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Optimization of Protein Extraction from Fermented and Non Fermented Perah Seed by using Response Surface Methodology

Hamimah Satirah Mazlan[a], Ida Idayu Muhamad[a,b*], Nor Diana Hassan[a], Nuraimi Azlan Hadi Tan[a]

[a]*Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[b]*Cardiovascular Engineering Centre IJN-UTM (Biomaterial Unit), Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: idayu@cheme.utm.my

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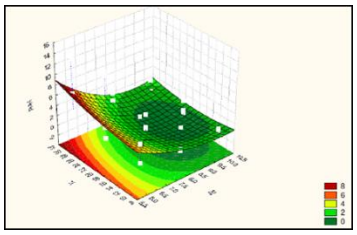
Graphical abstract



Abstract

The optimum protein extraction from fermented and non-fermented Perah seed (*Elateriospermumtapos*) was investigated using the response surface methodology (RSM). A box-behnken design with three independent variables which are NaOH concentration (6, 8 and 10%), extraction time (10, 20 and 30 minutes) and solvent/meal ratio (50:1, 100:1 and 150:1, v/w) was used to study the response of protein yield. A second-degree equation for independent and response variables was produced from simulation to obtain the contour plot graphs. The best protein extraction procedure from both fermented and non-fermented Perah seed was obtained at 5.5% of solvent extraction, 40:1 ratio of solvent/meal and at 32 minutes of reaction time. Surface concentration and ratio of solvent/meal were found to influence the protein yield from fermented seed; meanwhile only the solvent concentration influenced protein yield from non-fermented seed. The maximum protein yields for both fermented and non-fermented Perah seeds were 18.0 g/100g and 5.0 g/100g seed meal respectively.

Keyword: Box-behnken; third degree equation; surface concentration; protein extraction; Perah seed

1.0 INTRODUCTION

With the rapid growth and development of Science and Technology, protein's impact and importance in many sectors such as the food, pharmaceutical and cosmetics industry has intensified. Protein is one of the essential nutrients in the human diet where it serves as the major structural component of muscle and other tissues in the body. It is also used to produce hormones, enzymes, haemoglobin and as energy [1]. Hence, many studies have been conducted in order to get good quality and high protein extraction. Perah seed (*Elateriospermum tapos*) has been introduced as a potential protein source due to its high content of nutritional value [2]. The Perah fruit usually measures about 5 to 6.4 centimeters long. It has a big woody capsule. Inside the capsule, the fruit is split into 6 parts with a seed in each part. Meanwhile, the Perah seed usually measures 4.5 centimeters long with a shining brown coat and a faint ridge on each side. The seed is coated by a thin endocarp [3]. Perah seeds are found abundantly in South-East Asia. As previous studies conducted by researchers indicate that the Perah seed contains 59.32 g crude protein per 100 g of seed [2]. Protein extraction from non-fermented and fermented Perah seeds using liquid-solid extraction was carried out at room temperature from ground seed samples. Yield of extraction can be maximized by increasing the surface area (small particle size). However, if the particles are too small, the extraction process may lose its efficiency and decrease the yield of extraction [4].

In order to obtain the best protein extraction, certain variables need to be considered. Minimization of moisture content in seed is needed to avoid the alteration of product shelf life [5]. On the other hand, longer extraction time allows enough time for the reaction between solvent and meal to transpire, thus increasing the yield of extraction. However, at a certain reaction time, the product yield decreased as the reaction time increased because the diffusivity of the solvent and the meal has ceased to transpire. Ratios of solvent/meal were investigated by adding a selected portion of solute into a selected portion of solvent. A series of different ratios of solvent/meal were developed to investigate the driving force for the mass transfer of product into the solvent [6]. The type of solvent is crucial for extraction, especially for the extracts to be used in the food industry. The suitability and extractability of the sample is equally important. The solvent chosen was based on low cost, efficiency and solubility which subsequently depended on the molecular structure of the molecule. Solvent concentration plays a major role in the extraction process as it has been described as one of the important variables used as investigation factors. A dilution at certain ratios has given different solvent concentrations that will influence the efficiency of the extraction process between solvent and sample [7]. Therefore, the determination of the maximum or minimum values of extraction can be assessed by manipulating multiple variables during the response surface methodology (RSM). RSM is a statistical technique for optimizing complex processes and has been successfully applied to such extraction

processes [8-9]. RSM involves two stages, which are conducting the experiments in some points of the region and obtaining the results and making predictions of the results which can be obtained by evaluating the parameters of the variables.

