Effects of Pretreatment on the Yield of Peanut Oil and Protein Extracted by Aqueous Enzymatic Extraction and the Characteristics of Emulsion

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Abstract

Peanut was crushed by dry comminution and wet comminution, and effects of comminution on peanut particle size and yield of peanut oil and protein were analyzed. The properties (surface protein concentration, particle size, and ξ-potential) of emulsion were compared. Moreover, different demulsification methods were used to investigate the stability of the emulsion. Results showed that yield of peanut oil and protein reached highest (87.23% and 82.05%, respectively) at dry comminution 72 s. At wet comminution 120 s, yield of peanut oil and protein was 89.91% and 84.70%, respectively, which were both higher than that of dry comminution significantly ($P < 0.05$). The surface protein concentration and ξ-potential of emulsion made by dry comminution (DCE) was 7.02 mg/m2 and 12.08 mV, respectively, and that was 10.71 mg/m2 and 15.25 mV of emulsion made by wet comminution (WCE), which were significantly higher than that of DCE ($P < 0.05$). The volume average particle size (D(4,3)) of DCE was 3.41 μm, which was significantly higher than D(4,3) of WCE (3.18μm, $P < 0.05$). Collectively, results of emulsion properties indicated stability of WCE was higher than DCE. Further, demulsification rate of DCE was significantly higher than that of WCE treated by freeze-thawing, pH, Papain, and Phospholipase A2 ($P < 0.05$). Demulsification effect of Alcalase 2.4L was best in these five demulsification methods, and demulsification rate of DCE reached 92.77%, slightly higher than WCE (92.67%), further illustrated stability of WCE was higher than DCE.

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Abstract: Peanut was crushed by dry comminution and wet comminution, and effects of comminution on peanut particle size and yield of peanut oil and protein were analyzed. The properties (surface protein concentration, particle size, and ξ-potential) of emulsion were compared. Moreover, different demulsification methods were used to investigate the stability of the emulsion. Results showed that yield of peanut oil and protein reached highest (87.23% and 82.05%, respectively) at dry comminution 72 s. At wet comminution 120 s, yield of peanut oil and protein was 89.91% and 84.70%, respectively, which were both higher than that of dry comminution significantly ($P < 0.05$). The surface protein concentration and ξ-potential of emulsion made by dry comminution (DCE) was 7.02 mg/m^2 and 12.08 mV, respectively, and that was 10.71 mg/m^2 and 15.25 mV of emulsion made by wet comminution (WCE), which were significantly higher than that of

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Keywords: 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 that enzymatic hydrolysis of peanut is conducted on the basis of mechanical comminution to promote release of oil and protein, and oil and non-oil components (protein and carbohydrate) can be separated utilizing the difference of affinity of non-oil components and the difference of specific gravity between oil and water (Li, Chen, & Hao, 2017). Though AEE is a promising strategy, its industrialization is hampered. The fundamental reason is 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. Comminution pretreatment before AEE could assist the cell wall breakage. Serious damage of peanut cell wall structure 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 (Tan C & Yuan, 2006; Nyam, Tan, & Lai, 2009). Moreover, the extracting 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 for these surface-active material and agitation during extraction and centrifugal separation, limiting the release of oil. Whereas, too large comminution degree of oil seeds will promote the formation of stubborn emulsion and increase the difficulty of demulsification in the subsequent process (Li, Gasmalla, & Liu, 2016). Within certain limits, it was beneficial to extract oil with the decrease of particle size. ROSENTHAL A et al. (2001) found that the yield of soybean oil increased with the increase of comminution degree during extracting oil 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 reduced the release of free oil and increased the difficulty of demulsification (Ramón, Kim, & Zhang, 2008; Wu, Johnson, & Jung, 2009). Zhu K et al. (2012) studied the effect of peanut comminution degree on the yield of peanut oil. Research found that the yield of total oil rate and hydrolyzed protein reached highest (88.8% and 77.5%, respectively) when the average size of peanut decreased to 28 μm. However, further reduction of the particle size increased the stability of the emulsion, and reduces the yield of oil and protein.na

