Optimization of Microwave-Assisted Extraction of Palm Kernel Cake Protein

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Abstract. Palm kernel cake (PKC) is an abundant by-product of the palm oil industry. It is used as an ingredient in feed due to the high amount of protein and fiber content. In order to increase the value of PKC, the PKC protein can be extracted and may be able to be used as an alternative protein for plant-based food. This study aims to optimize the PKC protein extraction using the microwave-assisted extraction (MAE) method with a response surface methodology (RSM). MAE is a green extraction method due to less chemicals needed, less time and less energy consumption when compared to the traditional thermal extraction method. The experiment was designed by the Box-Behnken method with 3 factors; microwave power (A), extraction time (B) and solid-liquid ratio (C). The optimum condition was at the microwave power of 700.16 W, extraction time of 543.08 s and the solid-liquid ratio of 1:7.73 g PKC/ ml water resulting in a theoretical yield of protein extraction of 32.46%.

Keyword. Palm kernel cake, Microwave-assisted extraction, Protein, Response surface methodology

1 Introduction

Protein is an essential nutrition, which can be obtained from animals, insects, plants, and microorganisms. Nowadays, protein consumption is continuously increasing while protein sources are limited. In order to solve this problem, the by-products or wastes from food and agro-industry become alternative protein sources for human foods and feeds. Utilization of by-product as the protein source is not only add additional value to the by-product but also relieves the environmental problems.

Thailand is one of the top five palm oil-producing countries which can annually produce 16.40 million tons and the production rate is expected to be higher [1]. One thousand kilograms of oil palm produces 250 kilograms of oil which includes 200 kilograms of crude palm oil and 50 kilograms of crude palm kernel oil. Therefore, the by-products and waste from palm oil process are approximately up to 750 kilograms. Palm kernel is the pulp within the oil palm seed which is composed of lipid up to 49%. The residue after the palm kernel oil extraction process is called palm kernel cake (PKC). The PKC consists of carbohydrate 50.3%, fiber 16.7%, protein 14.8%, lipid 7.9%, moisture 6.4% and ash 3.9% [2].

Normally, it is used as a feed supplement for ruminants due to the high amount of protein and fiber [3-5]. Thus, the PKC is an interesting protein source. Moreover, PKC contains essential amino acids around 6.98%, such as arginine, leucine, valine, phenylalanine and threonine [6]. Therefore, PKC protein is an interesting alternative source for future food. Currently, there are several research works investigating the optimization of PKC protein extraction. One of the most popular methods of protein extraction is the chemical extraction method, which uses solvent, such as water, alkali, acid, and organic solvent. The alkaline extraction method is widely used to extract protein from plant material due to its high protein yield and easy operation. However, there are some problems including brown color from Maillard reaction, lack of some functional properties and protein denaturation caused by a high concentration of alkaline [7].

The microwave-assisted extraction (MAE) is an environmental-friendly extraction technique, which is the combination of the chemical extraction method and the physical extraction method. Microwave generates heat by two simultaneous mechanisms: ionic conduction and dipole rotation, thus the whole sample is heated up at the same time. The rotation of dipolar molecules can break the weak hydrogen bonds. Moreover, microwaves can reduce the viscosity and induce the solvent to penetrate the matrix and release the target product [8]. There are many advantages of MAE, such as short extraction time, small amount of solvent needed, and low cost of energy. The applications of MAE are typically used for the extraction of some natural products, such as essential oil [9-11], flavonoid [12-14], phenolic compounds [15], alkaloids [16-17], saponins [18-20], polysaccharide [21-22] and protein, etc. Many studies have also reported the applications of MAE technique for protein extraction from plant feedstocks, such as rice bran [23-24], sesame bran [25], coffee silverskin [26] and watermelon seeds [27]. Some of the main factors affecting the efficiency of MAE are sample characteristics, temperatures, microwave powers, extraction times, solid-liquid ratios, and solvents [23,28]. According to the previous studies the MAE was applied to extract rice bran protein by optimizing the microwave power, extraction time and solid-liquid ratio. The results showed that the protein yield obtained from the optimal condition (1000 W of microwave power, 90 s of extraction time and 0.89 g of rice bran/ 10 mL of water) was 1.45-fold higher than the yield of alkaline extraction [23].

