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# Characterization and demusification of cream emulsion from aqueous extraction of peanut

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ABSTRACT

In this study, the effect of aqueous extraction processing (AEP) and enzyme-assisted aqueous extraction processing (EAEP) on peanut oil yield was investigated. Only 34.53 ± 4.07% of free oil was recovered when using AEP without demulsification. In the case of EAEP, 89.31 ± 0.90% of free oil was obtained (1.5% w/w Alcalase 2.4L) without demulsification. Meanwhile, the protein yields of AEP and EAEP were similar, 84.10 ± 0.22% and 80.62 ± 0.92%, respectively. The efficiencies of enzymes (Protex 6L, Protex 7L, Protex 50FP, Alcalase 2.4L, Papain and Lecitase® Ultra) and pH of the released oil from peanut pastes were evaluated. Papain and Protex 50FP were effective in destabilizing the cream emulsion (93.10 ± 0.79% and 94.89 ± 0.19%, respectively). Adjusting the pH of the cream emulsion to the isoelectric point of peanut proteins also destabilized the cream with 85.71% of free oil recovery. Furthermore, the changes in the microstructure of cream emulsion during enzymatic demusification were observed by confocal laser scan microscope (CLSM). The interface tension decreased with the increase in enzymatic treatment time. For surface hydrophobicity (*H0*), cream emulsions treated by Protex 50FP and Papain had a remarkable decrease. Papain treated cream emulsion had a smaller *H0* than that of Protex 50FP due to the protein aggregations formed during adjusting pH value to 4.5. This might have hindered the efficiency of protein hydrolysis and left more basic arachin (MW 18–24 kDa) which have more hydrophobic groups.


## 1. Introduction

Aqueous extraction processing (AEP) and enzyme assisted aqueous extraction processing (EAEP) are developed for protein extraction and release of the oil in a separate phase through processes such as centrifugation (Morales Chabrand et al., 2008; Dickey et al., 2011; Hanmoungjai et al., 2002; Latif and Anwar, 2009; Mat Yusoff et al., 2015). In comparison with solvent and high temperature screw pressing extraction methods, AEP and EAEP are environmentally friendly and extract edible oil at moderate temperatures resulting in by-products such as protein and fiber with high quality functional properties with no toxins (Mat Yusoff et al., 2015). In addition, they have lower capital investment and energy consumption than traditional extraction methods (Wu et al., 2009). So far, EAEP and AEP have been employed to extract oil from several oilseeds such as soybeans (Campbell et al., 2011; de Moura et al., 2009), coconut (Kwaku and Ohta, 1997), peanut (Wang et al., 2008), sunflower seed (Latif and Anwar, 2009), mustard seeds (Tabtabaei and Diosady, 2013) and rapeseed (Zhang et al., 2007). Besides the advantages, application of AEP or EAEP is restricted by formation of a stable emulsion during extraction and the cost of enzyme. Therefore, it is necessary to reduce the amount of formed cream emulsion and enzyme.

The cream emulsions obtained from AEP and EAEP can be oil-in-water (O/W) consisting of peanut oil globules suspended in an aqueous phase, and stabilized by emulsifiers such as protein, phospholipids, small debris and other ingredients absorbed at the interface. In the preliminary tests, the proportion of the main emulsion composition was found to change with the variety of comminution types, extraction conditions such as dry or wet milling of peanut kernel, extraction with or without enzymes, high speed stirring and other extraction parameters. Many researchers have focused on cream emulsion demulsification (Campbell and Glatz, 2009; Wu et al., 2009; Zhang et al., 2013a, 2013b). These

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studies were generally carried out by comparing free oil recovery yields after enzymatic hydrolysis or physical treatment or a combination of both methods. Although, the demusification rate improved, the addition amount of enzyme is still high. Moreover, physical methods had different levels of demulsifying capacity for the emulsion formed during AEP and EAEP such as thermal treatments and freeze-thaw, but their efficiency and the amount of oil recovery was unsatisfactory (Morales Chabrand et al., 2008; Zhang et al., 2011). However, a few studies have been done on the effects of enzymatic hydrolysis on the properties of the naturally occurring emulsions from AEP.

In this study, in order to better understand the difference between AEP and EAEP, the oil and protein fraction distribution and the amount of enzymes involved in both processing (AEP and EAEP) were investigated. For AEP, the formed cream emulsion needed additional demulsification steps. Commercial enzymes were used to demulsification and the effect of pH on the stability of cream emulsion was studied. The microstructural changes of cream emulsion during the demusification was determined by confocal laser scanning microscopy (CLSM). Also, the emulsion proteins extracted at various enzymatic demulsification stages were further determined for their molecular weight, surface hydrophobicity and interfacial tension.

