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Aqueous Enzymatic Extraction of Oil and Protein Hydrolysates from Roasted Peanut Seeds

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Abstract To evaluate the effects of the roasting process on the extraction yield and oil quality, peanut seeds were roasted at different temperatures (130–220 °C) for 20 min prior to the aqueous extraction of both oil and protein hydrolysates with Alcalase 2.4 L. Roasting temperatures did not significantly affect the yields of free oil, whereas the temperature of 220 °C led to a reduced recovery of protein hydrolysates. The color and acid values of peanut oils did not change significantly with roasting temperatures. The enzyme-extracted oil with roasting at 190 °C had a relatively low peroxide value, a strong oxidative stability, and the best flavor score. Using the same seed-roasting temperature (190 °C), quality attributes such as color, acid and peroxide values, phosphorus content and oxidative stability of the enzyme-extracted oil were better than those of the oil obtained by an expeller. After the peanut seeds were roasted at 190 °C for 20 min, with a seeds-to-water ratio of 1:5, an enzyme concentration of 2%, and an incubation time of 3 h, the yields of free oil and protein hydrolysates were 78.6 and 80.1%, respectively. After demulsification of the residual emulsion by a freezing and thawing method, the total free oil yield increased to 86–90%.

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Introduction

Peanuts are one of the most important oilseeds in the world. In China, the production of peanuts exceeds 14,000,000 tons per year. Peanut seeds contain 25–29% (w/w) protein and 40–50% (w/w) oil. The conventional processing of peanuts in industry involves the mechanical pressing or solvent extraction (generally with hexane), which yields two products, oil and a low-valued meal.

Due to growing environmental concerns over the use of hexane to extract edible oil from oilseeds, aqueous (enzymatic) extraction processing has gained attention. This alternative process uses an aqueous (enzymatic) extraction of the comminuted materials, followed by a centrifugal separation of the slurry into oil, emulsion, and the aqueous and solid phases. Protein may be recovered in the aqueous or solid phase, depending on the conditions selected. In the last few decades, aqueous (enzymatic) extraction has been attempted to extract oil/protein from many oil-bearing materials, such as coconut [1], corn germ [2], sunflower kernels[3], rice bran [4], rapeseeds [5], soybeans [6, 7]. Recently, Moura et al. [8] have developed two-stage countercurrent aqueous enzymatic extraction process for soybeans, which significantly reduced the amount of water used and achieved slightly higher oil and protein extraction yields than standard single-stage aqueous enzymatic extraction. As for peanut seeds, Rhee et al. [9] simultaneously extracted oil and protein in an aqueous system without use of enzymes and obtained more than 95% oil and protein yields, respectively. Lanzani et al.

[10] reported a 74–78% recovery of peanut oil by an enzyme-assisted aqueous extraction using protease, cellulase, and α-1,4-galacturonide glycano-hydrolase either separately or in combination. Sharma et al. [11] used multi-activity proteases from *Aspergillus flavus*, Protizyme™, to extract peanut oil and recovered 86–92% of it. More recently, Jiang et al. [12] prepared oil and protein hydrolysates from raw peanut seeds with Alcalase 2.4 L, and reported that the yields of free oil and protein hydrolysates were 79.32 and 71.38%, respectively. However, after the residue and emulsion were further hydrolyzed by the protease As1398, the yields were increased to 91.98 and 88.21%, for the oil and protein, respectively. The varied oil and protein yields among the above studies were mainly caused by the diverse grinding methods or extraction parameters selected.