To obtain the maximum yield of protein extraction, statistical process optimization is required to reduce the number of experiments and to predict the response using the mathematical model. The experiments can be designed by many methods such as full factorial design, Taguchi and response surface methodology. Full factorial design is used for optimizing the effects of each factor which suits a low number factor experiment. This method is one of the most precise methods because of the number of experimental runs. On the other hand, the more factors or levels are studied, the more runs are generated which consume a lot of time and labor [29]. Taguchi method or robust design method can estimate the most effective factor with a minimum amount of experiment which can save time and resources. It can be divided into 4 phases including planning, conducting, analysis and validation. However, the main advantage of this method is the bias of the studies which require testing all relationships of all factors, because it does not test all combinations [30-31]. Response surface methodology (RSM) is a well-known optimization method. It can identify the factors and the combinations, which have an effect on the response. The linear regression model shown in equation 1 is generated based on the experiment data to predict the response.

$$S=a_0+\sum_{i=1}^{k}a_ix_i+\sum_{i=1}^{k}a_{ii}x_i^2+\sum_{i,j=1,\, j\neq i}^{k}a_{ij}x_ix_j \qquad (1)$$

where S is a response, a_0 is an average of responses; a_i, a_{ii} and a_{ij} are response coefficients [32].

Comparing to full factorial design and Taguchi method, RSM is more compromise due to the number of experimental runs which is not too much like the full factorial design or too less like the Taguchi method. Furthermore, RSM can study the effect of a combination of variables on the response.

Overall, the aim of this study was to extract the protein from PKC using microwave-assisted extraction technique which is an eco-friendly method. The optimal condition was obtained by a response surface methodology based on Box-Behnken design. Three variables including microwave powers, extraction times and liquid-solid ratios were studied.

2 Material and method

2.1. Sample preparation

Defatted PKC from a palm oil factory in Southern Thailand was ground and sieved in order to get the PKC size between 250-500 µm (Figure 1). The protein content of PKC was analyzed by the Kjeldahl method and the moisture content was obtained using a moisture analyzer (Halogen Moisture Analyzer HE53, Mettler-Toledo Ltd., Ohio, United States) before extraction.

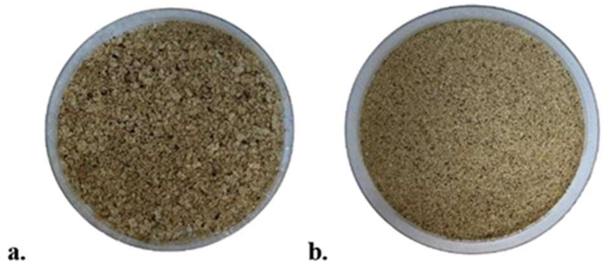

Figure 1. Defatted PKC before (a.) and after (b.) grinding and sieving

2.2. Experimental design and statistical analysis

The response surface methodology (RSM) based on the Box-Behnken design (BBD) was used to optimize the PKC protein extraction using MAE method due to the BBD requiring a small number of experimental runs and suitable for the limitation of microwave power generated by the machine. A three-level-three-factor BBD generated a response surface model using the Design-Expert software (V.7.0.0, Stat- Ease, Inc., Minneapolis, USA). The independent variables for this experimental design are microwave power (A), extraction time (B) and liquid-solid ratio (C) which are some of the main extraction factors. The range of values of the variables used in the experimental design from MAE was presented in Table 1 which was chosen according to the preliminary experiment. The levels of each parameter coded -1, 0 and +1 were the lowest, the middle and the highest values, respectively.

The experimental design consisted of 17 experimental runs which were 5 replicates of the center point shown in Table 2. The protein extraction yield (%) was selected as the response (Y).

Table 1. Values range of the variables used in the PKC protein extraction by MAE method.

Parameters	Codes	Levels		
		-1	0	+1
Microwave power (W)	A	100	450	800
Extraction time (s)	B	30	315	600
Liquid-solid ratio (ml/g)	C	3:1	6.5:1	10:1

Table 2. Box-Behnken design (BBD) and PKC protein extraction yield.

