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Protein Extraction from Palm Kernel Meal

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Abstract: Palm Kernel Meal (PKM) is a byproduct of palm kernel oil industry. In the last few years, Malaysia produced over two million tons of PKM annually and the byproduct is available throughout the year. Palm kernel meal contains biomaterials such as protein, cellulose and organic acids. Generally, palm kernel meal contains about 20% protein. Palm kernel protein can be extracted and purified and used as animal feed supplement or as raw material for other processes. However, with the current trend of green processes and products, palm kernel protein can be utilized for the production of formaldehyde- free wood glue. Palm kernel protein based wood glue is non-toxic as compared to conventional wood glue which is based on melamine-urea-formaldehyde resin. The extraction and utilization of palm kernel protein will definitely enhance the current usage of palm kernel meal. In this study, palm kernel protein extraction and purification were studied. The extraction of palm kernel protein was conducted using saline and alkali treatment method. For saline treatment, the extraction of protein was done under various conditions such as variation of solvent to palm kernel meal ratio, pH and salt concentration. For alkaline treatment, variation of solvent to palm kernel ratio, extraction time and extraction temperature was applied. Central composite designs of response surface methodology were used for identification of the best condition and extraction yield optimization. Result shows that over 80% of palm kernel protein can be extracted. Alkaline treatment produces better extraction yield compared to saline treatment.

Key words: Palm kernel protein, saline treatment, alkali treatment, central composite design

INTRODUCTION

Interest in alternative plant protein sources has grown due to the increasing demand in food and non-food industries. Since the soy protein, legume protein and wheat gluten protein are comparative attractive in food industry, the protein extraction from agriculture byproduct has received a lot of researchers attention in order to fulfill the demand of industries. There is a potential for conversion of agriculture byproducts into useful products or even as raw material for other industries. The utilization of agriculture byproduct as a source of functional ingredients is a talented field which indirectly solve the environment problems caused by disposal of agricultural byproduct.

Palm Kernel Meal (PKM) is a byproduct of palm kernel oil extraction from the meat in the nut of the palm oil fruits, Elaeis guineensis. Malaysia produces 2.15 million tons of PKM per year (Malaysian Palm Oil Board, 2007). The crude protein content of PKM ranges from 14 to 21% (Boateng *et al*., 2008). Since PKM contain valuable crude protein content, it is natural to try to transform these byproducts into new and non-conventional source of proteins. The protein isolate produced from agriculture byproduct may be used in conventional applications of protein isolates, such as, protein fortification of processed foods, emulsification of oils and as body formers in baked products. Furthermore, the protein isolate may be formed into protein fibres, which is useful in meat analogs, probably as an egg white substitute, or as extender in food products. The PKM protein isolate may be used as nutritional supplements. Protein extracted can be substitute into chemical products to fabricate 'green' composites. This will probably increase the market value of PKM.

Since, extractable protein determines the amount of protein that can be made available from particular source for food and non-food application, one of the preliminary factors that determine whether or not a protein source could be adopted for commercial exploitation is the protein extraction efficiency of such protein (Liu, 1997). Protein extraction usually involves the use of acid, alkaline and saline solution (Eromosele *et al*., 2008). Different type of protein contained in the raw material will favor certain treatment.

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The effectiveness of protein extraction depends on the solubility of protein and other operating parameter such as pH, temperature, time, solid/solvent ratio, solvent type and extractant ionic strength.

In order to effectively and efficiently study the extraction processes, the results analysis and optimization process were based on Response Surface Methodology (RSM) technique (Arifin *et al*., 2007). Response surface methodology is a statistical-mathematical method which uses quantitative data in an experimental design to determine and simultaneously solve, multivariate equations, to optimise processes or products (Giovanni, 1983). The objective of this study was to perform the comparison between protein extraction using saline and alkaline solution.

The RSM's central composite design was utilized in order to study the effect of saline concentration, alkaline concentration, pH, liquid to solid ratio and temperature on the extracted protein concentration and on the percentage of protein recovery.

MATERIALS AND METHODS

Palm Kernel Meal (PKM) preparation: Palm kernel (Elaeis guineensis var. tenera) was provided by Borneo Samudera Lumadan mill, Beufort, Sabah. After being washed and then dried at 60°C in oven for 16 h, the palm kernel was ground to pass through 0.3-0.425 mm mesh sieve to obtain fine powder. The oil of palm kernel was extracted with iso-propanol in a soxhlet extractor for 8 h and the process repeated in order to ensure the oil was fully removed from the powder. The oil free Palm Kernel Meal (PKM) fiber was then air-dried and stored in refrigerator.

Proximate analysis: The protein content of PKM samples were determined by Kjeldahl method. The crude protein content of each sample was calculated by multiplying the nitrogen content with a factor of 6.25.

Experimental design: The experimental design was conducted using Design Expert software (version 6.10, Stat Easy Inc., Minneapolis, USA). For saline treatment, the effect of three independent variables i.e., NaCl concentration, pH and solvent to meal ratio were investigated. The constraint of component proportion is shown in Table 1. For alkaline treatment, the effect of three independent variables temperature, NaOH concentration and liquid to solid ratio were investigated. The range of the variables is shown in Table 2.

Protein extraction: Protein extraction method from PKM was modified based on Wani *et al*. (2008). Ten grams of PKM was used to extract the protein in conjunction of different levels of independent variables. The protein extraction was carried out with saline or alkaline solution in water-jacketed bottles which is connected to a temperature controlled water bath. The solution was continuously stirred using a magnetic stirrer for a selected period of time. The supernatant was then filtered through Whatman filter paper No. 1. The supernantants were then dried in an oven at 50°C. The soluble protein content was determined accordingly.