Oil seeds comminution methods are usually divided into dry comminution and wet comminution. Peanut, as a kind of material with high oil content, is prone to produce the phenomenon of the oil leakage, material viscosity, and temperature rise during the dry comminution process, which resulted in poor comminution effect, difficulty in the outlet of oil and transfer, and screen blockage (Nyam, Tan, & Lai, 2009). Whereas the wet comminution has small energy consumption and large processing capacity, but a large stable emulsion produced by treating oil seeds with high oil and protein content, which is difficult to break. Therefore, it is a crucial pretreatment operation unit for AEE to destroy oil seeds by proper comminution method and comminution time. The effect of dry comminution and wet comminution on peanut particle size, peanut oil yield, and protein yield were compared in this study, and the characteristics (surface protein concentration, particle size, and ξ-potential) of emulsion extracted in AEE process were further studied. At the same time, the demulsification rate of emulsion using different methods were evaluated to discusses the stability of the emulsion to provide theoretical basis for the choice of the ways of comminution.

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 until used. Viscozyme® L (main ingredients: cellulase, hemicellulase, and arabinase), Papain, Alcalase 2.4L, and Phospholipase A2 were purchased from Novozymes (Novo, China).

2.2. Determination of Main Components of Peanut

The protocols set for national food safety standards and issued by the Ministry of Agriculture, People's Republic of China, were used to determinate different parameters. 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 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

According to the method of Li Y et al. (2017) to measure particle size of peanut, with some modifications. One gram peanut power obtained by dry comminution and wet comminution were diluted 100 times with deionized water and then dispersed evenly by swirling shock for 1min. Peanut diluted liquid were instilled the sample pool of laser particle size distribution instrument (BT-9300H, Dandong baite instrument co. LTD, Dandong, China) to measure the average particle size (D(4,3)), median particle size(D50), and characteristic particle size (D90).

2.5. Preparation of Peanut Oil and Protein

Ten grams skinless peanut seeds were ground by dry comminution (according to 2.3., and then dispersed in deionized water with 1:4 (wt/vol) solid-liquid ratio) and wet comminution (according to 2.3.), respectively. 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 after adding 1.25% Viscozyme$^{(r)}$ L. Subsequently, the enzyme was deactivated by 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 x 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 diagram of AEE of peanut oil and protein is shown in Fig. 1.