RSM provides the best conditions for the variables used and consequently the maximum value of protein yield. Thus, the objective of this study is to investigate the effect of independent variables (solvent concentration, reaction time and solvent/meal ratio) on the production of protein from fermented and non-fermented Perah seeds by using RSM. The response surface will be further extended to get the mathematical model that fits the experimental data and the determination of the best condition of process variables. The main purpose of this research was to study the response pattern and to determine the optimum combination of the variables.

2.0 EXPERIMENTAL

2.1 Protein Extraction

Perah seeds were obtained from the Perah farm at Hulu Terengganu. The seeds were separated into two categories which were the non-fermented and fermented Perah seed. The shell were peeled, cleaned and dried at 50^{O}C for 24 hours in the oven. Then, a drying blender was used to set the seed into powder. Then, the size of the powdered seeds was homogenized by using mesh wire where the size was set at 1 millimeter. The powdered seeds were then stored in screw cap bottles and kept at room temperature for further analysis. A total of 1.0 gram of the sample was diluted in 50 ml of 6% NaOH and stirred for 10 minutes. 25 ml of the solution was poured into the centrifuge tube and centrifuged at 700 rpm/hr. Then the supernatant was taken for the protein determination by using a spectrophotometer at 540 nm. This was then compared to the data of the standard curve to get the protein extraction values. The steps were then repeated for different NaOH concentration at 8% and 10%, different solvent/meal ratio of 100:1 and 150:1 v/w and different reaction time of 20 and 30 minutes.

2.2 Design of Experiment

The Response Surface Methodology was used to study the effects of the independent variables X_1 (NaOH concentration, *Sc*), X_2 (liquid:solid ratio, *Rt*) and X_3 (reaction time, *Ti*) at three variation levels in the extraction process which is shown in Table 1. The experiments were designed by using Statistica software version 6.0. From Table 1, low (-1), middle (0) and high level (+1) was selected for each independent variable. The experimental design used was the 3^3 full factorial Box-Behnken of Centre Composite Rotatable Designs.

The number of experimental runs was determined by Equation (1),

$$N = 3^k \quad (1)$$

where, N = number of experimental runs, k = number of independent variables. Therefore 27 experiments of extraction were required to cover all possible relation of variable levels as presented in Table 1, in order to determine the optimum parameters to produce the highest protein extract yield for this study.

Table 1 Independent variable values of the process and their corresponding levels

Independent variable	Symbol	Range and levels		
		−1	0	1
NaOH concentration (%)	X_1	6	8	10
Liquid:solid ratio (v/w)	X_2	50:1	100:1	150:1
Reaction time (min)	X_3	10	20	30

3.0 RESULTS AND DISCUSSION

3.1 Protein Extraction

A study of protein yield extracted from fermented and non-fermented Perah seeds was evaluated. The Response Surface Methodology (RSM) was used to observe the optimum condition of protein yield extracted between those three independent variables (solvent/meal ratio, NaOH concentration and reaction time) by Box-Behnken model. A mathematical model was developed by Statistica Software Version 6.0 and the response surface of protein yield extracted between those three variables was investigated.

3.2 Mathematical Model

The mathematical model for yield of protein is shown in Equations (2) and (3) for fermented seed and non fermented seed respectively.

$$Y_1 = 94.95 - 20.4138X_1 + 1.07083X_1^2 - 1.20672X_2 + 0.00273X_2^2 + 0.29516X_1X_2 - 0.000776X_1X_2^2 - 0.016416X_1^2X_2 + 0.000045X_1^2X_2^2 + 5.19916X_3 - 0.110583X_3^2 - 0.942083X_1X_3 + 0.0184583X_1X_3^2 + 0.056249X_1^2X_3 - 0.0011249X_1^2X_3^2 - 0.0332416\ X_2X_3 + 0.00091X_2X_3^2 + 0.000157\ X_2^2X_3 - 0.0000043X_2^2X_3^2 \quad (2)$$

$$Y_2 = 18.20959 - 6.73750X_1 + 0.37986\ X_1^2 + 0.97733\ X_2 - 0.061022\ X_2^2 - 0.18621\ X_1X_2 + 0.0012325\ X_1X_2^2 + 0.11938\ X_1^2X_2 - 0.000077917\ X_1^2X_2^2 - 1.78444\ X_3 + 0.053778\ X_3^2 + 0.85375\ X_1X_3 - 0.023150\ X_1X_3^2 - 0.053333\ X_1^2X_3 + 0.0014583\ X_1^2X_3^2 - 0.033750\ X_2X_3 + 0.0007625\ X_2X_3^2 + 0.00015917\ X_2^2X_3 - 0.000036167\ X_2^2X_3^2 \quad (3)$$

