut particle size and yield of peanut oil and protein were analyzed. Additionally, the emulsion properties (surface protein concentration, particle size, and ξ-potential) were compared. Moreover, different demulsification methods were used to investigate the emulsion stability. Results showed that the yield of peanut oil and protein was highest (87.23% and 82.05%, respectively) after dry comminution for 72 s. Upon wet comminution for 120 s, the yields of peanut oil and protein were 89.91% and 84.70%, respectively, which were both significantly higher than that obtained after dry comminution (p < 0.05). The surface protein concentration and ξ-potential of emulsion made by dry comminution (DCE) were 7.02 mg/m² and 12.08 mV, respectively, and those of emulsion made by wet comminution (WCE) were 10.71 mg/m² and 15.25 mV, respectively, which were significantly higher than that of DCE (p < 0.05). The volume average particle size of DCE was 3.41 µm, which was significantly higher than that of WCE (3.18 µm, p < 0.05). Collectively, these results indicated that WCE was more stable than DCE. Further, the demulsification rate of DCE was significantly higher than that of WCE when treated by freeze-thawing, pH, papain, and phospholipase A2 (p < 0.05). Demulsification effect of Alcalase 2.4L was the best among these five demulsification methods treated, and the demulsification rate of DCE reached 92.77%, which was slightly higher than that of WCE (92.67%), further illustrating the higher stability of WCE.

Key words: peanut, aqueous enzymatic extraction, characteristics of emulsion, demulsification, pretreatment

1 Introduction
The basic principle of aqueous enzymatic extraction (AEE) of peanut oil and protein is the enzymatic hydrolysis of peanut via mechanical comminution promotes the release of oil and protein, and oil and non-oil components (protein and carbohydrate) can be separated based on the difference in affinity of non-oil components and the difference in specific gravity between oil and water. Though AEE is a promising strategy, its industrialization is hampered due to the low yield potential of oil and protein, which could be attributed to the fact that the peanut cell wall is not broken completely and the oil released from the cells does not aggregate into large oil droplets, but directly mixed with water forming stable emulsion. Communion pretreatment before AEE could assist with the cell wall breakage. Breaking of peanut cell wall structure effectively can increase the contact area between oil seeds and enzymes, expand the diffusion rate of enzymes in the feed solution, and promote the enzymatic hydrolysis reaction. Moreover, the extraction solution of AEE is rich in protein, phospholipids, and tiny cell fragments with good surface activity. Therefore, a large amount of stable emulsion cannot avoid these surface-active material and agitation during extraction and centrifugal separation, limiting the release of oil. Whereas, too large a comminution degree of oil seeds will promote the formation of stubborn emulsion and increase the difficulty of demulsification in the subsequent process. Within certain limits, it is beneficial to extract oil with reduced particle size. Rosenthal et al. found that the yield of soybean oil increased with an increase in comminution degree during oil extraction by AEE, and the yield of oil increased by 31% when the particle size dropped from 400 µm to 100 µm. If the particle size was too small, which would reduce the release of free oil and make demulsification more difficult. Zhu et al. studied the effect of peanut comminution degree on the
yield of peanut oil and found that the yield of total oil and hydrolyzed protein was highest (88.8% and 77.5%, respectively) when the average size of peanut was decreased to 28 μm. However, further reduction of the particle size increased the stability of the emulsion, and reduced the yield of oil and protein.

Oil seed comminution methods are usually divided into dry comminution and wet comminution. Peanut, as a high oil content material, is prone to display the phenomenon of oil leakage, material viscosity, and temperature rise during the dry comminution process, resulting in poor comminution effect, difficulty in the outlet of oil and transfer, and screen blockage. Whereas the wet comminution method requires small energy consumption and large processing capacity, however, a large stable emulsion that is difficult to break is produced by treating oil seeds with high oil and capacity, however, a large stable emulsion that is difficult to break is produced by treating oil seeds with high oil and protein content. Therefore, pretreatment operation by proper comminution method and comminution time to break oil seeds is crucial for effective AEE. In this study, the effect of dry comminution and wet comminution on peanut particle size, peanut oil yield, and protein yield were compared, and the characteristics (surface protein concentration, particle size, and ζ-potential) of the emulsion extracted in the AEE process were further evaluated. Concomitantly, the demulsification rate of the emulsion was determined to evaluate the stability of emulsion and provide a theoretical basis for choosing the appropriate comminution method.