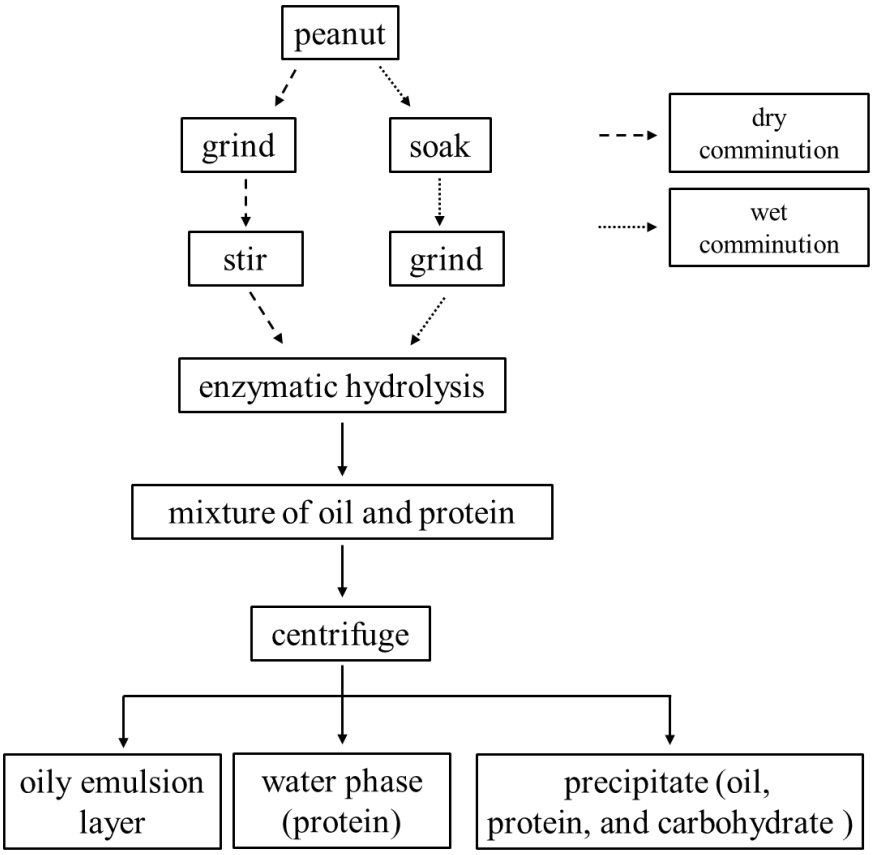


Fig. 1 Process diagram of AEE of peanut oil and protein.

The lower precipitation 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 with an Automatic Kjeldahl Apparatus (K1100; Jinan Haineng Instrument Inc., Shandong, China). Yield of peanut oil and protein calculated using the formulas (1) and (2):

$$\text{Yield of peanut oil}\,(\%) = \left(1 - \frac{\text{oil content of residue}\,(g)}{\text{oil content of peanut}\,(g)}\right) \times 100 \qquad (1)$$

$$\text{Yield of peanut protein}\,(\%) = \frac{\text{protein content of water phase}\,(g)}{\text{protein content of peanut}\,(g)} \times 100 \qquad (2)$$

2.6. Determination of the Main Composition of Emulsion

Moisture content of emulsion were determined by referring to GB 5009.3-2016. Soxhlet extraction was performed to determinate oil content of 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 of Agboola et al. (1998) and Chabrand et al. (2008), with some modifications, and computing method uses the formula (3).

$$\Gamma = \frac{M_{P/O}}{\mathrm{SSA}} \quad ; \qquad SSA = \frac{6}{D_{3,2}} \times \frac{1}{\rho_{\mathrm{oil}}} \tag{3}$$

$\Gamma(\mathrm{mg/m^2})$ represents surface protein concentration; $\mathrm{M_{p/o}}(\mathrm{mg/g})$ represents mass ratio of protein of emulsion surface to oil; SSA is the specific surface area of the emulsion; D3,2(μm) represents the surface-area averaged particle size of the emulsion; ρ_{oil} represents density of peanut oil and ρ_{oil}= 0.91 $\mathrm{g/cm^3}$.

2.8. Determination of Particle Size and ξ-Potential

One gram 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 X et al. (2016) and Puppo M C et al. (2008), with some modifications. 0.01% Nile red (soluble in anhydrous ethanol) was used to stain fat and makes it fluoresces strongly in red, while proteins can be stained with 0.1% FITC (soluble in acetone) and fluoresces green. Two milliliters of the emulsion were mixed with 10 μL Nile red and 10 μL FITC evenly, then dropped them 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 zoom.

2.10. Demulsification of Emulsion

The stabilities of emulsions were examined by demulsifying using the following methods.

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.

Isoelectric demulsification : The pH of the emulsion was adjusted to 4.5 and stirred at a constant temperature of 50 for 30 min, then centrifuge it at 5000 x *g* for 20 min.

Enzymatic demulsification : The alkaline protease Alcalase 2.4L, Papain and Phospholipase A2 were used to demulsification of the emulsion. Ten 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 x *g* for 20 min.

$$\text{Demulsification rate}\,(\%) = \frac{\text{boiled oil after demulsification}}{\text{oil content of emusion}} \times 100 \tag{4}$$

2.11. Statistical Analysis

All measurements were repeated at least three times using duplicate samples, and the results were given as means ± standard deviations. The data were statistically analyzed using the software of Design Expert 8.05b, Origin 8.5, and SPSS 19.0. Significance of differences was defined at $p < 0.05$.