The acceptance of the model depends on the regression squared value, where the model is considered as accepted if the regression squared is greater than 80%. The acceptance of the model is proved by performing a variance test or ANOVA. The ANOVA results from the model is shown in Table 2 for fermented and non-fermented seeds where it includes values of regression sum of squares (SSR), error sum of squares (SSE) and total sum of squares (SST) to perform ANOVA. An analysis of variance was used in order to determine the acceptance of the model derived from the experiment by comparing the F-calculated value and the F tabulated value. At 95% of confidence level, the value of F-tabulated that $F_{(18,\ 8,\ 0.05)}$ was 3.17. The acceptance model was assumed to be where the F-calculated is bigger than F-tabulated. Moreover, the F-calculated was in the acceptance region of the F distribution curve and the models were significant. The F-calculated or variance ratio was 21.734 which is greater than F-tabulated, hence the model is accepted.

Table 2 ANOVA results for fermented seed and non fermented seed

FS	COC	DOF	Sum squared	Mean squared	VR	R^2
	R	18	363.711	19.143	21.734	0.981
	Error	8	7.046	0.881		
	Total	26	370.757			
NFS	**COC**	**DOF**	**Sum squared**	**Mean squared**	**VR**	**R^2**
	R	18	30.239	1.592	4.196	0.909
	Error	8	3.034	0.379		
	Total	26	33.273			

FS: fermented seed, NFS: non fermented seed, COC: cause of change, VR: variance ratio, R: regression

Meanwhile, the value of regression coefficient is 98% for the fermented seed and 91% for the non-fermented seed whereby the value is greater than 80%. The regression coefficient results imply that the predicted and experimental values are in agreement and the adequacy of the model for fermented and non-fermented seeds as shown in Figures 1 and 2 respectively.

3.3 Effect of Variables for Fermented and Non Fermented Seed

The analysis of variables by Statistica Software was illustrated in the response surface graphs and the contour plots. The fermented seed and non-fermented seeds produced three response surfaces and three contour plots due to the response yield from three variables. Figures 3 and 4 show the response surface plot for the effect of solvent concentration and reaction time to the yield of protein extracted from fermented and non fermented seed respectively with 100 v/w solvent/meal ratios. The extraction process was carried out in different concentrations of NaOH (6%, 8% and 10%).

The maximum protein yield (8.9%) from the fermented seed was achieved by using 5.5% of solvent. Maximum protein yield from non-fermented seed was 4% that also occurred at 5.5% solvent concentration. The observation of the plots show that high solvent concentration results in low protein yield. This is partly due to degradation of the protein as the solvent concentration increased [10, 11]. The extraction of polysaccharides shows the similar trend as a response to the concentration of NaOH [10-11]. According to a previous study, the maximum product yield measured to be 4.26% [8]. This is close to the result obtained from this study where the maximum protein yield was at 5.5% (fermented seed) and 4% (non-fermented seed).

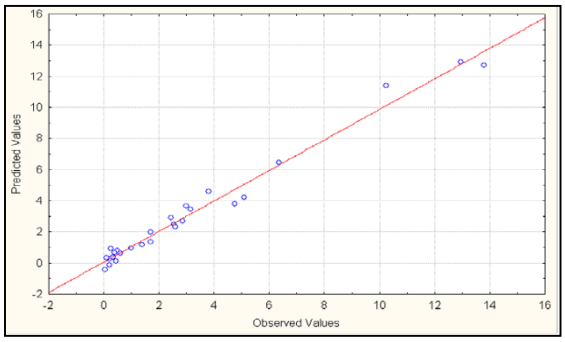


Figure 1 Observed and predicted values of the protein yield for fermented seed

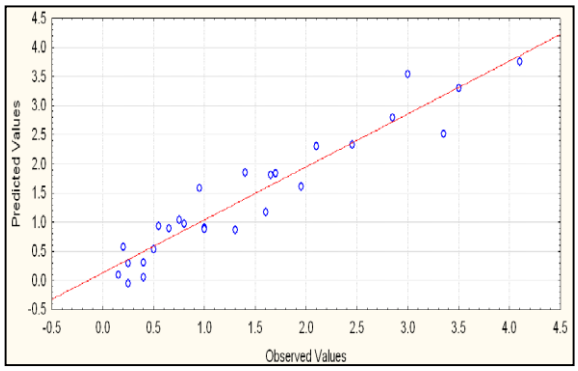


Figure 2 Observed and predicted values of the protein yield for non-fermented seed