Run	Factors			Y: Yield (%)
	A: Microwave power (W)	B: Extraction time (s)	C: Liquid-solid ratio (ml/g)	
1	100	30	6.5:1	21.08
2	800	30	6.5:1	23.59
3	100	600	6.5:1	28.08
4	800	600	6.5:1	30.07
5	100	315	3:1	23.87
6	800	315	3:1	23.98
7	100	315	10:1	23.61
8	800	315	10:1	30.86
9	450	30	3:1	24.40
10	450	600	3:1	25.31
11	450	30	10:1	21.52
12	450	600	10:1	31.80
13	450	315	6.5:1	27.35
14	450	315	6.5:1	31.12
15	450	315	6.5:1	31.36
16	450	315	6.5:1	29.28
17	450	315	6.5:1	32.29

2.3. Protein extraction using MAE method

The PKC protein extraction was carried out using a home-used microwave oven (Samsung ME711K, Thai Samsung Electronics Co., Ltd., Thailand). Five grams of PKC were mixed with the distilled water with the liquid-solid ratio of each run in a 50 ml-centrifuge tube, and put the tubes in a microwave oven. The PKC protein was extracted following the condition in Table 2. Afterward, the distilled water was added to the slurry to get the final volume of water at 50 ml. Then, the supernatant was separated by centrifugation (Centrifuge 5804R, Eppendorf Co., Ltd., Hamburg, Germany) at 10,000 rpm at a temperature of 4 °C for 10 min and the soluble protein in the supernatant was determined. The experiment was replicated for each run.

2.4. Protein determination

The soluble protein content in the supernatant was determined by the modified Folin-Lowry method [33] for 96-well plate. The difference concentration of albumin (BSA) (Bio Basic Canada Inc., Ontario, Canada), including 0, 1, 5, 25, 125, 250, 500, 1000, 1500 and 2000 µg/ml, was prepared and used for the standard. Forty µl of standard and diluted samples were added to each well of 96-well plate. The Lowry reagent was made from the combination of reagent A (2% sodium carbonate and 0.4% sodium hydroxide), 1% copper sulfate and 2% potassium sodium tartrate with the ratios of 100:1:1, and added 200 µl of the reagent into the samples. The mixtures were shaken for 30 s and incubated at room temperature for 10 min. Then, 20 µl of 1X Folin-Ciocalteu reagent was added immediately to each well. The mixtures were shaken for 30 s and incubated at room temperature for 30 min. The absorbance was detected at 750 nm. In order to calculate the protein concentration, the standard curve was plotted between the BSA concentrations and wavelengths, and the equation was used to calculate. All experiments were triplicated. The extraction yield (%) was calculated by equation 2:

$$\text{Yield (\%)} = \frac{\text{Protein concentration} \times \text{Extract volume}}{\text{mg of PKC} \times \text{Raw protein content}} \times 100 \quad (2)$$

3 Results and discussion

Microwave-assisted extraction (MAE) was performed to extract the PKC protein. The effect of factors, including microwave power (A), extraction time (B) and liquid-solid ratio (C) on the protein extraction yield (%) were studied. Box-Behnken design (BBD) of response surface methodology (RSM) was us as a statistical tool for optimization. The crude protein of PKC before extraction determined by the Kjeldahl method was 18.49% ± 0.05 (w/w). The experimental conditions were generated including 17 experimental runs presented in Table 2. The lowest extraction yield was obtained in the first run with 21.08% (w/w) and the highest yield was 32.29% (w/w) from the run number 17.

The statistical analysis of the model was performed in the form of analysis of variance (ANOVA) with backward elimination regression shown in Table 3. The p-value of this model was 0.0011 which implied the model was significant.

The coefficient of determination or R-squared (R2) value of 0.9216 represented a strongly fit of the model with the experimental data. Moreover, the lack of fit was not significant at the p-value of 0.9342, which could clearly confirm that the model was acceptable.

