ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 6 (4): 447-455, 2007
ISSN 1682-296X

The use of Response Surface Methodology in Predicting Sesame (*Sesamum indicum* L.) Protein Extractability with Water and the Analysis of the Protein Extracted for it Amino Acid Profile

[1,2]Philip John Kanu, [1]Hui Ming Zhou, [2]Jestina Baby Kanu,
[1]Ke-Xue Zhu, [1]Ke-Rui Zhu and [1]Haifeng Qian
[1]State Key Laboratory of School of Food Science and Technology,
Jiangnan University, Lihu Road No. 1800, Wuxi, 214122 Peoples's Republic of China
[2]Milton Margai College of Education and Technology-Affiliated to the University of Sierra Leone,
Goderich Campus, Freetown, Sierra Leone

Abstract: The aim of the study was to investigate the effects of pH, temperature, extraction time and flour/water ratio, monitored the combined effects of the above parameters through the use of Response Surface Methodology (RSM) during sesame seeds (*Sesamum indicum* L.) protein extraction, analyzed the highest protein recovered from the extraction for it amino acid profile. All the four parameters have influence on the extraction process of sesame seeds protein when they were varied individually and combined. The maximum recovery of protein during the extraction process was 94% at 12, 45°C, 45 min and 6/100 g mL^{-1} (w/v) of pH, temperature, time and ratio of sesame flour to water, respectively. The recovery was described by the model relationship given as Recovery (R) (extraction) = 51.33 + 2.75A + 1.25B + 2.00C - 3.58D +8.02A^2+ 2.27B^2+ 4.15C^2- 0.48D^2+ 4.50AB + 0.000AC-1.00AD-1.00BC -1.00BD -0.75CD. The model showed good fit, since the R^2 indicated that 96.47 % of the variability within the range of values studied could be explained by the model. The analysis of amino acids revealed the essential amino acids present in the highest extract were methionine, lysine, isoleucine, leucine, threonine, histidine, phenylalanine and tryptophan as 3.6, 2.6, 3.4, 7.1, 3.6, 2.2, 4.6 and 2.1, respectively. The extraction process was successfully done as RSM provided a good method in extracting protein from sesame seeds and obtains the optimum extraction conditions with manageable experimental runs and thus saved time and resources.

Key words: Sesame, protein extraction, response surface methodology, amino acid

INTRODUCTION

Plant protein plays significant roles in human nutrition, particularly in developing countries where the average protein intake is less than that required (Makri *et al*., 2005). Because of inadequate supplies of food protein, there has been a constant search for legumes and oil seeds, as new protein sources, for use as both functional food ingredients and nutritional supplements (Onweluzo *et al*., 1994). Plant protein products are gaining increased interest as ingredients in food systems throughout many parts of the world particularly in Africa; the final success of utilizing plant protein as additives depends greatly upon the favorable characteristics that they impart to foods. Therefore, the interrelationships of protein quality and processing parameters, that affect the amino acids and the functional performance of protein products, is worthy of an extensive investigation. Unlike soybean and allied oilseed proteins, the protein from other oil seeds has not been studied in great detail. However, these oil seeds like sesame play a significant role in the diets of a majority of the world's population particularly in Asia and Africa (Salunkhe *et al*., 1991). There is the need to understand details of the biosynthesis, physicochemical and functional properties and changes in processing of the proteins from sesame.

Sesame seed is one of the world's most important and oldest oilseed crop known to man (Suja *et al*., 2004). Sesame, also known as Sesamum, gingelly, benniseed, simsim and till, is an important annual oilseed crop. It has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein (Salunkhe *et al*., 1991). India, China, Sudan and Burma are

Corresponding Author: Hui Ming Zhou, State Key Laboratory of School of Food Science and Technology, Jiangnan University, Lihu Road No. 1800, Wuxi, 214122 People's Republic of China
Tel: +86-51085912709 Fax:+86-510 85864291

the major producers of sesame seeds, contributing approximately 60% of the total world production (Salunkhe *et al*., 1991).

Factorial design of a limited set of variables is advantageous in relation to the conventional method of the manipulation of a single parameter per trial since as such an approach frequently fails to locate the optimal conditions for the process due to its failure to consider the effect of possible interaction between factors. The factorial design also makes it possible to take advantage of practical knowledge about the process during the final response surface analysis (Cheison *et al*., 2007). RSM has important applications in the design, development and formulation of new products, as well as in the improvement of existing product design. RSM is a useful tool applied towards the optimization of several food processing operations (Bas and Boyac, 2007).