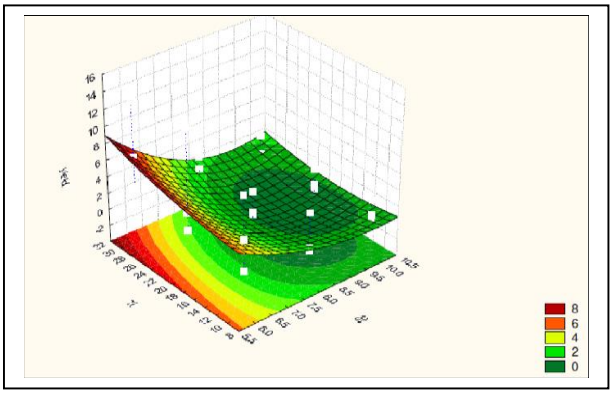


Figure 3 Response surface effects of solvent concentration to reaction time on yield of protein extraction from fermented seed with solvent/meal ratio of 100v/w

As for the response of yield of protein extraction from the reaction time, it was observed that the protein yield increased with increased reaction time. The longest reaction time of 32 minutes correlated with the highest protein yield extracted. Longer time enhances the diffusivity of the solvent to extract the protein and extraction time enhances the protein yield [7]. A linear relationship between protein yield and increased reaction time has been reported by other researchers [8]. Similar results were reported by other studies where reaction time had positive effects on polysaccharides extracted by NaOH [11, 12].

Figures 5 and 6 show response surface plots for the effect of protein yield to the ratio of solvent/meal and solvent concentration for fermented and non-fermented seed respectively. The figure shows that the highest protein yield was 18% for fermented seed and 5% for non fermented seed at a ratio of 40:1, which decreased as the ratio increased. The decrease of protein yield at a certain ratio is due to the decrease of the driving force for the mass transfer of the protein into the solvent [6].

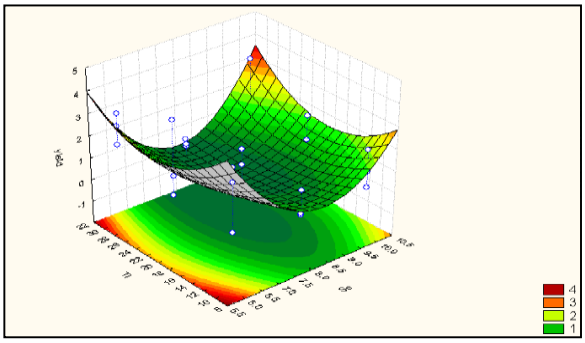


Figure 4 Response surface effects of solvent concentration to reaction time on yield of protein extraction from non fermented seed with solvent/meal ratio of 100v/w

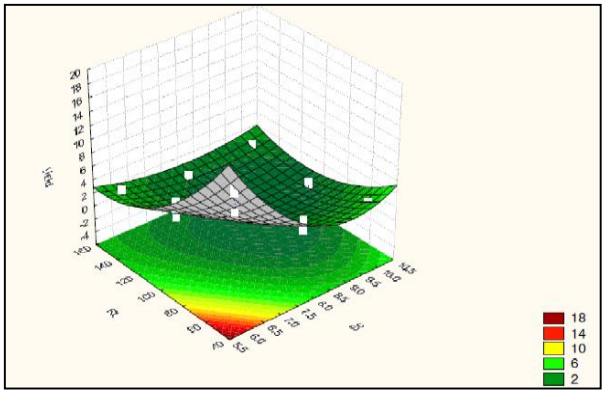


Figure 5 Response surface effects of solvent concentration to solvent/meal ratio on yield of protein extraction for fermented seed