## 2. Materials and methods

### 2.1. Materials

Blanched peanuts were prepared from a variety of Haihua peanuts (Shandong Province, China) harvested in 2015. The peanut paste was cracked by a roll crusher (Model; Changzhou Zili Chemical Machinery Co. Ltd., China). The oil content of the paste was 51.64% and the crude protein content was 22.71%.

All enzymes, Lecitase® Ultra (E 3.1.1.3, phospholipase $A_1$, optimal pH 5.0, optimal temperature 50 °C) and Alcalase 2.4 L (alkaline serine endopeptidase from *B. licheniformis*, optimal pH 8.0, optimal temperature 50 °C) were purchased from Novozymes (Novo, China); Protex 6L (alkaline serine endopeptidase, optimal pH 8.0, optimal temperature 50 °C), Protex 50FP (acid fungal endopeptidase exopeptidase complex, optimal pH 4.5, optimal temperature 50 °C), and Protex 7L (neutral metallo endopeptidase, optimal pH 7.0, optimal temperature 50 °C) were purchased from Genencor Division of Danisco (Wuxi, China); Papain (EC 3.4.22.2, optimal pH 7.5, optimal temperature 60 °C) was purchased from Nanning pangbo biological engineering co. ltd (Nanning, china).

### 2.2. Procedures of AEP and EAEP and cream fraction preparation

The peanut paste was weighed and put into a jacketed reactor connected to a thermostat circulating water bath (MP-501A, shanghai Yi Heng Scientific Instruments co., ltd., China) and dispersed in distilled water at 1:5 (w/v). The pH of the slurry was adjusted to 9.0 with 2 mol/L NaOH, followed by incubation at 60 °C with constant stirring at 70 r/min. The difference between EAEP and AEP was the presence or absence of added Alcalase 2.4L after 30 min alkaline extraction (at original pH 9.0), then incubate at 60 °C and pH 8.5 with constant stirring at 70 r/min for 2 h. The incubated slurry was then centrifuged at 3000×*g* for 15 min at room temperature. Free oil was carefully collected using an autopipettor, then weighed and considered as the free oil recovered. The rest of skim and insoluble fractions were collected and sampled for determination of protein and oil contents.

The cream emulsion was prepared following the method described by Wu et al. (2009) with some modifications. In brief, the slurry of AEP (after incubation for 2 h) was poured into a glass separating funnels and allowed to settle overnight at 4 °C to separate the insoluble residues and skim from the cream emulsion fraction.

### 2.3. Determination of oil, protein and phosphorous contents

Total oil contents of peanut pastes and insoluble fractions were determined by the Soxhlet extraction method (AOAC Method, 995.19), while the oil contents of residual cream and skim were determined by Mojonnier methods (AOAC Method, 989.05). The protein contents in skim, residual cream and insoluble fractions were determined by the Kjeldahl method (AOCS Method Ac 4-91). The nitrogen values were multiplied by 5.46 to estimate protein content. The phosphorus content of (oil + cream) fraction was determined according to AOCS method Ca 12–55. All experiments were carried out in triplicate, the mean and standard deviation for each of the determinations were calculated and reported. The total yields of free oil and protein were calculated using Eqs. (1) and (2), respectively.

$$\%\text{Total oil extraction yield} = \frac{\text{Free oil(g)}}{\text{Total oil in peanut paste(g)}} \times 100 \quad (1)$$

$$\%\text{Total protein extraction yield} = \frac{\text{Protein in Skim Phase(g)}}{\text{Total protein in peanut paste(g)}} \times 100 \quad (2)$$

### 2.4. Demulsification procedure

The cream emulsion fraction from slurry obtained by AEP was carefully collected as the starting material for demulsification. Aliquots of 40 g of cream emulsion fraction were added into a jacketed reactor equipped with a shaft stir. Demulsification was carried out by adding enzymes at the optimal pH value and temperature as recommended by the manufacturer. The control was treated in the same conditions without adding enzyme. The effect of pH on emulsion stability was carried out by adjusting the pH value from 3 to 9 by adding 2 mol/L HCL and 2 mol/L NaOH. The supernatant oil was then carefully collected and weighed as destabilized oil. Petroleum ether was used to wash the remaining free oil adhering to the jacketed reactor. The solvent was evaporated and the weight of oil was determined. Oil contents in residual cream was also determined. Protein contents in the residual cream and other AEP fractions were determined as described above. The free oil yield of demulsification was calculated using Eq. (3).

$$\%\text{Free oil yield} = \frac{\text{Free oil(g)} + \text{Petroleum ether washed oil(g)}}{\text{emulsion(g)} \times \text{oil content in(oil + cream) fraction}} \times 100 \quad (3)$$

### 2.5. Particle size distribution

Cream samples were analyzed immediately after funnel separation and enzymes treated samples were analyzed right after incubating prior to centrifugation. The emulsions were diluted with water to obtain an obscuration of about 11–14%. The mean volume diameter ($d_{0.5}$) of samples was measured in triplicate for particle