Table 1: Constraint for saline treatment

Parameters	Low limit	High limit
NaCl concentration (M)	0.2	0.4
pH	7	9
Solvent/meal ratio (g g^{-1})	40	60

Table 2: Constraint for alkaline treatment

Parameters	Low limit	High limit
NaOH concentration (M)	0.03	0.06
Temperature (°C)	35	45
Liquid/solid ratio (g g^{-1})	30	50

RESULTS AND DISCUSSION

The experimental results obtained for protein yield, percentage of protein recovery, NaCl concentration, pH and solvent/meal ratio, NaOH concentration and effect of temperature using the proposed experimental design was presented in Table 3 and 4, respectively. The predicted values of protein yields were calculated using regression model and compared with experimental values. The most compatible estimation model among the mean, linear, quadratic and cubic expressions of each response variable were identified based on all the statistical analysis which includes sequential model sum of squares, lack of fit tests and the model summary statistics.

Saline treatment

Relationship between variables and concentration of extracted protein: Results in Table 3 show that the experimental concentration of extracted protein varied from 9.4 to 32.9%. In general, the best results in terms of protein extraction in filtrate were obtained using low NaCl concentration and relatively high pH and solvent/meal ratio. (32.9% of protein in run No. 9 and 27.3% of PKM in run No. 2).

The statistical model, representing the concentration of extracted protein as a function of the independent variables within the region under investigation, can be expressed by the following quadratic equation:

$$\text{Concentration of extracted protein} = 12.92 + 0.29A - 6.03B - 1.83C + 0.12A2 + 2.86B2 + 1.05C2 - 1.56AB - 0.36AC + 0.85BC$$

Table 3: Experimental design with the respective and their response function in the extraction of protein from PKM

		Variables (actual level)		Response function	
Run	pH	NaCl concentration (M)	Solvent/Meal ratio	Concentration of extracted protein (% g protein/g filtrate)	Percentage of protein recovery (%)
1	8	0.3	50	12.5571	32.22
2	9	0.2	40	27.3114	74.44
3	8	0.3	50	12.6459	33.39
4	8	0.3	50	12.4241	32.09
5	8	0.3	50	12.5975	32.80
6	9	0.4	40	11.4963	28.39
7	8	0.3	66.82	12.6497	69.72
8	7	0.4	60	11.5969	81.98
9	8	0.13	50	32.9406	84.95
10	9	0.4	60	9.3917	49.62
11	8	0.3	33.18	19.9421	47.76
12	8	0.47	50	9.8866	53.10
13	6.32	0.3	50	14.3444	49.57
14	7	0.2	60	17.7685	54.03
15	8	0.3	50	14.4056	47.75
16	8	0.3	50	12.7450	34.47
17	9	0.2	60	21.6323	88.38
18	7	0.4	40	12.4202	37.81
19	7	0.2	40	21.8213	35.87
20	9.68	0.3	50	12.9715	35.83

Table 4: Experimental design with the respective and their response function in the extraction of protein from PKM

		Variables (actual level)			Response function	
Run	pH	Temperature (°C)	NaOH concentration (M)	Solvent/Meal ratio	Concentration of extracted protein (% g protein/ g filtrate)	Percentage of protein recovery (%)
1	11.73	40	0.04	40	10.1561	16.4409
2	11.74	40	0.04	60	7.6855	15.6894
3	11.69	40	0.04	40	10.3913	18.8422
4	11.55	35	0.03	50	14.2373	21.6043
5	11.71	40	0.04	40	10.6741	18.5253
6	11.67	40	0.04	40	11.5155	19.0386
7	11.71	45	0.03	50	36.5843	67.6908
8	11.85	35	0.06	50	5.1618	10.4731
9	11.67	40	0.04	20	30.8228	44.2004
10	11.81	35	0.06	30	34.2125	65.3744
11	11.70	50	0.04	40	31.4215	59.6567
12	11.97	45	0.06	50	18.6105	41.5003
13	11.71	40	0.04	40	11.7912	18.1708
14	11.62	45	0.03	30	30.4446	43.3928
15	11.82	40	0.07	40	17.1983	36.1413
16	10.96	40	0.02	40	38.3036	69.4269
17	11.84	30	0.04	40	32.6500	62.1334
18	11.64	35	0.03	30	39.9822	74.7736
19	11.94	45	0.06	30	12.1079	21.7767
20	11.71	40	0.04	40	10.4676	15.2971

where, A, B and C were the coded variables for pH, concentration of NaCl and solvent/meal ratio respectively.

Figure 1 shows values of concentration of extracted protein by varying pH and NaCl concentration while fixing the values of solvent/meal ratio at 50:1. The variation in pH and salt concentration revealed that maximum concentration of extracted protein was obtained at pH of 9 and NaCl concentration was 0.2M. Even though an increase in pH increased the concentration of extracted protein, but as NaCl concentration increase, the reverse effects take place.

If the salt concentration was kept at 0.30 M and the solvent/meal ratio was reduced to 40:1 (Fig. 2), then, the maximum concentration of extracted protein was obtained at pH equal 9. The results revealed that increase in pH shown an increasing trend for concentration of extracted protein while an increase in solvent/meal ratio showed a decreasing trend for extractable protein.