In addition to being a hexane-free process and enabling simultaneous extraction of oil and protein, aqueous (enzymatic) extraction can produce high-quality oil that requires less refining. Many of the quality attributes such as color, peroxide value, free fatty acid and phosphorus contents of oils prepared by the aqueous enzymatic extraction were better than those for oils obtained using traditional technology [2, 13]. In fact, the flavor of fats and oils is one of the most critical factors influencing quality. In the Orient, the oil aroma is especially desirable when cooking or frying foods with certain oils. Conventionally, to produce edible or condiment oils with a pleasant nut-like flavor, seeds are roasted at an appropriate temperature for an appropriate time, followed by an extraction of the roasted seeds with a mechanical press or expeller [14, 15]. During the roasting process, a pleasant aroma develops which is also transferred along with the oil during extraction. Moreover, the oxidative stability of the oil can be enhanced by seeds-roasting. Among lots of reported studies on aqueous enzymatic extraction of edible oils, few have focused on the flavor of the oils. According to our sensory evaluation of peanut oils, the aroma of enzyme-extracted oil according to the conditions provided by Jiang et al. [12] was significantly weaker than that of the commercial oil prepared by roasting and pressing peanut seeds. Therefore, to further improve the quality of peanut oil obtained by the aqueous enzymatic extraction, a feasible approach seems to be roasting the peanut seeds at an appropriate temperature prior to the enzymatic extraction.

The objective of this study was to investigate the effect of different seed roasting temperatures on the quality characteristics such as color, acid and peroxide values, phosphorus content, flavor, and oxidative stability of peanut oil prepared by an aqueous enzymatic extraction, and to optimize the enzymatic extraction process after peanut seeds have been roasted.

Materials and Methods

Materials

Peanut seeds were obtained from the local market. The dehulled peanut seeds contained 49.66% oil and 27.82% protein, on a dry basis. Alcalase 2.4 L (EC 3.4.21.62, *Bacillus licheniformis*) was purchased from Novo-Nodisk A/S (Bagsvaerd, Denmark). All the other reagents used were of analytical grade.

Preparation of Roasted and Unroasted Peanut Oils and Demulsification

Peanut seeds (500 g) were roasted in an oven equipped with a stirrer and a temperature controller. The peanut seeds were roasted with constant stirring for 20 min at 130, 160, 190 and 220 °C, respectively. The seeds were cooled and then dehulled by hand. The dehulled peanut seeds were milled into a fine particle-sized meal which was consistently sticky using a high-speed universal grinder (model FW-200; Beijing Zhongxinweiye Instrument Co, Ltd, China). Then, 60 g of the meal was transferred into a covered 500-mL conical flask and dispersed in distilled water at 1:4 (w/v). The pH of the slurry was adjusted to 9.5 with 4 N NaOH, followed by incubation at 55 °C for 1 h in a thermostated oscillator (model SHZ-82; Jiangsu Jintan Eltong Electric Corp, China) with a shaking speed of 120 rpm. Alcalase 2.4 L (0.6 mL) was then added and the slurry was incubated for 1 h with a shaking speed of 80 rpm. Subsequently, the suspension was heated at 90 °C for 10 min followed by a centrifugation at 1,819*g* (3,000 rpm) for 15 min. The upper oil phase was carefully collected using an auto-pipettor. The residual oil and the emulsion were transferred into a microcentrifuge tube and further centrifuged as above. The oil collected from both centrifugations was pooled, weighed and considered as the free oil recovered. The aqueous phase was collected and sampled for the determination of protein hydrolysates content. A control was run under the same conditions but without the roasting process.

To improve the yield of the total free oil, the residual emulsion was demulsified according to a freezing and thawing method [16]. The emulsion was frozen at −18 °C for 20 h and thawed in a water bath (35 °C) for 2 h, and then centrifuged at 8,694*g* for 15 min. The upper oil phase was carefully collected, weighed and considered as the free oil recovered.

For comparison of the quality of oils prepared by different methods, the peanut seeds roasted at 190 °C for 20 min were dehulled and the oil was then extracted using an expeller (model GY-78A; Guangzhou Guoyan Machinery Making Co, Ltd, China). The extracted peanut

oil was filtered to remove any particles. The oil then stayed for 2 days at room temperature and the clear top layer was collected and used as oil.