The chemical and physicochemical studies of sesame seeds available in the literature are mostly concerned with its solubility at different pHs, oil extraction and the antioxidant properties of sesame oil and the seed coat (Khalida *et al*., 2003; Lee *et al*., 2002; Shahidi *et al*., 2006; Suja *et al*., 2004). Information on the combination of various important factors that interplay in the aqueous extraction and characterization of sesame protein is scarce. There are, however, a number of reports on the isolation and fractionation of other oil seeds such as soya bean, cowpea, broad beans and pigeon peas (Lawal, 2004; Makri *et al*., 2005; Sathe and Salunkhe, 1981).

Thus, the objectives of present study were to investigate the effects of pH, temperature, time and ratio to water individually, monitored their effects when combined through the use of response surface methodology during sesame protein isolation. Analyzed the highest protein recovered from the extraction for it amino acid profile.

MATERIALS AND METHODS

Materials: White dehulled sesame seeds (*Sesamum indicum* L.) were purchased from a supermarket in Wuxi, People's Republic of China. The chemicals and reagents used were of food grade quality from the chemical store of Jiangnan University Wuxi, People's Republic of China bought from Sinopharm Chemical Reagent Co., Ltd. (SCRC) Shanghai People's Republic of China.

This research was conducted in the State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, People's of Republic China between September, 2006 to March, 2007.

Protein extraction: The dehulled sesame seeds were ground in a miller (DFY 600-20062103, YMAC. Co, Tokyo, Japan). The sesame flour was defatted using the soxhlet method with diethyl ether anhydrous for 8 h with 60 mL for every 10 g at 40-45°C. The procedure was repeated three times. The flour was sieved through a 100 mesh sieve. The defatted flour was stored in a 10°C reached in freezer until needed for further analysis.

The total crude protein (N×6.25), moisture and ash contents of the defatted flour were analyzed in triplicate using AOAC methods (1995). The carbohydrate content was estimated by subtracting the sum of percentage of moisture, fat, protein and ash contents from 100%.

Experimental design: Response surface methodology was used to determine the effect of protein recovery for pH (A: 4-12), temperature (T, B: 15-75°C), extraction time (t, C: 15-75 min) and the ratio of defatted sesame flour to water (DSF:W, D: 2-10 w/v) as the independent variables. The percentage of protein recovery during the extraction process was monitored as the dependent variable. Twenty seven experiments were carried out according to the model prescription as shown in Table 1. The optimization experimental design was done using RSM-Central Composite Rotable Design (CCRD) according to Myers and Montgomery (2002).

Table 1: The effect of the variables on the dependent response for the protein recovery using pH, temperature, time and ration of flour to solvent

pH	T (°C)	t (min)	DSF:W (g mL^{-1})	Recovery (%)
-1	-1	1	1	60
0	0	2	0	70
1	1	1	0	80
0	0	0	0	41
0	0	0	0	42
1	-1	1	0	74
-1	1	-1	1	55
1	1	-1	-1	88
0	-2	0	0	63
1	-1	-1	-1	57
-1	-1	-1	1	59
0	2	0	0	58
0	0	0	0	71
1	1	-1	1	60
1	-1	1	1	56
0	0	0	-2	43
-1	1	-1	-1	62
1	-1	-1	1	52
0	0	-2	0	66
0	0	0	2	56
-2	0	0	0	73
-1	1	1	1	57
1	1	1	1	67
-1	-1	1	-1	75
-1	-1	-	-1	69
-2	0	0	0	94
-1	1	1	-1	75

T = temperature, t = Time, DSF = Defatted Sesame Flour, W = Water
Note: The values under ratio of flour to water were dissolved in 100 mL of water for all of them

Table 2: Coded and natural units for the recovery of protein from defatted sesame flour variables and experimental design levels for response surface methodology

	Actual levels of factors			
Code	(A) pH	(B) T(°C)	(C) t (min)	(D) DSF:W(g mL^{-1})
+2	4	15	15	2
-1	6	30	30	4
0	8	45	45	6
+1	10	60	60	8
-2	12	75	75	10

T = temperature, t = time, DSF:W = Defatted Sesame Flour to water ratio, Coded = Represent the actual levels in the effect of the variables on the dependent response for the protein recovery

The variables as shown in coded and natural units (Table 2) were varied and studied at five levels. The pH was adjusted using 1.0 N NaOH and/or 1.0 N, HCl with a Hanna Precision pH meter (Model pH 212, SIGMA- USA). An electric steel stirrer (KIKA-WERKE KMO2 Produced by M-TEDIA- USA) was used to stir the solution of flour at a speed of 800 rpm in a 250 mL enzyme reactor bottle to the required working time according to the experimental design. After stirring, the suspension was centrifuged in a Beckman Coulter Centrifuge (Avanti J-26XPI, USA) at 4500 rpm for 20 min at room temperature (23-25°C). The supernatant was decanted and the precipitate was washed with deionized water (50 mL) and the solution brought to the working pH and stirred for 15 min then centrifuged again as described earlier.