2 Materials and Methods
2.1 Materials
Peanut samples (Yuhua 23) were purchased from Henan Academy of Agricultural Sciences (Zhengzhou, China) and stored at 4°C until used. Viscozyme® L (main ingredients: cellulase, hemicellulase, and arabinase), Papain, Alcalase 2.4L, and Phospholipase A2 were purchased from Novo-zymes (Novo, China).

2.2 Determination of main components of peanut
The different parameters were determined based on the protocols set for national food safety standards and issued by the Ministry of Agriculture, People’s Republic of China. In this study, GB 5009.3-2016, GB 5009.4-2016, GB 5009.5-2016, GB 5009.6-2016, and GB/T 5009.10-2003 were used to measure the moisture content, ash, protein, fat, and fiber of peanut, respectively.

2.3 Comminution pretreatment of peanut
Dry comminution: Skinless peanut seeds were ground by a high-speed universal grinder (FW-100; Beijing Ever Bright Medical Treatment Inc., Beijing, China), and comminution times were 8 s, 24 s, 48 s, 72 s, 96 s, and 120 s.

Wet comminution: Skinless peanut seeds were added with deionized water at 1:4 and placed in a refrigerator at 4°C for 18 h. Subsequently, the peanuts were ground at 10 s, 30 s, 60 s, 90 s, 120 s and 150 s by a multifunctional food processor (C022E, Joyoung Co., Ltd., Shandong, China).

2.4 Particle size analysis of peanut after grinding
The particle size of peanut was measured according to the method reported by Li et al. with some modifications. Briefly, one gram of peanut powder obtained by dry comminution and wet comminution was diluted 100 times with deionized water and then dispersed evenly by swirling shock for 1 min. The diluted peanut liquid was then instilled into the sample pool of laser particle size distribution instrument (BT-9300H, Dandong Baite Instrument Co., LTD, Dandong, China) to measure the average particle size (D4,3), median particle size (D50), and characteristic particle size (D90).

2.5 Preparation of peanut oil and protein
Ten grams of skinless peanut seeds were ground by dry comminution (according to section 2.3), and then dispersed in deionized water in the ratio of 1:4 (wt/vol; solid-liquid ratio) and wet comminution (according to section 2.3). The enzymolysis of the mixture was conducted in digital water-bathing constant temperature vibrator (THZ-82; Jintan Huafeng Instrument Inc., Changzhou, China) for 2 h at 50°C after adding 1.25% Viscozyme® L. Subsequently, the enzyme was deactivated by keeping in boiling water bath for 5 min. The cooled solution was transferred into a centrifuge tube and was centrifuged (DZ267-32C6; Anting Scientific Instrument Factory, Shanghai, China) at 5000 × g for 20 min. The floating oily emulsion layer (oil), water phase (protein), and the lower precipitation (residual oil, protein, and carbohydrate) were separated. The process flowchart of AEE of peanut oil and protein is shown in Fig. 1.