Determination of Oil and Protein Contents

The oil content of dehulled peanut seeds was determined by the Soxhlet extraction method [17]. The protein contents of dehulled peanut seeds and aqueous phases obtained from the enzymatic extraction process were determined by the Kjeldahl method (N × 5.46) [17]. The free oil and protein hydrolysates yields were expressed using Eqs. 1 and 2, respectively.

$$\text{Free oil yield } (\%) = \frac{[\text{free oil}]}{[\text{total oil in peanut seeds}]} \times 100\% \quad (1)$$

$$\text{Protein hydrolysates yield } (\%) = \frac{[\text{protein in aqueous phase}]}{[\text{total protein (in peanut seeds} + \text{enzyme)}]} \times 100\% \quad (2)$$

Evaluation of Oil Quality

Acid values, peroxide values and phosphorus contents of the oil samples were determined by AOCS official methods Cd 3d-63, Cd 8-53 and Ca 12-55, respectively [18]. To study the oxidative stability of unroasted and roasted peanut oils, 50 g of oil were transferred, in duplicate, to a 100-mL capacity glass beaker. The samples were stored in a forced-draft air oven at 60 °C for 18 days. The oxidative stabilities of oils were studied by measuring the increase in the peroxide value. To evaluate the oil flavor, samples were submitted to a trained panel consisting of eight persons. The panelists were asked to rank the aroma score of the oil sample on a 10-point hedonic scale in which 10 = 'extremely like' and 1 = 'extremely dislike'. The average score for each oil sample was reported.

Optimization of the Enzymatic Extraction Process

The peanut seeds were roasted at 190 °C for 20 min and then dehulled. The oil and protein hydrolysates were extracted using Alcalase 2.4 L in the aqueous system as described above. The seeds-to-water ratio (1:3–1:7), enzyme concentration (0–3%, v/w), and hydrolysis time (0–5 h) were independently varied, keeping other parameters fixed, so as to obtain the best conditions for extraction.

Statistical Analysis

Values represented are the means and standard deviations for at least two replicates. The data were analyzed by ANOVA and Duncan's multiple range test (Duncan's test). Significance of differences was defined at $p < 0.05$.

Results and Discussion

During the aqueous enzymatic extraction of oil and protein from roasted peanut seeds, only the roasting process was carried out at a high temperature. Therefore, the roasting temperature is a critical factor that probably affects extraction yield and oil quality. As shown in Fig. 1, the roasting temperatures did not significantly affect the yield of free oil; however, the yield of protein hydrolysates was reduced greatly when peanut seeds were roasted at 220 °C. According to many previous studies on the color of oils prepared by an expeller or press, such as sesame [19], rice germ [20] and safflower oils [15], with an increase in roasting temperatures, the oils became darker, which was assumed to be due to the Maillard reaction between reducing sugars and amides. The brown substances formed are transferred into the oils during extraction. However, there was no obvious color difference by visual examination of the oil samples recovered from the enzymatic extraction process with different roasting temperatures. The color of peanut oil prepared using the expeller (named as expeller oil below) was a little darker than that of the enzyme-extracted oils. It was evident that the color of aqueous phases obtained at higher roasting temperatures was significantly darker, suggesting that the color substances formed due to Maillard reaction can easily dissolve in water during enzymatic extraction. This could explain the inconsistent experimental results on the relationship between oil color and roasting temperatures.

Table 1 shows the quality attributes of enzyme-extracted peanut oils at different roasting temperatures and the expeller oil roasted at 190 °C. There was no significant

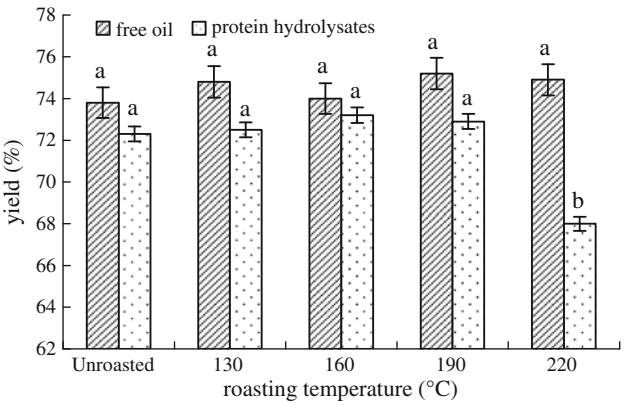

Fig. 1 Effects of roasting temperature on the yields of peanut free oil and protein hydrolysates. Values ($n = 2$) for a given product sharing the same superscript are not significantly different ($P < 0.05$)