Determination of protein recovery: Twenty seven experiments were carried out with the protein recovery experiment as the index. An aliquot of the supernatant was taken to determine the protein concentration in each of the supernatants by the Kjeldahl method (AOAC, 1995), using 6.25 as conversion factor:

$$R\,(\%) = \frac{N_s}{N_0} \times 100$$

Where:

R(%) = Percentage of protein recovery
N_S: = Amount of nitrogen in supernatant (mg).
N_0: = Nitrogen in initial sample (g).

Protein concentration was expressed as percentage of extracted protein recovered.

Determination of amino acids: The amino acids content from the highest extraction was determined according to the method described by Benjakul and Morrissey (1997) with minor modification. Sixty microliter of the defatted sesame protein isolate was mixed with phosphate buffer (pH 8.2) followed by 1.0 mL of 0.01% trinitrobenzenesulfonate (TNBS) solution. The samples were kept at 50°C for 30 min in the dark. The reaction was stopped by adding 2.0 mL of 0.05 M sodium sulphite and cooled to room temperature. Ampoules with sesame protein isolate suspension (0.5 mL) and 4.4 mL of 6 m HCl were vacuum sealed and heated at 100°C for 24 h.

The method utilizes *p*-toluenesulfonic acid as the catalyst for hydrolysis and the amino acid analyzer for the quantitative estimation of tryptophan the calculation was done according to Matheson (1974). The sample was filtered (Whatman No. 1) and neutralized with 6 M NaOH before amino acid determination. The amino acids were separated and quantified by injecting 50 μL of the sample into a Hitachi Amino acid Analyzer (Model 835) equipped with a 2.6×150 mm ion exchange column coated with resin 2619#. The column temperature was 53°C.

Statistical analysis: The experimental design and analysis of data were done using Design-Expert® Version 6.0.11 (State-Ease, Inc., Minneapolis, MN). The quadratic response surface analysis was based on the multiple linear regression analysis taking into account the main, the quadratic and the interaction effects, according to Eq. 1. As four factors were varied, 27 b-coefficients were to be estimated

$$R = \beta_o + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum\sum \beta_{ij} X_i X_j + E \qquad (1)$$

Where:

R = The measured response variable,
β_o, β_i, β_{ii}, β_{ij} = regression coefficients of the model,
X_i, X_j = The coded levels of independent variables i and j.

Based on RSM, this equation was used to evaluate the linear, quadratic and interactive effects of independent variables on the chosen response. For the model, the calculation from the linear and cross regression was performed. The R^2 value, the residual error, the pure error (calculated from the repeated measurements) and the lack of fit were calculated.

The results were also subjected to statistical analysis of variance (ANOVA), using a Statistical Analysis System (SAS Institute, Inc. 2002). The Significant of difference between means were determined by Duncan's Multiple Rang Test (DMRT), where $p<0.05$ was considered for significant difference.

RESULTS AND DISCUSSION

Effect of extracting conditions/factors: The defatted sesame flour was analyzed before the extraction process as shown in (Table 3), the results obtained was in accordance with previous results of Kahyaoglu and Kaya (2006), they studied the modeling of moisture, color and

texture changes in sesame seeds during the conventional roasting. The difference between their results and our results was not significant ($p<0.05$).

The preliminary extraction process to ascertain the effect of the extraction parameters individually (pH, temperature, time and ratio of sesame flour to water) was carried out and results are summarized in Fig. 1 a-d. In doing that, other factors were held constant only varying the parameter to be studied. This was done to know at what level to fix the various parameters to be studied when fixing the design. For a CCRD, it's good to work within a range. This will show the lower, mid and up limits of a parameter particularly when combined for the RSM.