The lower precipitate obtained from AEE was freeze-dried (LGJ-25; Beijing Sihuan Scientific Instrument Inc., Beijing, China) for 24 h to remove water and measure the oil content in the residue. The peanut oil content was measured by the Soxhlet extraction method, and protein content was measured using an Automatic Kjeldahl Apparatus (K1100; Jinan Haineng Instrument Inc., Shandong, China). The yield of peanut oil and protein was calculated using the formulas (1) and (2):

\[ \text{Yield of peanut oil (\%) } = \left( 1 - \frac{\text{oil content of residue (g)}}{\text{oil content of peanut (g)}} \right) \times 100 \tag{1} \]

\[ \text{Yield of peanut protein (\%) } = \frac{\text{protein content of water phase (g)}}{\text{(protein content of peanut (g))}} \times 100 \tag{2} \]
2.6 Determination of the main composition of emulsion

The moisture content of the emulsion was determined by referring to GB 5009.3-2016. Soxhlet extraction was performed to determine the oil content in oily emulsion layer after vacuum drying according to GB 5009.6-2016. The protein and phospholipid contents were determined according to GB 5009.5-2016 and GB/T 5537-2008, respectively.

2.7 Determination of Surface Protein Concentration

Surface protein concentration was calculated according to the method reported by Agboola et al. (10) and Chabrand et al. (11), with some modifications, and computing method uses the formula (3).

\[ \Gamma = \frac{M_{pro}}{SSA} ; \quad SSA = \frac{6}{D_{12}} \times \frac{1}{\rho_{oil}} \]

\[ \Gamma (\text{mg/m}^2) \text{represents surface protein concentration; } M_{pro} (\text{mg/g}) \text{represents mass ratio of protein of emulsion surface to oil; } SSA \text{is the specific surface area of the emulsion; } D_{12} (\text{μm}) \text{represents the surface-area averaged particle size of the emulsion; } \rho_{oil} \text{is the density of peanut oil and } \rho_{oil} \text{is 0.91 g/cm}^3. \]

2.8 Determination of particle size and ξ-potential

One gram of emulsion to be measured was diluted 100 times by deionized water, and the evenly dispersed liquid was quickly absorbed by a plastic straw and dripped into the sample pool of the laser particle size analyzer to determine particle size. The emulsion sample was diluted by 10 times with deionized water, and ξ-potential was determined by Zeta potential analyzer (Zetasizer Nano ZSP, Marvin instrument co., LTD., Marvin, England).

2.9 Laser confocal analysis of emulsion

The fluorescent dyes and staining methods were selected following the methods of Sui et al. (12) and Puppo et al. (13), with some modifications. Briefly, 0.01% Nile red (soluble in anhydrous ethanol) was used to stain fat, making it fluoresce strongly in red, while proteins were stained with 0.1% FITC (soluble in acetone), which fluoresces green. Two milliliters of the emulsion were mixed with 10 μL Nile red and 10 μL FITC evenly, then they were dropped onto fluted slide and covered with a cover glass. The distribution and microstructure of emulsion were observed with a laser confocal microscope (Germany Carl Zeiss co., LTD., Jean, Germany) at the excitation wavelength of 488 nm and 40X magnification.

2.10 Demulsification of emulsion

The stability of the emulsions was examined by demulsifying using the following methods.

2.10.1 Freeze-thawing

The emulsion was frozen at -20°C for 24 h, then thawed at 40°C for 40 min and centrifuged at 5000 × g for 20 min.

2.10.2 Isoelectric demulsification

The pH of the emulsion was adjusted to 4.5. Then, the emulsion was stirred at a constant temperature of 50°C for 30 min, followed by centrifugation at 5000 × g for 20 min.

2.10.3 Enzymatic demulsification

The alkaline protease Alcalase 2.4L, Papain and Phospholipase A2 were used to demulsify the emulsion. Briefly, 10 grams of emulsion were taken and diluted 3 times with deionized water. The enzymes were added at the optimal pH and temperature of the enzyme following the manufacturer’s instructions and stirred 30 min, then centrifuged at 5000 × g for 20 min.

\[ \text{Demulsification rate(%) = } \frac{\text{boiled oil after demulsification}}{\text{oil content of emulsion}} \times 100 \]

2.11 Statistical analysis

All measurements were repeated at least three times using duplicate samples, and the results are reported as means ± standard deviations. Statistical analysis was performed on the obtained results using one-way analysis of variance (ANOVA) analysis and Tukey’s test at a significance level of p < 0.05 using the software of SPSS 19.0.