Table 1 Chemical and sensory properties of unroasted and roasted peanut oils obtained by the aqueous enzymatic extraction and an expeller

	Enzyme-extracted oils with different seeds-roasting temperatures					Oil extracted by an expeller with seeds-roasting at 190 °C
	Unroasted	130 °C	160 °C	190 °C	220 °C	
Acid value (mg KOH/g)	0.41 ± 0.02^{a}	0.38 ± 0.01^{a}	0.39 ± 0.01^{a}	0.42 ± 0.01^{a}	0.41 ± 0.01^{a}	0.61 ± 0.02^{b}
Peroxide value (meq/kg)	10.65 ± 0.49^{a}	10.25 ± 0.35^{a}	10.81 ± 0.42^{a}	2.55 ± 0.35^{b}	2.35 ± 0.21^{b}	8.50 ± 0.71^{c}
Phosphorus (ppm)	Trace	Trace	Trace	Trace	Trace	25 ± 2.8
Aroma score	3.9 ± 0.83^{a}	3.6 ± 0.92^{a}	5.8 ± 0.53^{b}	8.4 ± 0.27^{c}	2.5 ± 0.93^{d}	8.6 ± 0.26^{c}

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($P < 0.05$)

difference of acid value among the enzyme-extracted oils prepared by seed-roasting under 220 °C. Yen [21] reported that the acid value of sesame oils obtained by a press increased markedly when the seeds-roasting temperature was more than 200 °C. A possible reason explaining the similar and low acid value in the enzyme-extracted oils is the common use of alkali during the extraction which can neutralize free fatty acids. Therefore, compared with the enzyme-extracted oils, the expeller oil had a higher acid value.

The peroxide value of the enzyme-extracted oils was almost the same as that of oils with roasting temperatures under 160 °C. However, this value reduced sharply when the roasting temperature was increased to 190 °C (Table 1). This may be due to the products produced by the Maillard reaction at the elevated roasting temperatures having strong antioxidant activities [22, 23]. The peroxide value of the expeller oil was much higher than that of the enzyme-extracted oil roasted at 190 °C, the reason could be that (even though both had the same roasting process, thus the same effect of Maillard reaction) the antioxidant activities of protein hydrolysates during aqueous enzyme extraction can retard the oxidation of enzyme-extracted oil.

As shown in Table 1, the phosphorus content was a trace in the enzyme-extracted peanut oils while it was 25 ppm in the expeller oil. Kim et al. [20] reported that the phosphorus content was 70 ppm in unroasted rice germ oil prepared by a press, which significantly increased as the preheating temperature of the oilseeds was increased. Lee et al. [15] also pointed out that the phosphorus content in the expeller oils was enhanced with the increased roasting temperatures of safflower seeds. However, in our present study the phosphorus content of the enzyme-extracted oils with roasting at different temperatures was kept at a low level. This may be because in an aqueous medium hydrophilic phospholipids absorb water and swell, thereby are easily separated from the oils after centrifugation. The low level of phospholipids virtually helps to improve the oil stability.

The peanut oils prepared by the enzyme or expeller when seeds were roasted at 190 °C, as shown in Table 1, had remarkably higher flavor scores than other oil samples. Both the oils prepared by the two different methods had a pleasant nut-like aroma and their flavor scores were not significantly different. The aroma was assumed to be a result of the pyrazines formed during the seeds-roasting process [24] since pyrazines impart a nut-like aroma in different types of foods when roasted or otherwise thermally processed [25, 26]. However, when roasted at an even higher temperature (220 °C), a burnt odor developed, which may lead to the oil's low flavor score.