The results also revealed that increase in NaCl concentration and solvent/meal ratio resulted in a decreasing trend for concentration of extracted protein. Maximum concentration of extracted protein was

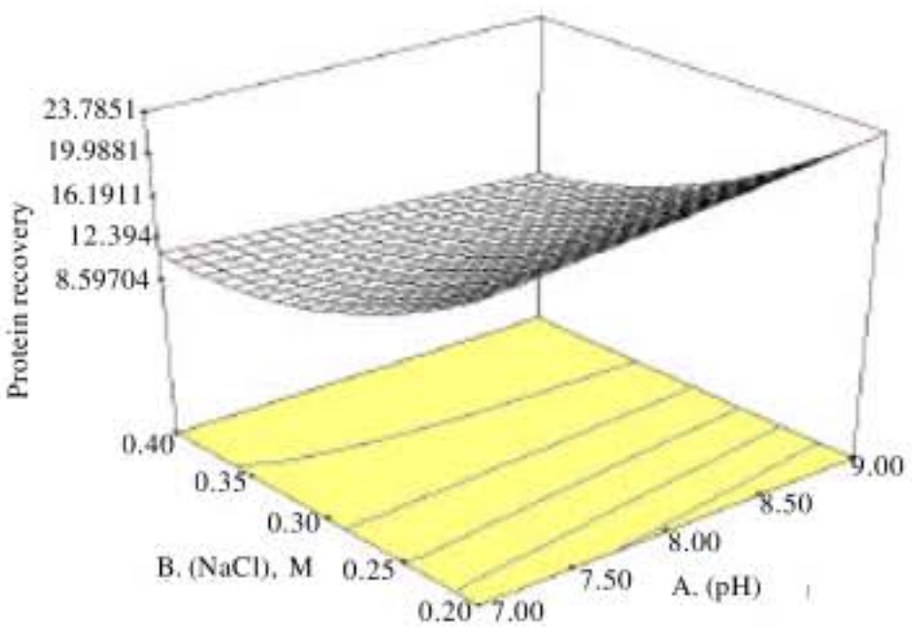

Fig. 1: Effect of pH and NaCl concentration on concentration of protein extracted

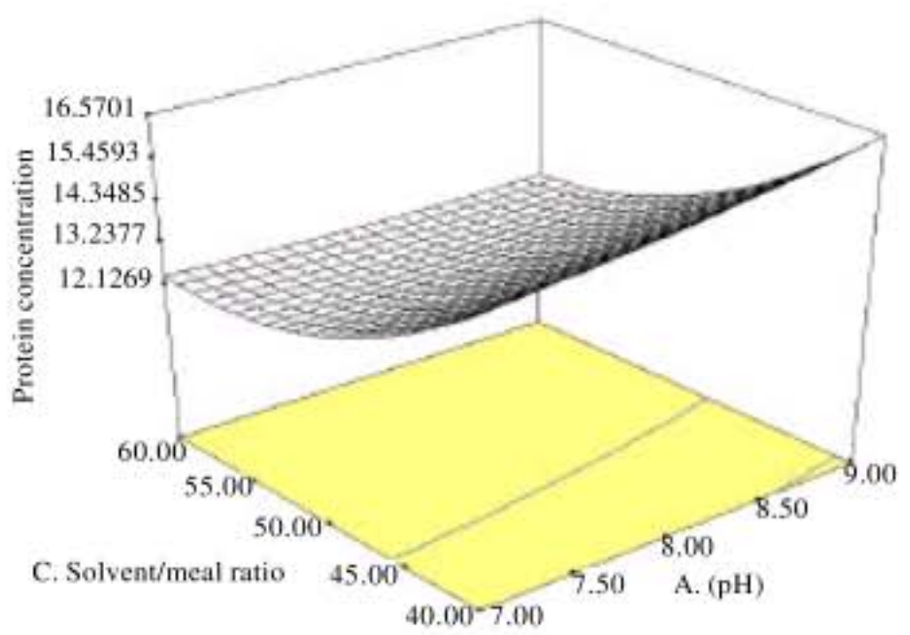

Fig. 2: Effect of pH and solvent/meal ratio on concentration of protein extracted

obtained when NaCl concentration was kept at 0.2 M and solvent/meal ratio was 40:1, while pH was kept at 8 (Fig. 3).

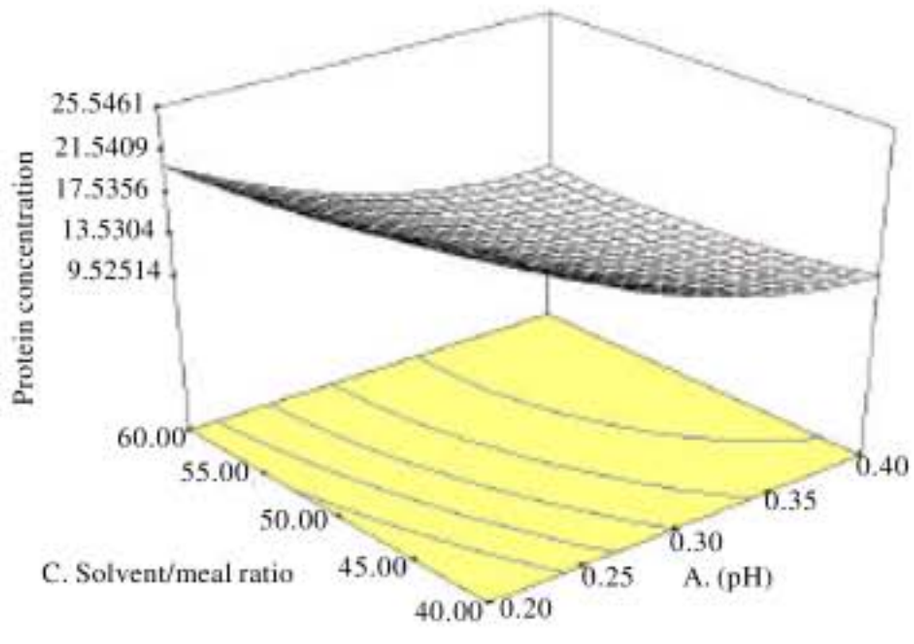

Fig. 3: Effect of NaCl concentration and solvent/meal ratio on concentration of protein extracted