3. Results and Discussion

3.1. Analysis of Main Components of Peanut

Analysis result of the main component of peanut is shown in Table 1. Peanut is rich in oil and protein. Fat content of peanut kernel was 51.43%, which second only to sesame, whereas protein content was 24.16%.

Peanut oil has rich nutritional value, and the fatty acids are mainly unsaturated fatty acids, of which oleic acid and linoleic acid account for about 80% (Zhou, Zhou, & Jiang, 2012). 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 is easy to be 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 form a large amount of stable emulsion.

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

3.2. Effect of Different Comminution Methods and Time on Peanut Particle Size

After peanut crushed by dry comminution, the relationship between peanut particle size and comminution time is shown in Fig. 2. Median particle size (D50), volume average particle size (D(4,3)), and typical particle size (D90) of peanut decreased rapidly during comminution time increase 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 increased 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, D(4,3) was 31.06 μm, 28.46 μm, and 27.84 μm, respectively. The decrease rate of D90 was higher than that of D50 and D(4,3), indicating that the larger peanut particles crushed were significantly decreased and peanut particle size tended to be uniform.

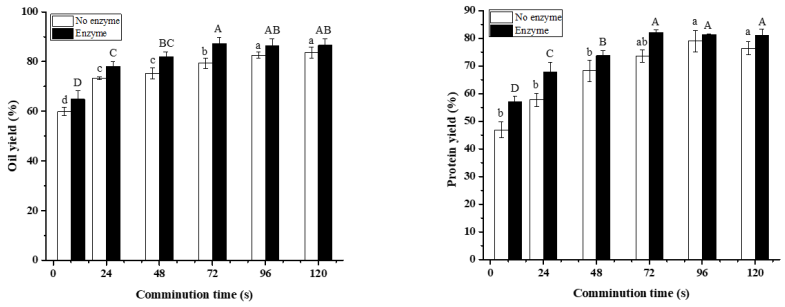
Fig. 2 Relationship between peanut particle size and dry comminution time.

As shown in Fig. 3, the change trend of 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 during comminution time increased 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. The findings of the present study indicated that the selection of the comminution method is crucial to obtain the best particle size.

Fig. 3 Relationship between peanut particle size and wet comminution time.

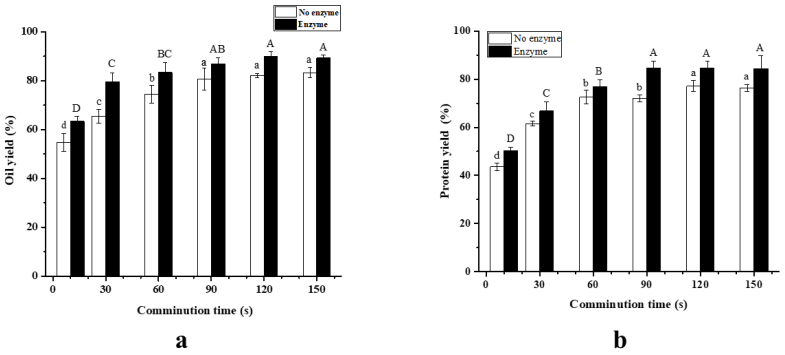
3.3. Effects of Different Comminution Methods and Times on Yield of Peanut Oil and Protein

Peanut oil and protein were extracted according to the AEE process of 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. 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 were higher than that of the blank control group at all the time points of the wet comminution.



Note: significance analysis was carried out on the data of the enzyme group and the non-enzyme group, and those marked with different letters indicated significant difference (P <0.05).

Fig. 4 Relationship between yield of peanut oil and protein and dry comminution time.