The extraction effects of pH, temperature, time and ratio

Effect of pH: Extraction of sesame protein was affected by the pH (Fig. 1a) whereby the protein shows an isoelectric point between pH 4-5. Although sesame protein exhibits a characteristic pH dependent solubility, it is clear from Fig. 1a that even at the isoelectric point (pI) about 10-15% of protein was still extractable. This does not agree with the data previously reported for other seed protein (Lawal, 2004) who found that the oil seeds they studied showed a 5-10% of protein extraction within their pI and the difference was significant ($p<0.05$). But present results supported the report made by Khalida *et al.* (2003) they found a solubility of 9-15% while working with sesame seed as influenced by pH. Unlike sesame most of the plant protein reported like in the case of legumes, showed a protein extractability of only 10% or less at their isoelectric point (Makri *et al.*, 2005). This high extractability at the isoelectric pH may be due to the composition of the proteins since some proteins albumins and globulins may precipitate at the isoelectric pH (Khalida *et al.*, 2003).

Table 3: Chemical composition (%) of dehulled sesame flour

Protein	Fat	Carbohydrate	Moisture	Ash
19.3±0.52[a]	52.3±0.27[a]	17.7±0.08[b]	5.4±0.24[a]	5.3±0.50[b]

[a]: Values represent means of three replicates±SD; Means values with different letter (s) in the same column are significant at level ($p<0.05$)

Effect of temperature: Defatted sesame flour protein showed a characteristic extraction curve when the temperature was changed from low (20°C) to high (80°C) (Fig. 1b). The extraction was observed to increase from 20 to 40°C after that temperature a sharp fall of the protein

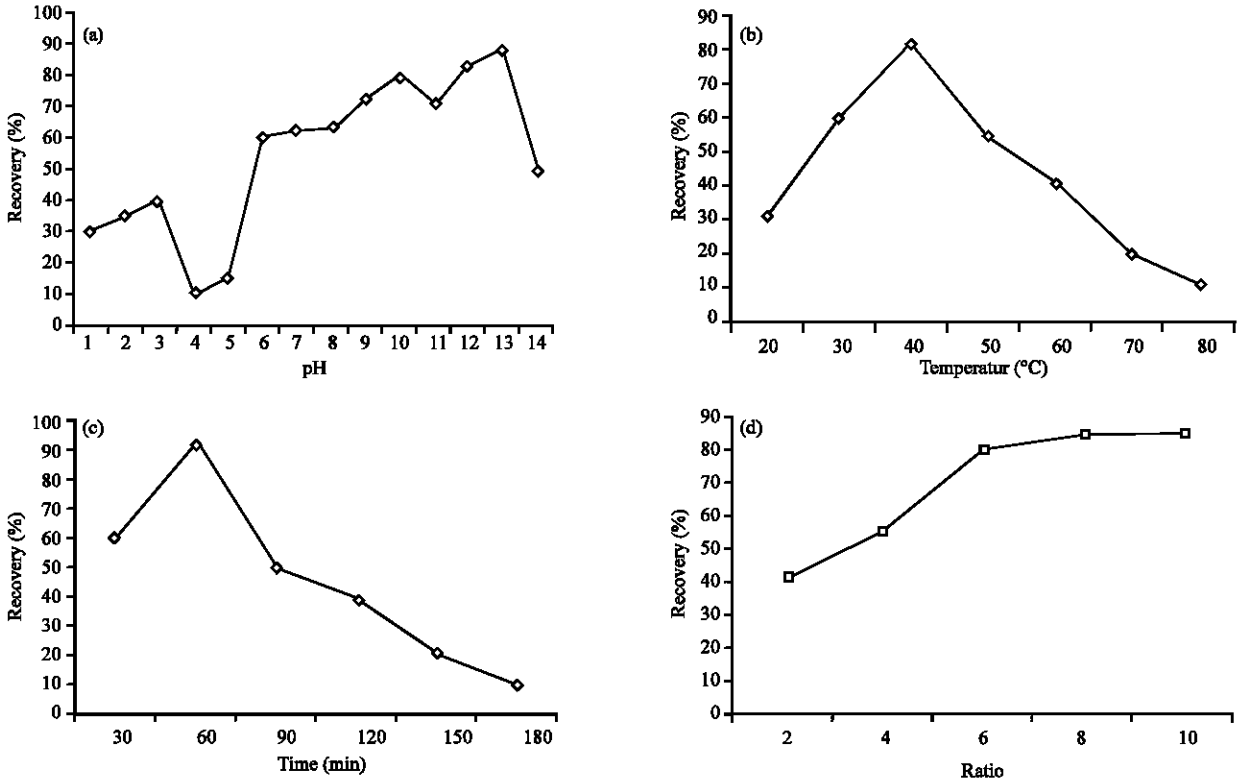

Fig. 1: The effect of (a) pH (b) temperature (c) time and (d) ratio of sesame flour to water, on protein extractability of defatted sesame flour