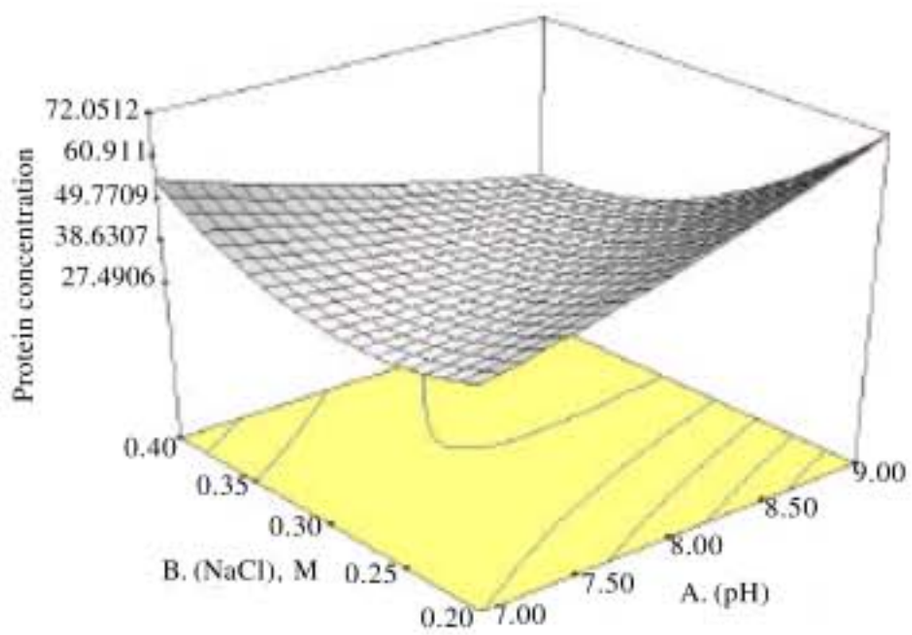

Fig. 4: Effect of pH and NaCl concentration on percentage of protein recovery

Relationship between variables and percentage of protein recovery: Table 3 shows that the experimental protein recovery varied from 28.4 to 88.4%. As can be seen, in general, the best results in terms of protein extraction in filtrate were obtained at the region of low NaCl concentration and relatively high pH and solvent/meal ratio. (88.4% of protein in run No. 17 and 84.95% of PKM in run No. 9).

The statistical model, representing the percentage of protein recovery from palm kernel meal as a function of the independent variables within the region under investigation, can be expressed by the following quadratic equation:

$$\text{Percentage of protein recovery} = 35.51 + 0.59A - 7.94B + 9.84C + 2.18A2 + 11.49B2 + 7.85C2 - 14.34AB - 3.40AC + 4.16BC$$

where, A, B and C were the coded variables for pH, concentration of NaCl and solvent/meal ratio, respectively.

Figure 4 shows values of percentage of protein recovery by varying pH and NaCl concentration while fixing the values of solvent/meal ratio at 50:1. Variation in pH and salt concentration revealed that maximum concentration of extracted protein was obtained when pH was 9 and NaCl concentration was 0.2M. An increase in pH at low NaCl concentration increased the percentage of protein recovery while concentration of extracted protein decreases as NaCl concentration increase at high pH.

Maximum percentage of protein recovery was obtained when pH was 7 and solvent/meal ratio was 60:1,

while salt concentration was kept at 0.30 M (Fig. 5). The results revealed that increase in pH and solvent/meal ratio shown an increasing trend for percentage of protein recovery.

The results revealed that an increase in NaCl concentration caused a decreasing trend of recovery whilst an increase of solvent/meal ratio shown an increasing trend for percentage of protein recovery. Maximum concentration of extracted protein was obtained when NaCl concentration was kept at 0.2 M and solvent/meal ratio was 60:1, while pH was kept at 8 (Fig. 6).

Considering all the responses, it is evident that pH, NaCl concentration and solvent/meal ratio had a significant effect on protein yield. Thus maximum concentration of extracted protein was obtained using 0.2M NaCl concentration, 60:1 w/w solvent/meal ratio and pH of 9.

Some researchers reported a broad pH range of minimum nitrogen solubility at pH 3.8-4.6, 3.0-6.0, 3.0-4.0 and 3.0-7.0 for defatted linseed, demucilaged, defatted dehulled linseed, defatted E. variegate and pumpkin seed flour, respectively (Dev *et al*., 1986; Jyothirmayi *et al*., 2006; Lazos, 1992; Madhusudhan and Singh, 1983). This trend follows the accepted protein monomer aggregation principle in which protein aggregates into an insoluble mass at the isoelectric point due to decrease in electrostatic charge repulsion between the particles as the net charge tends to zero. As the particles come closer, columbic forces between positive and negative charges of the protein residues; Van der Waals attraction and hydrogen bonding would then hold the mass together against dispersing forces. But as pH increases, the net negative charge increases and thus desegregation (solubility) progressively increases (Boulet *et al*., 2000).