Note: significance analysis was carried out on the data of the enzyme group and the non-enzyme group, and those marked with different letters indicated significant difference (P <0.05).

Fig. 5 Relationship between yield of peanut oil and protein and wet comminution time.

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, the greater the degree of oil seeds comminution, the higher the yield of oil. The cotyledon cells are crushed to a certain extent and the cell wall structure are destroyed 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 hydrolysis(Wang, 2008; TEIXEIRA, MACEDO, & MACEDO, 2013; Mat Yusoff M, Gordon, & Niranjan, 2015). 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 did not always increase with the extension of comminution time when peanut particle size decreased to a certain extent. During dry comminution time increase from 8 s to 72 s, yield of peanut oil and protein increased rapidly with decreasing rapidly of peanut particle size. At dry comminution time 72 s, the yield of peanut oil and protein reached the highest. The decrease rate of peanut particle size tended to slow down during dry comminution time increased 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%, with no significant difference from the protein highest yield (84.74%). Combined with 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 damage to the cell structure of peanut than the dry comminution, result that 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 DCE and 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 (Wu, Johnson, & Jung, 2009). 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, which indicated that comminution pretreatment had a great influence on the composition of emulsion.

Table 2. Main ingredients 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^{a}	1.12 ± 0.03^{a}	22.05 ± 0.19^{a}

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

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 (Γ) 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, and the greater the stability of the emulsion (Castellani, Belhomme, & David-Briand,2006). Tcholakova et al. (2003) concluded that when the protein concentration on the surface of oil droplets was 1-2mg/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 stability of WCE was higher than that of DCE.

In the emulsion system, protein molecules themselves have the ionizable groups, thus providing electric charge to the emulsion droplets which were surrounded by a multi-layer protein membrane. The electrostatic repulsion between the emulsion droplets keeps them relatively stable without aggregation and condensation (Sarkar, Kelvin, & K. T. G., 2009; Hong, Mc Clements, & D. J, 2007). With a higher absolute ξ-potential value of emulsions, the repulsive forces exceed the attractive forces, resulting in a relatively stable system.

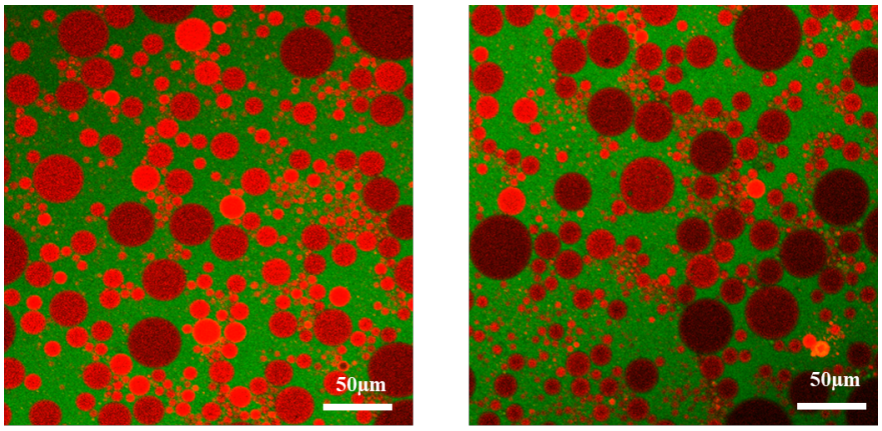
On the contrary, a smaller absolute ξ-potential value leads to an increase in emulsion particle size because of the lack of electrostatic repulsion (Li, Gasmalla, & Liu, 2016). Therefore, emulsion particle size and ξ-potential absolute value are often used to characterize the stability of the emulsion. In the present study, the D(4,3) and ξ-potential absolute value of DCE was 3.41 μm and 12.08 mV, respectively. The D(4,3) of WCE was 3.18 μm, which significantly lower than DCE. Moreover, ξ-potential absolute value of WCE was 15.25 mV, significantly higher than that of DCE. Results of particle size and ξ-potential absolute value showed that stability of WCE was higher than DCE.