The test of oxidative stability clearly showed that, as the roasting temperature increased, the oxidative stability of enzyme-extracted peanut oils increased significantly. Figure 2 shows the changes of peroxide values in unroasted and roasted peanut oils during storage at 60 °C. After 18 days of storage, the peroxide values in the unroasted enzyme-extracted oils and the expeller oils were much increased, being 85.6 and 91.3 mequiv, respectively; however, the peroxide values of enzyme-extracted oils roasted at 190 and 220 °C were increased slowly, being 56.5 and 52.2 mequiv, respectively. The obvious differences of oxidative stability between unroasted and roasted enzyme-extracted oils were mainly attributed to the Maillard reaction occurring at high roasting temperatures,

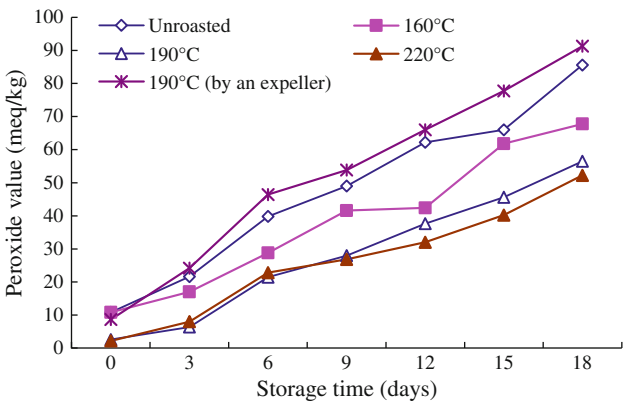

Fig. 2 Changes of peroxide values in unroasted and roasted peanut oils obtained by the aqueous enzymatic extraction and an expeller during storage at 60 °C

which could produce antioxidant substances to stabilize the oils. The weaker oxidative stability of the expeller oil should be related to its higher phosphorus content. Our observation was in accordance with previously reported results for sesame [14], soybean [27], and safflower oils [15] prepared by an expeller.

From the above results, it could be concluded that the peroxide value, flavor, and oxidative stability of enzyme-extracted peanut oils were improved when seeds were preheated at an appropriate temperatures. Therefore, considering both the extraction yield of protein hydrolysates and oil quality, the optimum seeds-roasting temperature is 190 °C for the simultaneous extraction of oil and protein hydrolysates from peanuts when the roasting time was fixed at 20 min. The extraction parameters such as seeds-to-water ratio, enzyme concentration, and hydrolysis time were then optimized.

Figure 3 indicates that there was no significant difference in the free oil yield when the seeds-to-water ratio varied from 1:3 to 1:6, though the yield obviously decreased as the slurry was further diluted (1:7). However, the seeds-to-water ratio significantly influenced the extraction yield of protein hydrolysates. At the ratio of 1:5 the yield of protein hydrolysates was the highest (73.9%), as shown in Fig. 3. During the roasting process, peanut protein in the seeds may be partly denatured leading to a decrease in solubility. The protein yields, hence, depended more on the enzyme hydrolysis rather than the amount of solvents used. The seeds-to-water ratio of 1:5 seemed to be the best for enzyme hydrolysis in this study, which may be true since concentrated suspensions (1:3 for instance) could prevent effective penetration of the enzymes while in dilute suspensions (1:6 and1:7) the interactions between enzymes and their substrate molecules may be weak. Jiang et al. [12] also reported that 1:5 was the optimal seeds-to-water ratio

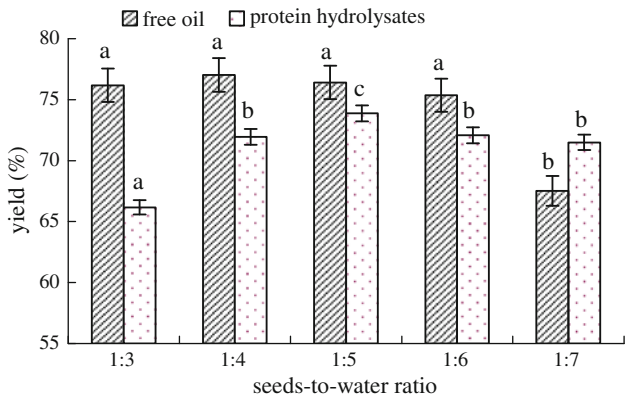

Fig. 3 Effects of seeds-to-water ratio on the yields of peanut free oil and protein hydrolysates. Conditions: 1% concentration (v/w) of Alcalase 2.4 L; 1-h incubation. Values ($n = 2$) for a given product sharing the same superscript are not significantly different ($P < 0.05$)