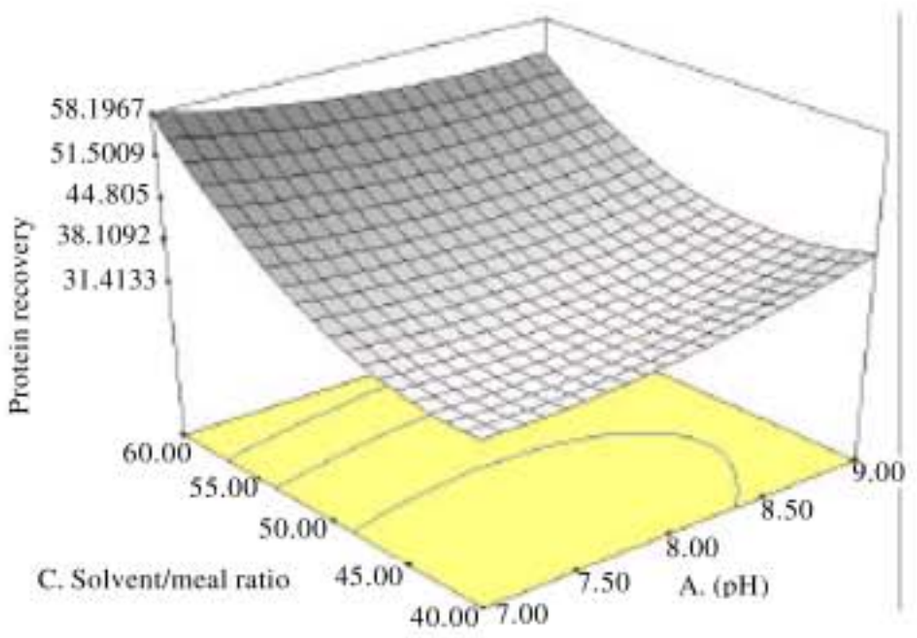

Fig. 5: Effect of pH and solvent/meal ratio on percentage of protein recovery

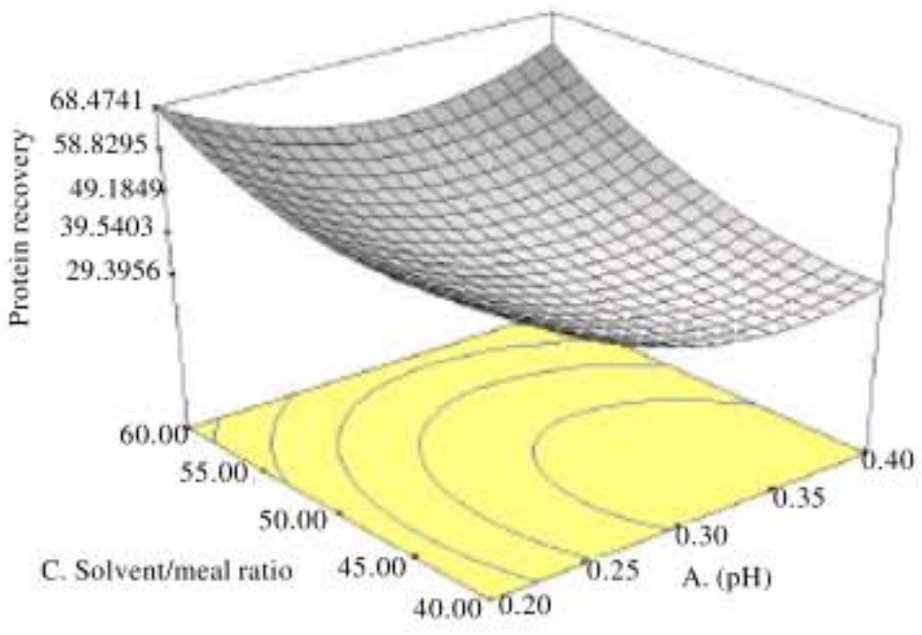

Fig. 6: Effect of NaCl concentration and solvent/meal ratio on percentage of protein recovery

Other researchers have reported a lower and a higher concentration range of NaCl solution other than that observed in this study. For extraction of vegetable protein; 0.3 M NaC was found to be optimal for winged bean and faba bean (Mccurdy and Knipfel, 1990; Okezie and Bello, 1989); 0.8M for tomato seed (Liadakis and Tzia, 1998), 1 M for coconut and sunflower (Chakraborty,1985; Venktesh and Prakash, 1993) and up to 2.0 M gave the optimal (82%) extractable sesame protein (Prakash, 1986). At low concentrations of salt, solubility of the proteins usually increases slightly (salting in). But at high concentrations of salt, the solubility of the proteins drop sharply (salting out). Proteins are surrounded by the salt counter ions (ions of opposite net charge) and this screening result in decreasing electrostatic free energy of the protein and increasing activity of the solvent, which in turn, leads to increasing solubility. On the other hand, at high salt concentrations, the abundance of the salt ions decreases the solvating power of the salt ions decreases the solubility of the proteins decreases and precipitation results.

Oomah *et al*. (1994) and Jyothirmayi *et al*. (2006) reported optimal protein extractability at a solid-liquid ratio of 1:40 and 1:30 from defatted flax seed and Erythrina variegate flour, respectively. Decrease in extractable protein was observed at higher ratio. Nilo-Rivas *et al*. (1981) also reported a decrease in nitrogen extractability when a large excess of solvent was employed. Other studies on winged bean, tomato seeds, flax seeds and pigeon pea proteins were in agreement with the current study that the increase in solvent/meal ratio and pH resulted in higher protein yield (Liadakis and Tzia, 1998; Wanasundara and Shahidi, 1996; Mizubuti *et al*., 2000).

Alkaline treatment

Relationship between variables and concentration of extracted protein: From Table 4, the extracted protein concentration was 5.1-39.9%. Independent variables were analyzed to get regression equations that could predict the response under the given range.

The statistical model, representing the concentration of extracted protein as a function of the independent variables within the region under investigation, can be expressed by the following quadratic equation:

$$\text{Extracted protein concentration} = 10.80 + 0.11A - 6.34B - 5.53C + 5.27A + 5.92B2 + 2.08C2 - 2.68AB + 8.43AC - 0.37BC$$

where, A, B and C were the coded variables for Temperature, NaOH concentration and liquid/solid ratio.

Figure 7 showed that varying NaOH concentration and temperature on extracted protein concentration when the liquid/solid ratio fixed at 50:1. The optimum condition for extracted protein concentration is 0.03 M, 35°C and liquid/solid ratio 50:1. This revealed that increase in NaOH concentration showed a decreasing trend however, increasing temperature showed a positive linear effect on extracted protein concentration.

The maximum value for extracted protein concentration was obtained when it is in 0.03 M, 30:1 ratio and 35°C (Fig. 8). This revealed that NaOH concentration has a decreasing trend however liquid/solid ratio has a negative linear effect on extracted protein concentration.