yield was noticed and the fall was significant ($p<0.05$). Protein extraction with temperature has been discussed with respect to whey proteins (De-Wit and Hontelez-Backx, 1988). Generally, at room temperature, the protein extraction is lower but increases with increasing temperature. Thus, the increase in protein extraction with increasing temperature may be related to the increased solubility. However, much higher temperatures lead to protein matrix rearrangement, denaturation and aggregation (Wilde and Clark, 1996) this will release more protein during the extraction process.

The highest extraction was observed when the temperature was 40°C and 81% was observed, our results supported the findings of Nilo-Rivas *et al.* (1981), they reported 83% of protein extraction at 40°C when studied nitrogen extractability of sesame seed and the preparation of two protein isolates. The sharp fall of the yield might be due to the fact that most plant protein could not withstand high temperature particularly above 45°C (Basha and Pancholy, 1982). When subjected to high temperature the protein structure will be denaturated. Hermansson (1972) reported a similar curve for a commercial soybean protein extract. She offers a physicochemical explanation for these changes, which could be extended to sesame protein. Thus, the sharp decrease in the recovery at high temperature may be due to the formation of ionic bonds (a) within the protein molecules and (b) between adjacent protein molecules leading to the formation of protein aggregates (De-Wit and Hontelez-Backx, 1988).

Effect of extraction time: The protein extraction ranged between a low amount of 10% of protein recovery obtained at time (180 min) to a high amount of 92% of protein recovery obtained at 60 min. Preliminary experiments using time as a factor revealed that extraction time had a very marked effect on protein extractability (Fig. 1c). The highest yield on protein extractability was obtained at 60 min, beyond which there was a reduction in the protein extracted and the decrease was significant ($p<0.05$). Present experiment corroborated with the research reported by Sefa-Dedeh and Stanley (1979) who found out that at 60 min they observed an extraction of cowpea protein of 90% their result was not significantly different ($p<0.05$) with present. The decrease which was significant ($p<0.05$) was observed in results experiment after 60 min might be due to the denaturation and coagulation of the protein arising from the formation of foam during the prolonged extraction. The data in Fig. 1c indicated that 92% of the total protein in sesame flour is extractable within 60 min during this experiment.

Effect of the flour: water ratio: The protein recovery extractable at the lowest ratio (2/100 w/v) was 40%. While the one obtained at the highest ratio (10/100 w/v) was 81% (Fig. 1d). The protein recovery observed when the ratio of defatted sesame flour to water was varied; 6 g of defatted sesame flour to 100 mL of water gave us a satisfactory recovery of 79% as compared to the other ratios. Interestingly the percentage of protein recovered increased when the ratio was increased but the increase was not significantly different ($p<0.05$) from the 6/100 w/v. As when the ratio was increased from 6 g to 8/100 w/v brought a difference of 2% in the recovery while a further increase to 10 g did not affect the recovery, it was still 81%. The slow increase of the recovery with further increase in the ratio might be due to the insufficient amount of the water which might be the water was not enough for dispersing and extracting the protein in the same amount of water. Previous extraction report used flour:water ratio (6/100 w/v) for watermelon seeds protein to supplement cowpea protein, reported 80% recovery of protein (Akpapunam and Markakis, 1981) which is very similar to present findings. Present results point to an optimum ratio of around 6/100 w/v for the extraction of sesame seed protein.

The above results from the individual parameters were used to fix up the range (lowest and highest) for the RSM, since CCRD was used, as it needs to have a range of the parameters to be investigated to give a good combination for the plotting of the effect by the model.

Response surface modeling: The effect of the variables on the dependent response for the protein recovery using pH, temperature, time and ratio of flour to water was analyzed through the model. The regression coefficient showed pH, temperature, time and ratio to be significant variables for the model. The predicted equation for recovery showed a good fit with the experimental design, since the R^2 of 0. 9647 and the Adj R^2 is 0.9469 indicated that 96.47% of the variability within the range of values studied could be explained by the model. The Lack of Fit (F-value) of 0.31 implies the Lack of Fit is not significant relative to the pure error which is good as the model is adequate for this particular experiment, all of those factors were calculated by the model. The values used were the ones shown in Table 1 as in the coded form.