for aqueous enzymatic extraction of peanut seeds. Moura et al. [7] pointed out that 1:10 was the best in single-stage aqueous enzymatic extraction of soybeans and subsequently they [8] obtained a solids-to-liquid ratio of 1:5 to 1:6 for soybeans in two-stage countercurrent extraction process.

The concentration of Alcalase 2.4 L had a marked effect on the extractability of both oil and protein. Compared with the results of aqueous extraction without enzymes (the free oil and protein hydrolysates yields were only 18.6 and 30.5%, respectively), even a low concentration of enzyme (0.5%, v/w) can significantly increase the yields of free oil and protein hydrolysates to 68.8 and 70.0%, respectively. During the non-enzyme aqueous extraction, most of the oil was obtained only in an emulsified form and a protease was therefore needed for demulsification, as explained in our previous study [5]. Not only oil was liberated from the emulsion, but also many non-soluble peanut proteins (probably resulting from seed-roasting) in this study could be effectively extracted due to the protease. As the enzyme concentrations increased from 1 to 3%, the yield of free oil was not significantly increased. When the enzyme concentration was less than 2%, the yield of protein hydrolysates increased remarkably with the increase of enzyme amounts. Further addition of more enzymes only led to a slight increase in protein extractability. The amount of enzyme used should be a compromise between the improvement of protein recovery and the cost of enzyme. Therefore, 2% (v/w) of Alcalase 2.4 L was the optimal for simultaneous extraction of oil and protein hydrolysates. At this adding level of protease, the yields of oil and protein hydrolysates were 75.8 and 76.7%, respectively.

During the first 3 h, the oil extractability was markedly improved by the increment of enzyme treatment time, but it declined as the time was further extended. The decrease in the free oil yield was speculated to be due to re-emulsification of partially free oil and protein during the prolonged time. The yield of protein hydrolysates increased significantly with increased hydrolysis time during the first 3 h, thereafter reaching a plateau. Increasing the time up to 5 h did not provide any significantly higher extractability of protein hydrolysates as compared with 3 h, which may be due to the depletion of substrates and/or product inhibition of enzymes. Therefore, an enzyme treatment of 3 h was chosen for the aqueous enzymatic extraction, which was remarkably less than the reported result of Jiang et al. [12] with unroasted peanut seeds (5 h was optimal in their study).

In summary, when peanut seeds were roasted at 190 °C for 20 min, Alcalase 2.4 L was used for 3 h at a concentration of 2% (v/w), the yields of free oil and protein hydrolysates yields can reach 78.6 and 80.1% (seeds-to-water ratio = 1:5), respectively. The yield of free oil was

close to that (79.32%) of Jiang et al. [12], but the oil quality was improved by roasting. Additionally, the yield of protein hydrolysates was significantly higher than that (71.38%) reported by Jiang et al. [12]. Although Alcalase 2.4 L effectively facilitated the separation of free oil, a thin but distinctive layer of emulsion between the oil and the aqueous phase remained after centrifugation. The emulsion contained a considerable amount of oil (ca. 13% of the total oil) and a demulsification step was, therefore, required to improve the yield of total free oil. The freezing and thawing method has been used in an attempt to break the emulsions formed during aqueous extraction of oils by other researchers [16, 28], which proved to be simple and effective. After the residual emulsion in the present study was demulsified by the freezing and thawing method, the yield of total free oil was increased to 86–90%.

In conclusion, roasting peanut seeds prior to an aqueous enzymatic extraction is a viable approach to improve the oil quality. Good recoveries of peanut oil and protein hydrolysates implies that this environmentally friendly process has great potential for applications in industry, especially with currently increasing environmental concerns and requirements for vegetable proteins.

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