Hence, increasing temperature revealed a decreasing trend on extracted protein concentration at low liquid/solid ratio. However, it gave a proportional relationship with protein extraction concentration when liquid/solid ratio was high. Similar condition occurred when for increasing liquid/solid ratio. The optimum condition for protein concentration is 35°C, 30:1 liquid/solid ratio and 0.03 M (Fig. 9).

Relationship between variables and percentage of protein recovery: Table 4 showed that the percentage of protein recovery was 10.47% and maximum was 74.77%. The statistical model, representing the percentage of protein recovery from palm kernel meal as a function of the independent variables within the region under investigation, can be expressed by the following quadratic equation:

$$\text{Protein recovery} = 17.66 - 0.18A - 9.10B - 7.57C + 10.73A2 + 12.26B2 + 2.99C2 - 3.41AB + 19.01AC - 0.79BC$$

where, A, B and C were the coded variables for Temperature, NaOH concentration and liquid/solid ratio.

Figure 10 showed the relationship of NaOH concentration and temperature on percentage of protein recovery when the liquid/solid ratio fixed at 50:1. The maximum percentage of protein recovery is in 0.03 M, 45°C and 50:1 of liquid/solid ratio. The results revealed that increase in NaOH concentration showed a decreasing trend whereas increase temperature showed positive linear trend on protein recovery.

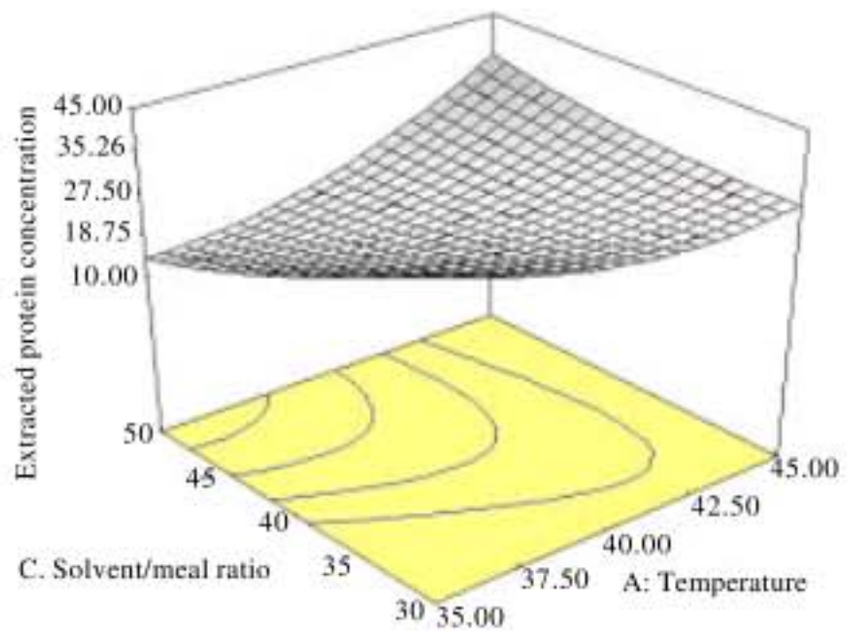

Fig. 8: Effect of NaOH concentration and liquid/solid ratio on extracted protein concentration.

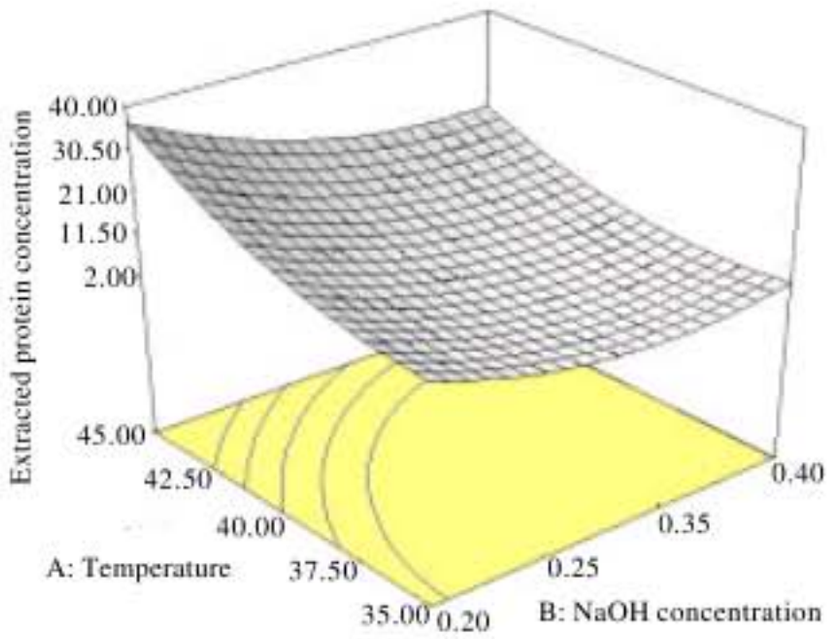

Fig. 7: Effect of NaOH concentration and temperature on extracted protein concentration