Table 3. ANOVA for the RSM quadratic model

Source	SS	df	MS	F value	p-value Prob > F
Model	216.18	8	27.02	11.75	0.0011
A	17.61	1	17.61	7.66	0.0244
B	76.04	1	76.04	33.06	0.0004
C	13.09	1	13.09	5.69	0.0441
AC	12.75	1	12.75	5.54	0.0464
BC	21.98	1	21.98	9.55	0.0149
A^2	23.78	1	23.78	10.34	0.0123
B^2	20.35	1	20.35	8.85	0.0178
C^2	22.73	1	22.73	9.88	0.0137
Residual	18.40	8	2.30		
Lack of Fit	2.88	4	0.72	0.19	0.9342
Pure Error	15.52	4	3.88		
Core Total	234.59	16			

In this case microwave power (A), extraction time (B), liquid-solid ratio (C), the interaction of microwave power and liquid-solid ratio (AC), the interaction of extraction time and liquid-solid ratio (BC) and the quadratic term of all variables (A2, B2 and C2) had an effect on the response value ($p<0.05$). The interaction of microwave power and extraction time (AB) was removed because it was the insignificant variable ($p>0.10$). The statistic model represented the relation of response variable (extraction yield) and the significant model terms, which could be expressed by the quadratic equation 3:

$$Y = 17.03441 + 0.012227A + 0.012594B + 1.43535C + 0.00145735AC + 0.00234987BC - 0.0000194006A^2 - 0.0000270646B^2 - 0.18968C^2 \quad (3)$$

where A, B, C and Y coded for microwave power, extraction time, liquid-solid ratio and extraction yield, respectively.

The three-dimensional response surface plots explained the interactive effects of the independent variables on the amount of protein extraction yields. Two variables were chosen to observe when the other variable was fixed at the middle level. These plots should indicate the optimal value of variables, which resulted in the highest response. Figure 2 and 3 were the three-dimensional response surface plot and contour plot showing the effect of variables on the response.

Figure 2 showed the effect of microwave power and liquid-solid ratio on extraction yield, when extraction time was fixed at 315 s. Increasing microwave power to around 600-700 W could increase the yield. It might be the effect of the microwave-induced dipole rotation and the penetration of water to the matrix which could help release the intracellular product. Moreover, the extraction yields slightly decreased when microwave power was higher than the optimal value. It was possibly caused by the denaturation of protein from the heat generated by microwaves [23].

a.

b.

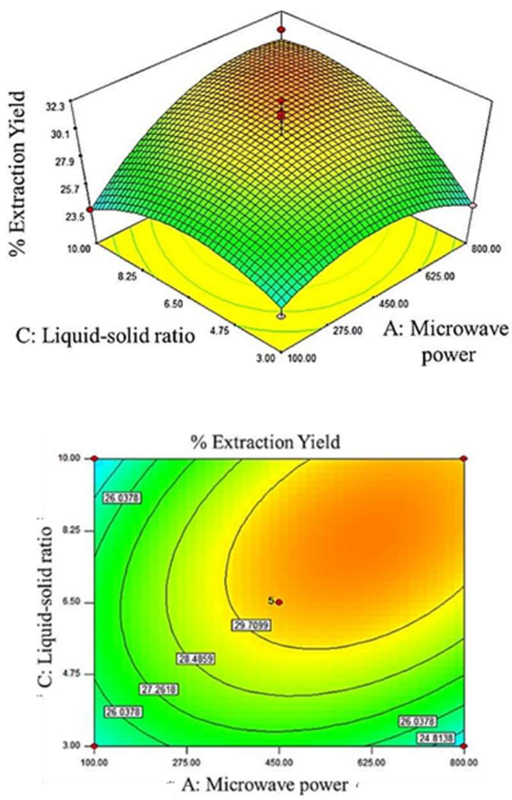

Figure 2. The three-dimensional response surface plot (a.) and contour plot (b.) show the effect of microwave power and liquid-solid ratio on extraction yield.

The effect of extraction time and liquid-solid ratio on extraction yield, when microwave power was fixed at 450 W was presented in Figure 3. The more extraction time was increased, the more extraction yield was obtained. The time increased from 30 to 600 s. showed the increasing extraction yield. It might be due to the duration of diffusion.

In both figures 2 and 3, the yield was increased when the liquid-solid ratio increased to the optimum, and the yield slightly decreased when the ratio was higher than the optimum. It might be due to increasing water not only containing more extract but also water could absorb more microwave power and dispersing microwave energy [19].