3 Results and Discussion

3.1 Analysis of main components of peanut

Analysis result of the main components of peanut is shown in Table 1. Peanut is rich in oil and protein. The fat content of peanut kernel was 51.43%, whereas the protein content was 24.16%. Peanut oil has rich nutritional value, and the fatty acids are mainly unsaturated fatty acids, with...
oleic acid and linoleic acid accounting for approximately 80%. Moreover, peanut protein contains 18 kinds of amino acids, including 8 kinds of essential amino acids required by human body. Peanut protein contains no cholesterol and can be easily digested and absorbed by human body, making it a highly nutritious plant protein resource. Protein, as an amphiphilic macromolecule, adsorbs at the oil-water interface during the extraction of peanut oil and protein from the aqueous phase, and inevitably forms a large amount of stable emulsion.

3.2 Effect of different comminution methods and time on peanut particle size

After crushing peanut by dry comminution, the relationship between peanut particle size and comminution time is shown in Fig. 2. The D50, D(4,3), and D90 of peanut decreased rapidly with an increase in comminution time from 8 s to 72 s, and D(4,3) of peanut decreased rapidly from 87.60 μm to 31.06 μm. During comminution time from 72 s to 120 s, the decrease rate of D50 and D(4,3) tended to slow down. At comminution time 72 s, 96 s, and 120 s, the D(4,3) was 31.06 μm, 28.46 μm, and 27.84 μm, respectively. The rate of decrease of D90 was higher than that of D50 and D(4,3), indicating that the larger peanut particles were significantly decreased and peanut particle size tended to be uniform.

As shown in Fig. 3, the trend of changes in peanut particle size crushed by wet comminution was the same as that of dry comminution. With the increase of comminution time, D50 and D(4,3) of peanut decreased rapidly at first and then slowed down, while the decrease rate of D90 was always higher than that of D50 and D(4,3). D(4,3) of peanut rapidly decreased from 94.23 μm to 23.87 μm with an increase in the comminution time from 10 s to 150 s, and D90 rapidly decreased from 137.02 μm to 39.76 μm, indicating the particle size tending to be uniform over time. These findings indicated that the selection of the comminution method is crucial to obtain the best particle size.

3.3 Effects of different comminution methods and times on yield of peanut oil and protein

Peanut oil and protein were extracted according to the AEE process described in section 2.5. In addition, for comparative analysis, both the oil and protein were extracted without enzymes as blank controls. The effects of dry comminution and wet comminution at different times on the yield of peanut oil and protein are shown in Fig. 4 and Fig. 5, respectively. As shown in Fig. 4, yield of peanut oil and protein increased rapidly at first up to 72 s, and then slowed down with the increase of dry comminution time. At comminution time 72 s, the yield of peanut oil and protein reached the highest (87.23% and 82.05%, respectively). In contrast, yield of peanut oil in the blank control group increased gradually with the increase of comminution time, reaching a maximum of 83.64%. The yield of peanut protein in the blank control group increased first and then decreased, and reached a maximum of 79.01% at 120 s. Effects of dry comminution on yield of peanut oil

### Table 1 Main components of skinless peanut.

| Component | Fat         | Protein     | Water       | Ash          | Cellulose    |
|-----------|-------------|-------------|-------------|--------------|--------------|
| Content (%) | 51.43 ± 0.86 | 24.16 ± 0.37 | 3.98 ± 0.03 | 2.47 ± 0.02  | 6.32 ± 0.36  |

![Fig. 2](image1.png) Relationship between peanut particle size and dry comminution time.