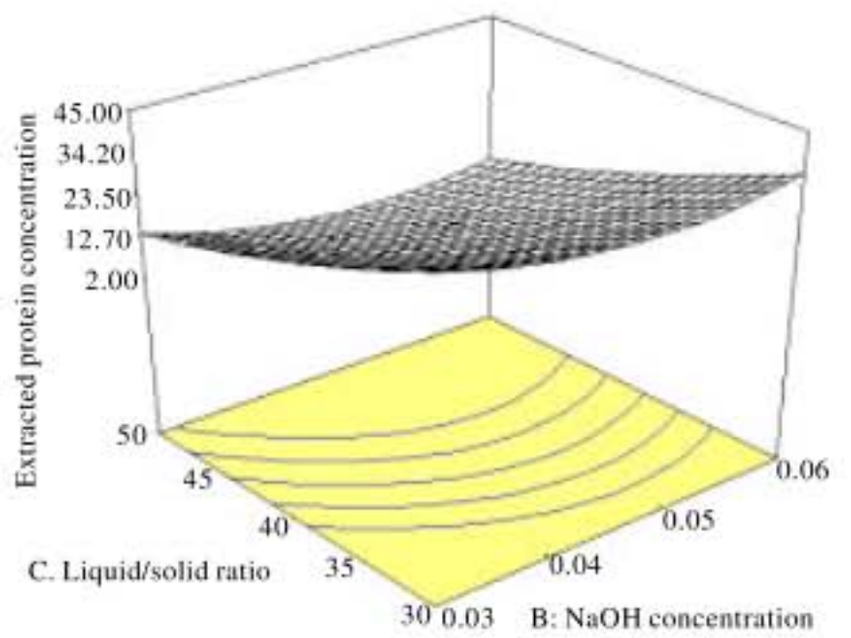

Fig. 9: Effect of temperature and liquid/solid ratio on extracted protein concentration

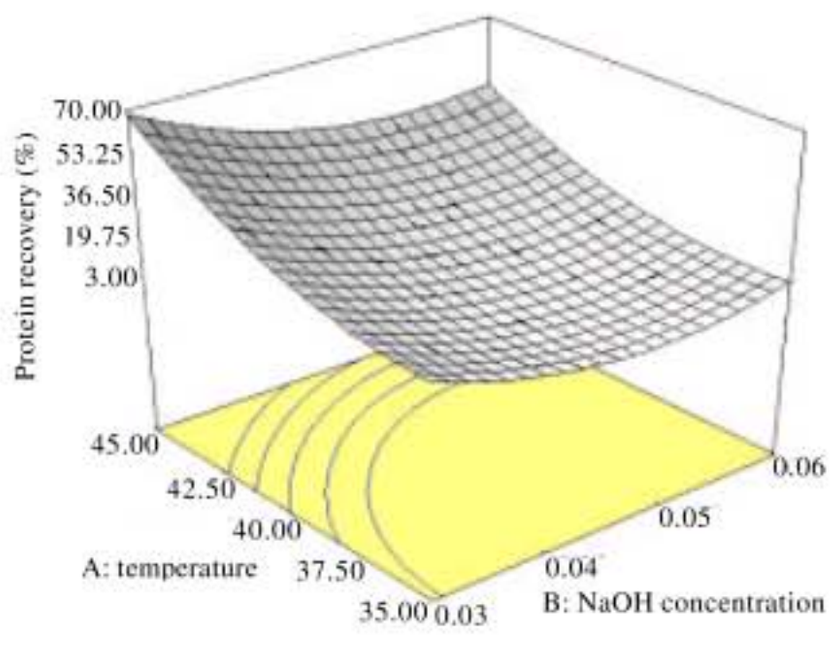

Fig. 10: Effect of NaOH concentration and temperature on percentage of protein recovery from PKM

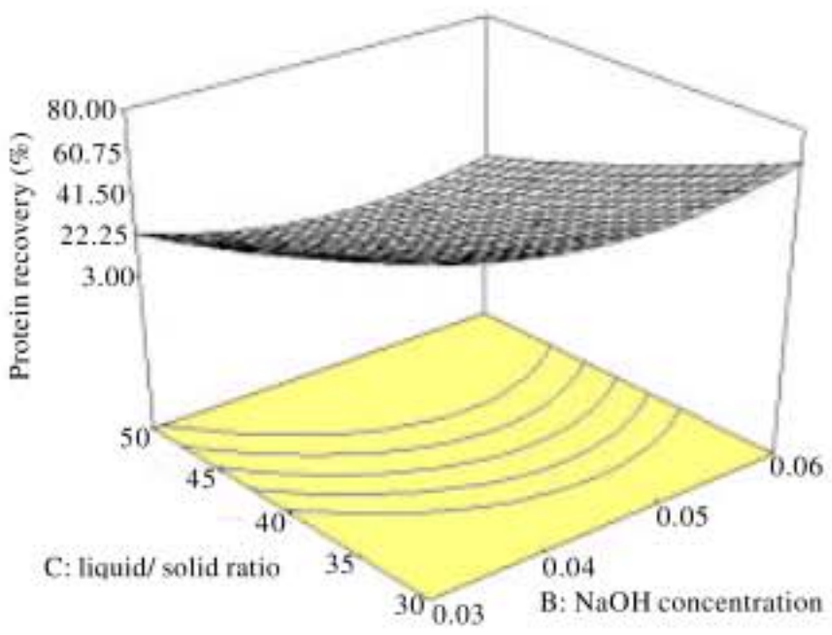

Fig. 11: Effect of NaOH concentration and liquid/solid ratio on percentage of protein recovery

The maximum percentage of protein recovery condition was in 0.03 M, 30:1 ratio and 35°C (Fig. 11). This results revealed that increase in NaOH concentration showed a decreasing trend whereas increase liquid/solid ratio gave a negative linear effect on protein recovery.

However, from Fig. 12, the optimum value of protein recovery was when the Temperature was 35°C, 30:1 ratio and 0.03 M NaOH concentration. This showed that increase in temperature showed a decreasing trend on protein recovery when at low liquid/solid ratio; increasing trend when liquid/solid ratio was high. Similar to liquid/solid ratio, it showed decreasing effect at low temperature and positive linear effect at high temperature on protein recovery.