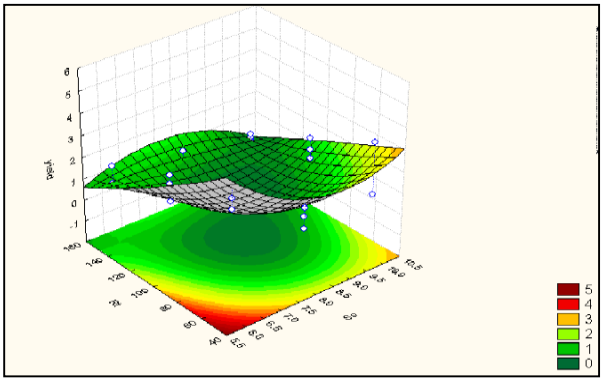


Figure 6 Response surface effects of solvent concentration to solvent/meal ratio on yield of protein extraction for non fermented seed with reaction time at 20 minutes

Similar findings have been reported where protein extracted from watermelon seed started to increase at 40 volumes per weight [13]. This is due to the sufficient solubility of the meal and the solvent in order to extract the protein. Similar findings have been reported for polysaccharides instances where the yield increased as the ratio solvent/meal increased [9, 10]. The series of ratio was developed from 10:1 to 50:1. The protein yield increased as the ratio moved from 10:1 to 40:1, when the yield stopped increasing. The result shows that the maximum product yield is at 40:1. Meanwhile, the solvent concentration had affected the protein yield as the highest protein yield was at 5.5% solvent concentration. The protein yield decreased as the solvent concentration increased, as discussed in Figure 3.

The response of reaction time and ratio of meal per solvent to the yield of protein was plotted as in Figures 7 and 8 for fermented and non fermented seed respectively.

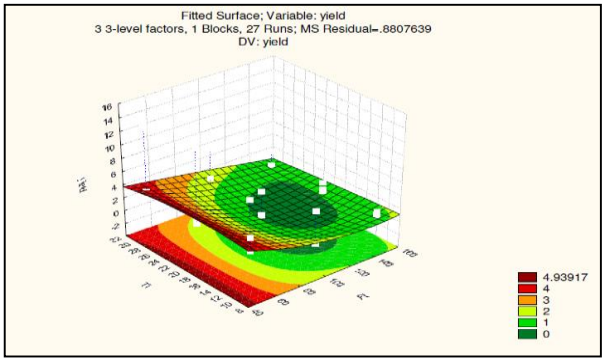


Figure 7 Response reaction time to solvent/meal ratio on yield of protein extraction from fermented seed with solvent concentration at 8%

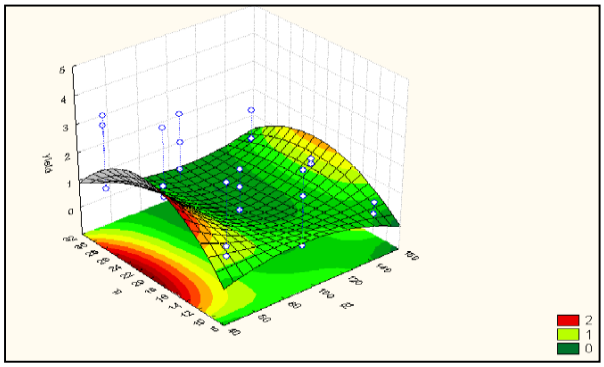


Figure 8 Response reaction time to solvent/meal ratio on yield of protein extraction from non fermented seed with solvent concentration at 8%

The highest protein yield was at 4.9% for fermented seed and 2% for non-fermented seed at the ratio solvent /meal of 40:1. The same case affected the ratio of meal per solvent where the protein yield decreased when the ratio was increased. Meanwhile, the reaction time did not significantly influence the protein yield. This is in agreement with the previous reports of protein extraction from watermelon seed, tomato seed meal and flaxseed and pigeon pea [8, 13, 14].

4.0 CONCLUSION

Response surface methodology is an effective way to organize and analyze experimental processes involving variables. The statistical analysis from the reaction time, solvent concentration and ratio of solvent/meal to the response of protein yield has been examined and was found to fit the third-order polynomial models with R^2 is 0.98 and 0.91 for fermented and non-fermented Perah seed respectively. Graphical response surface and contour plots shows that the best condition for protein yield extracted for both fermented and non-fermented Perah seed is at 40:1 for solvent/meal ratio, 5.5% of solvent concentration and at 32 minutes of reaction time with a maximum protein yield of 18.0 g/100 g and 5.0 g/100 g respectively. Solvent concentration and ratio of solvent/meal showed the most influence variable in protein yield extract from the

fermented seed. These results will contribute to the information of the productivity and quality of the protein yield extracted.

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