![Fig. 3](image2.png) Relationship between peanut particle size and wet comminution time.
and protein was similar to the research of Zhu et al.8, but preparation technology and enzyme are different. Protein obtained in this study was not hydrolyzed by Viscozyme® L and retained the original functional properties, however, protein obtained by Zhu et al. was hydrolyzed by Alcalase 2.4L. At the same time of dry comminution, yield of oil and protein extracted by AEE were both higher than that of blank control group. Wet comminution had the same effect on the yield of peanut oil and protein with dry comminution (Fig. 5). With an increase in the time of wet comminution, the yield of peanut oil and protein extracted by AEE increased rapidly at first and then slowed down after 90 s. Peanut oil yield reached up to highest (89.91%) at comminution time 120 s, while protein yield reached up to highest (84.74%) at comminution time 90 s. The yield of oil and protein extracted by AEE was higher than that obtained from the blank control group at all the time points of the wet comminution.

Peanut oil and protein are mainly concentrated in the oil-bearing cells of cotyledon which is 70 μm long and 40 μm wide. Peanut cell wall is composed of cellulose, hemicellulose and pectin, which is relatively tough and can prevent peanut oil and protein and other nutrients from spreading outward, while preventing the external solvent from penetrating into the cell. Therefore, comminution is an important pretreatment method for AEE and the degree of comminution has a significant effect on the yield of peanut oil and protein. Within certain limits, greater the degree of oil seeds comminution, higher the yield of oil. The cotyledon cells are crushed to a certain extent and the cell wall structure is broken by mechanical comminution, which promotes the release of the water-soluble components in the cell, increases the contact area of oil and enzyme, expands the diffusion rate of enzyme in the material liquid, and promotes the process of enzymatic hydrolysis14-16. In contrast, if the degree of comminution is too large, the formation of emulsion and the difficulty of demulsification will increase. Therefore, the yield of oil and protein does not necessarily increase with the extension of comminution time and decrease in peanut particle size beyond a certain extent. In this study, with an increase in dry comminution time from 8 s to 72 s, the yield of peanut oil and protein increased rapidly along with a rapid decrease of peanut particle size. The yield of peanut oil and

![Graph](image1)

**Fig. 4** Relationship between yield of (a) peanut oil and (b) protein and dry comminution time. Note: different capital letters indicated significant difference between the enzyme group ($p < 0.05$).

![Graph](image2)

**Fig. 5** Relationship between yield of (a) peanut oil and (b) protein and wet comminution time. Note: different capital letters indicated significant difference between the enzyme group ($p < 0.05$).
protein was highest at dry comminution time 72 s. The decrease rate of peanut particle size tended to slow down with increase of dry comminution time from 72 s to 120 s, while yield of peanut oil and protein decreased without significant difference. With the increase of wet comminution time, peanut particle size decreased rapidly at first and then slowed down, while yield of peanut oil and protein increased rapidly at first and then slowed down. At wet comminution time 120s, the yield of peanut oil reached the highest and the yield of protein was 84.70%, which was not significantly different from the highest protein yield (84.74%). Combining the effect of different comminution methods and comminution times on the particle size of peanut, it can be concluded that wet comminution caused more serious breakage of the cell structure of peanut than the dry comminution. Moreover, upon wet comminution, oil in the residue was more likely to dissociate out of the cell and the residual oil rate was lower. The dry comminution time 72 s and wet comminution time 120 s were selected as the comminution time for subsequent experiments.

3.4 Composition of the emulsion

Compositions of emulsion made by dry comminution (DCE) and emulsion made by wet comminution (WCE) obtained by AEE are shown in Table 2. The emulsion was mainly composed of oil, protein, phospholipid and water, of which protein and phospholipid were amphoteric substances, with both hydrophilic and hydrophobic groups. As macromolecular surfactants, they can significantly reduce interfacial tension and contribute to the formation and stability of the emulsion. In the present study, the compositions of DCE were determined to be 80.11% fat, 1.69% protein, and 1.02% phospholipid. Although protein and phospholipid content were low, they were still important contributors to emulsification. Compared with the DCE, the oil content of WCE was significantly lower, while the protein and phospholipid content were significantly higher, indicating that comminution pretreatment had a great influence on the composition of emulsion.