Table 3 Surface protein concentration, particle size, ξ-potential of emulsion

	surface protein concentration Γ(mg/m^2)	D(4,3) (μm)	ξ-potential(mV)
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^b	-15.25±0.44^b

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

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. Fig. 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.



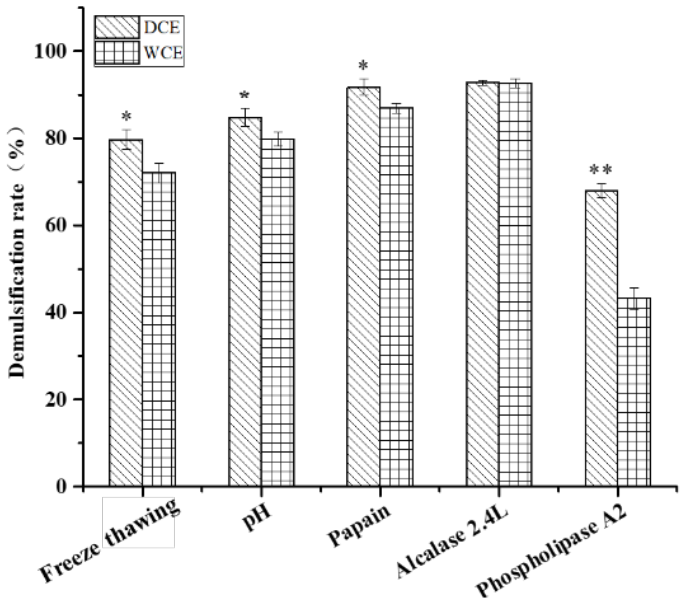
Note: (a) DCE (b) WCE

Fig.6 Microstructure of emulsion.

3.6. Analysis of Emulsion Stability

In order to further study the effect of dry comminution and wet comminution on the stability of emulsion, the physical method, chemical method, and enzymatic method were used to treat the emulsion and the stability of emulsion was compared. Effects of different demulsification methods on DCE and WCE stability are shown in 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 (Peng, Wang, &

Wang, 2014). 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 is almost zero when the pH value of the emulsion is close to the isoelectric point of peanut protein (pH 4.5), the electrostatic repulsion and the hydrophilicity of the protein are reduced, resulting in destroying the complete protein membrane structure on the surface of the oil drop and reducing the stability (Ramón, Kim, & Zhang, 2008). 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 (Sünder A. & Scherze I. 2001; Tzen J T & Huang A H, 1992). In this study, Papain, alkaline protease Alcalase 2.4L, and Phospholipase A2 were selected for demulsification of the emulsion. From Fig.7, demulsification rate of DCE was significantly higher than WCE treated by papain and phospholipase A2. Effect of alkaline protease Alcalase 2.4 L on emulsion stability was found to be best in these five demulsification methods, of which demulsification rate of DCE was as high as 92.77%, slightly higher than that of WCE (92.67%). Combine the demulsification rate of DCE and WCE treated by freeze-thawing, pH, Papain, alkaline protease Alcalase 2.4L, and Phospholipase A2, the stability of WCE was higher than that of DCE.



Note: significance analysis was carried out on the data of the same demulsification method, * stands for P <0.05, ** stands for P <0.01.

Fig. 7 Effects of different treatments on emulsion stability.

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 produces the phenomenon of oil infiltration, sticky material, and rise of temperature in the process of dry comminution, which resulting in crushing unevenly and physical transfer difficulties.Compared with dry comminution, wet comminution requires less equipment and less energy, so it can save cost in mass production. The yield of

peanut oil and protein both reached the highest (87.23% and 82.05%, respectively) at dry comminution time 72s. At wet comminution time 120s, 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 ξ- 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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