By considering all the responses, it was evidenced that NaOH concentration and liquid/solid ratio had a significant effect on protein extraction yield. However, temperature showed less significant on PKM protein extraction in this particular range. The relationship of

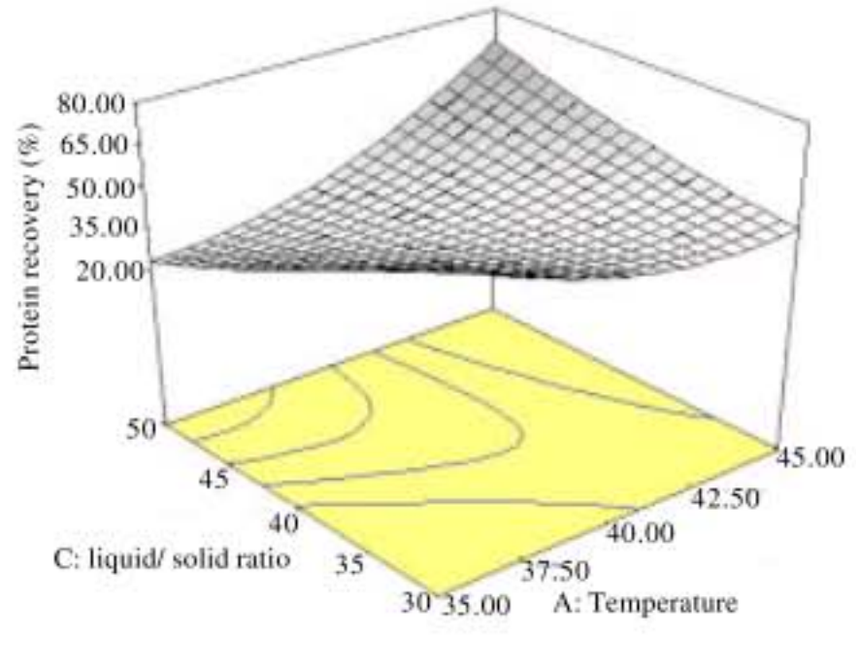

Fig. 12: Effect of temperature and liquid/solid ratio on percentage of protein recovery

these three variables on PKM protein extraction was proved and supported by other similar researches, which related to different plant or byproduct protein extraction.

For NaOH concentration, PKM protein extraction showed a decreasing trend when increase in concentration as proved by Wani *et al*. (2006) in her study indicating a decreasing trend as NaOH increased from 0.3 to 1.5%. Kain *et al*. (2009), stated protein solubility (%) was depending on pH and showed a gradual decreased as pH increase from 10 to 12. They also studied the peanut protein extractability on the effect of pH and proved that the yield decreases 84 to 78% when pH at 11 and 12. Eromosele *et al*. (2008) found that extractable African yam bean protein (%) decreased from 17.8 to 14.9% at 0.01 and 0.1 M NaOH. Similar report from Gonzalez-Quijada *et al*. (2003) showed an agreement that best extraction occurred at pH 8 to 11. Further increase in pH may cause decreased in yield.

For temperature effect, it can be shown by study of Shen *et al*. (2008) which studied the effect of various factors on tea protein extraction by using alkaline treatment. From his study, it is concluded that higher temperature would be beneficial for tea protein extraction. The most significant effect was observed between 35 to 40°C. It was in agreement with Ordonez *et al*. (2001) which revealed that temperature affected the result of extraction at pH 10. There was an increased of protein yeild from 13.95 to 17.83% when temperature were increase at 20 and 60°C.

Liquid/solid ratio relationship was indicated by Aguilera and Garcia (1989), stating that extraction methods involve the use of flour/solvent ratio between 1:5 and 1:30 have a significant relationship previous studies on extraction of watermelon seed protein also reported that the increase in solvent/meal ratio and NaOH

Table 5: Confirmation test for both treatments

Treatment	Extracted protein concentration (% g g^{-1})	Protein recovery (%)
Saline		
Experimental	25.81	78.59
Predicted	21.79	82.18
Alkaline		
Experimental	38.51	73.07
Predicted	41.21	75.30

concentration decreased protein recovery yield (Wani *et al*., 2008). Its optimum condition occurred at 70:1 solvent/meal ratio and 0.03 g L^{-1} NaOH concentration, while temperature and extraction time were kept at 50°C and 15 min. Similar research from Jyothirmayi *et al*. (2006) proved that optimal protein extractability at a liquid/solid ratio of 30:1 from defatted Erythrina variegata flour. According to Eromosele *et al*. (2008), there was decrease in extractable protein from African yam bean (Sphenostylis stenocarpa) when at higher ratio. This may be connected with the co-extraction of other components of the flour, which formed insoluble complex aggregate with the soluble protein.

Confirmative tests: For saline treatment, the optimization condition of independent variables (0.2 M NaCl, 60:1 solvent/meal ratio and pH 9) was carried out. However, for alkaline treatment, the optimization condition was selected at 0.03 M NaOH concentration, 35°C, 30:1 liquid/solid ratio. By referring to the Table 5, it showed that predicted value was slightly higher than experimental value. Therefore, the confirmative test validated the experimental results and the regression model.

By comparing both extraction methods, it was noticed that saline treatment was most suitable for PKM protein extraction, while NaOH treatment shows that PKM protein extraction was only suitable in low NaOH concentration (low pH). High NaOH concentration may denature the PKM protein.

CONCLUSION

For saline treatment, extracted protein concentration was in the range of 9.4 to 32.9% and its protein recovery was 28.39 to 88.38%. The optimized condition was at pH 9, 0.2 M NaCL concentration and 60:1 solvent/meal ratio. For NaOH treatment, the extracted protein concentration obtained was between 5.2-39.9% whereas percentage of protein recovery was 10.5-74.8%. The optimization condition for alkaline treatment was at 0.03 M NaOH concentration, 35°C, 30:1 liquid/solid ratio. In comparing both methods, it was noticed that saline treatment was most suitable for PKM protein extraction. NaOH treatment shows that PKM protein extraction was only suitable in low NaOH concentration (low pH). High NaOH concentration may denature the PKM protein.

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