3.5 Analysis of surface protein concentration, particle size, potential, and microstructure of emulsion

During the process of emulsion formation, the hydrophilic and lipophilic proteins adsorbed on the oil-water interface forming a layer or multi-layer protein film to prevent the aggregation of oil droplets and maintain the stability of emulsion. Surface protein concentration ($\Gamma$) is an important parameter determining the stability of the emulsion. A higher surface protein concentration leads to the higher of protein membrane coverage rate of oil droplet surface, the more conducive to reduce the interfacial tension of two phases, the stronger the protein emulsification ($\Gamma$), and the greater the stability of the emulsion. Tcholakova et al.\textsuperscript{19} concluded that when the protein concentration on the surface of oil droplets was 1-2 mg/m$^2$, a monolayer of protein could be formed to form stable emulsion. As can be seen from Table 3, the surface protein concentrations of DCE and WCE were 7.02 mg/m$^2$ and 10.71 mg/m$^2$, respectively, which were both several times of the minimum surface protein concentration required for oil droplets. The surface protein concentrations of DCE and WCE indicated that the oil drop interface of emulsion was formed by multi-layer protein membrane, which could enhance the stability of the emulsion. The surface protein concentration of WCE was significantly higher than that of DCE, indicating that the former was more stable.

In the emulsion system, protein molecules themselves have the ionizable groups, thus providing electric charge to the emulsion droplets that are surrounded by a multi-layer protein membrane. The electrostatic repulsion between the emulsion droplets keeps them relatively stable without

| Table 2 | Main Composition of the emulsion. |
|---------|----------------------------------|
| Ingredients | Fat (%) | Protein (%) | Phospholipid (%) | Water (%) |
| DCE | 80.11 ± 0.23$^a$ | 1.69 ± 0.03$^b$ | 1.02 ± 0.02$^b$ | 15.97 ± 0.10$^b$ |
| WCE | 73.26 ± 0.56$^b$ | 2.09 ± 0.03$^b$ | 1.12 ± 0.03$^b$ | 22.05 ± 0.19$^b$ |

Note: significance analysis was conducted for each column of data, and marked with different letters indicated significant difference ($p < 0.05$).

| Table 3 | Surface protein concentration, particle size, $\xi$-potential of emulsion. |
|---------|---------------------------------|
| surface protein concentration | D(4,3) (µm) | $\xi$-potential (mV) |
| $\Gamma$ (mg/m$^2$) | |
| DCE | 7.02 ± 0.21$^b$ | 3.41 ± 0.06$^a$ | -12.08 ± 0.12$^a$ |
| WCE | 10.71 ± 0.19$^a$ | 3.18 ± 0.04$^a$ | -15.25 ± 0.44$^a$ |

Note: significance analysis was conducted for each column of data, and marked with different letters.

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aggregation and condensation\textsuperscript{19,20}. With a higher absolute \(\xi\)-potential value of emulsions, the repulsive forces exceed the attractive forces, resulting in a relatively stable system. On the contrary, a smaller absolute \(\xi\)-potential value leads to an increase in emulsion particle size because of the lack of electrostatic repulsion\textsuperscript{4}. Therefore, emulsion particle size and \(\xi\)-potential absolute value are often used to characterize the stability of the emulsion. In the present study, the \(D(4,3)\) and \(\xi\)-potential absolute value of DCE was 3.41 \(\mu m\) and 12.08 mV, respectively. The \(D(4,3)\) of WCE was 3.18 \(\mu m\), which significantly lower than DCE. Moreover, \(\xi\)-potential absolute value of WCE was 15.25 mV, significantly higher than that of DCE. Results of particle size and \(\xi\)-potential absolute value showed that stability of WCE was higher than DCE.

The oil and protein in the emulsion were stained with Nile red and FITC, respectively, and the microstructure of the emulsion was observed by laser confocal microscopy. Figure 6 shows the microstructure of DCE and WCE. Fat stained with Nile red and fluoresces red, while proteins stained with FITC and fluoresces green. The oil droplets were tightly bound by protein interface membrane, which limits the accumulation of oil droplets. It could be seen intuitively particle size of DCE was smaller than that of WCE. Combined with the particle size and potential analysis results of the emulsion, the stability of the emulsion obtained by wet comminution was higher than that obtained by dry comminution.