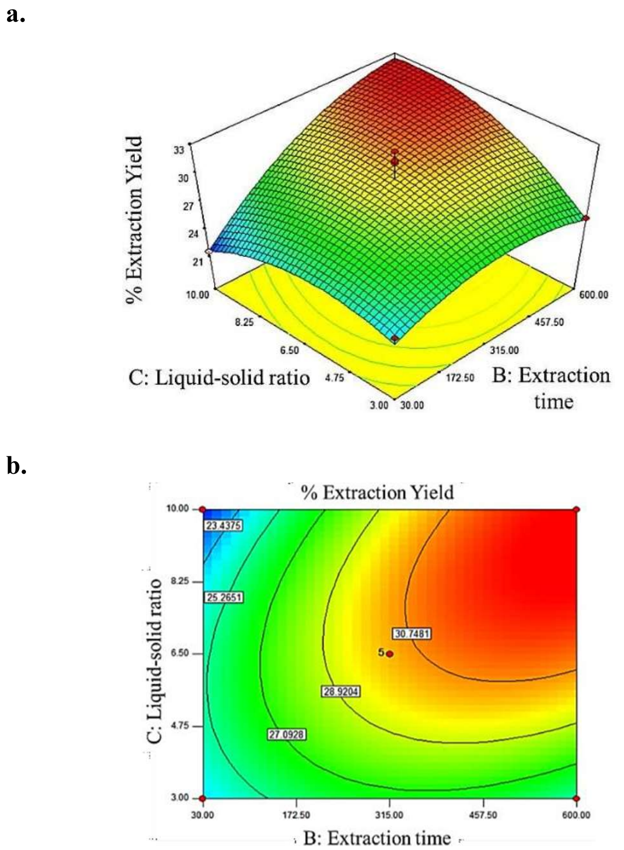

Figure 3. The three-dimensional response surface plot (a.) and contour plot (b.) showing the effect of extraction time and liquid-solid ratio on extraction yield.

The optimized model was validated by performing several experiments. The predicted and experimental values of optimum condition of PKC protein extraction using MAE method were presented in Table 4. The optimum condition was predicted with the maximum desirability of 32.4554%. The model validation was performed at microwave power of 700.00 W, extraction time of 543 s and liquid-solid ratio of 7.73:1. The protein extraction yield at 31.03% (w/w) was obtained from this condition, which missed from the prediction about 4.38%.

The experimental results were in a good correlation between predicted and actual response values. Thus, the response surface methodology could be applied to optimize the condition of palm kernel cake protein extraction using the microwave-assisted method.

Table 4. The optimal predicted and experimental values of variables.

Variables	Predicted values	Experimental values
Microwave power (W)	700.16	700.00
Extraction time (s)	543.08	543.00
Liquid-solid ratio (ml/g)	7.73:1	7.73:1
Extraction yield (%)	32.4554	31.03

From the previous studies, PKC protein was extracted by the chemical extraction method using saline and alkaline. The optimum condition for saline treatment was at pH 9, 0.2 M NaCl and liquid ratio of 60:1, and the optimum condition for alkaline treatment was at 0.03M NaOH, 35°C and liquid ratio of 30:1 gave the protein recovery yield of 78.59% and 73.07%, respectively [34]. In another study, PKC protein was extracted using the trypsin-assisted extraction method. The optimum condition was 50 °C, pH 9.5, PKC 1.1 g/ 100 ml and trypsin concentration 1.36 g/ 100 g which resulted in protein yield of 61.99 g/ 100 g [35].

According to previous studies, the high protein yield was got from the high pH value (9-9.5) condition. Moreover, they have to consume a lot of time and chemicals (NaCl, NaOH and phosphate buffer) which could cause a high production cost.

4 Conclusion

Response surface methodology (RSM) was used to optimize the condition of palm kernel cake (PKC) protein extraction using the microwave-assisted extraction (MAE) method. The PKC protein could be extracted by using water as a solvent with the MAE method because the microwave enhanced the penetration of water to the matrix to release the intracellular protein. The optimum condition was obtained from quadratic modeling. The extraction yield was 31.03% at a microwave power of 700 W, extraction time of 543 s and liquid-solid ratio of 7.73:1. However, further study of the functional properties of PKC protein will be investigated for functional food and feed applications.

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