3.6 Analysis of emulsion stability

To further study the effect of dry comminution and wet comminution on the stability of emulsion, physical, chemical, and enzymatic methods were used to demulsify the emulsion and thereby determine and compare the stability of the emulsion (Fig. 7). Freezing-thawing is a commonly used physical demulsification method. The principle of freeze-thawing is that fat crystals formed between adjacent oil droplets during the freezing process can puncture the interface membrane and accelerate the fusion of oil droplets during the thawing process\textsuperscript{21}. As shown in Fig. 7, the demulsification rate of DCE and WCE treated by freeze-thawing was 79.74\% and 72.05\%, respectively. Demulsification rate of DCE was significantly higher than that of WCE. The surface electrostatic charge becomes almost zero when the pH value of the emulsion is close to the isoelectric point of peanut protein (pH 4.5). Moreover, the

![Fig. 6 Microstructure of emulsion.](image)

Note: (a) DCE (b) WCE.
electrostatic repulsion and the hydrophilicity of the protein are reduced, resulting in breakage of the complete protein membrane structure on the surface of the oil drop and reduction of the stability\(^5\). As shown in Fig. 7, demulsification rate of DCE was 84.82\% treated by pH, which was significantly higher than that of WCE (79.84\%). Proteins and phospholipids on the surface of the emulsion have an important influence on its stability. Emulsion surface membrane will completely rupture and lose its stability when treated with proteases and phospholipases\(^{22, 23}\). In this study, papain, alkaline protease Alcalase 2.4L, and phospholipase A2 were selected for demulsification of the emulsion. As shown in Fig. 7, demulsification rate of DCE was significantly higher than WCE treated by papain and phospholipase A2. Among the five demulsification methods tested, alkaline protease Alcalase 2.4L was found have the best effect on emulsion stability with demulsification rate of DCE being as high as 92.77\%, which was slightly higher than that of WCE (92.67\%). Together, these results indicated that the stability of WCE was higher than that of DCE.

4 Conclusions

In this study, the effects of dry comminution and wet comminution pretreatment on the efficiency of AEE of peanut oil and protein and the stability of emulsion were investigated. The results showed that there were advantages and disadvantages of dry comminution and wet comminution. It is easy to produce the phenomenon of oil infiltration, sticky material, and rise of temperature in the process of dry comminution, resulting in crushing unevenly and physical transfer difficulties. Compared with dry comminution, wet comminution requires less equipment and less energy, so it can be cost effective in mass production. The yield of peanut oil and protein both reached the highest (87.23\% and 82.05\%, respectively) at dry comminution time 72 s. At wet comminution time 120 s, the yield of peanut oil and protein was 89.91\% and 84.70\%, respectively, which were both higher than that of dry comminution. By comparing the main composition, surface protein concentration, particle size and \(\xi\)-potential of the emulsion, and using different demulsification methods to demulsify the emulsion, which were found that the stability of the emulsion obtained by wet comminution was higher than that by dry comminution. Although the emulsion obtained by wet comminution was highly stable, it was found that the demulsification effect of alkaline protease Alcalase 2.4L on the emulsion was the best, and the demulsification rate of DCE and WCE were 92.77\% and 92.67\%, respectively, with no significant difference. Combining the advantages and disadvantages of the dry comminution and the wet comminution, the wet comminution was selected as the pretreatment method of AEE to extract peanut oil and protein. We believe that the findings of the present study could be useful in the selection of process parameters for AEE to optimize oil and protein yield.

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Author Contributions

C. Liu designed and conducted the experiments, performed data analysis, and wrote the manuscript. F. Chen supervised the study, helped to initiate the project, and revised the manuscript. R. Niu and Y. Gao helped to conducted the experiments.

Conflict of Interest

We declare that we have no conflict of interest.

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