Optimum conditions and the combined effects of the working parameters for extracting sesame protein: A regression equation ($R = 51.33 + 2.75A + 1.25B + 2.00C - 3.58D + 8.02A^2 + 2.27B^2 + 4.15C^2 - 0.48D^2 + 4.50AB + 0.000AC - 1.00AD - 1.00BC - 1.00BD - 0.75CD$) was obtained by fitting the results to predict the amount of

protein extractable under varying conditions according to the model using the coded values. The model explained a strong dependence of protein extractability on all the four factors as all of them showed a statistical significance ($p<0.05$). To illustrate the regression equation graphically, the independent variables pH, temperature, time and ratio (Fig. 2a and e), they were paired by the model to observe the recovery percentage of the protein. The combined effect of temperature and pH on protein extractability is shown in Fig. 2a which shows that when the temperature and the pH were increased the recovery also increased sharply. Our result was in agreement with other reports (Khalida *et al*., 2003; Prakash and Nandi, 1978) they reported on some oil and legume seeds, respectively to predict the extraction of protein. It implies that both the response and the two factors (%PR and T, pH, respectively) increased concomitantly. Intuitively, this is attributable to the high protein recovery depending on the increase of the pH and the temperature and hence the increased extractability. Conversely, reducing the pH and increase the temperature it will reduce the recovery of the protein.

When time and pH were combined (Fig. 2b) an increase in the recovery was also observed but when the time was longer than 60 min the increase was slower. The increase is seen to be in a linear after 45 to 60 min at pH 10-12. It was in agreement with Lawal (2004) who found that 45 min of extraction, 93% of protein recovery was observed when he studied the effect of pH in sesame solubility. For ratio and pH (Fig. 2c), it was observed that the increase of pH gave a positive increase in protein recovery but increase in the ratio brought out a low increase of protein recovery. Present findings supported the report of Krishna-Murti (1965) who studied the extraction of protein from sesame cake after an industrial extraction of the oil.

The combined effects of time and temperature was shown in (Fig. 2d) from which it was clear that an increase in the extraction time and temperature led to an increase in the protein recovery of sesame flour but according to (Fig. 2d) the two factors should not be prolonged beyond 60min and 60°C. Present result was in agreement with Onweluzo *et al*. (1994). It could be explained by the fact that when the extraction time is prolonged the probability of the protein mixture to foam due to the denaturation and coagulation of the protein matrix was high. At the same time, when the temperature is high and the extraction is carried out for a longer period a similar scenario will be true. So these two factors have to be fixed at (60 min for the time and 60°C for the temperature) when fixing them for the extraction of sesame protein. When ratio and temperature were combined for their effect, an increase of both gives a slow increase (8/100 w/v for the ratio and 60°C for the temperature) (Fig. 2e). Both the ratio and temperature axises were in a linear shape when both of them were increased. The increase of the two will not significantly increase the protein recovery percentage of the sesame flour. This could be explained by the effect of the high temperature on the protein leading to inactivation that will in turn lead to low extractability. Present result supported previous reports of other seed proteins and also sesame (Godfrey *et al*., 1976; Sefa-Dedeh and Stanley, 1979).

The combined effect of ratio and time revealed that an increase of recovery of protein was observed when the ratio was constant up to 60 min (Fig. 2f) after that time; the increase was not significant ($p<0.05$). Present result was conflicting with other previous reports on the effect of extraction time and ratio of flour to water on the percentage of protein recovered (Nath and Giri, 1957). Nath and Giri (1957), for instance, reported an optimum time of 120 min at a ratio of 6/100 g mL^{-1}. However present result was in accordance with Gheyassuddin *et al*. (1970) they reported on the extractability of sunflower protein.

Most of the parameters and the predicted results according to the model prescription were around the parameters that gave the highest results. The model gave the predicted results to assess if during the process an error was not made. To check the predictability of the regression equations, the extractions were repeated using the optimal conditions for example when pH 12.4, temp 44.7°C, time 45.9 min and ratio 6/100 w/v were estimated by the model to give a protein recovery of 93%. When those parameters were experimentally tested, they gave a protein recovery of 93.5%. The optimal conditions were calculated by the model. Ten duplicate experimental trials were made, the experimental values showed good agreement with the predicted values as the differences of those results were not significant ($p<0.05$). The regression analysis showed that using water, the maximum extractable protein was (94%) occurred at pH 12, at 45°C, 45 min and ratio of 6/100 w/v (Table 2). Thus, the regression equation was a useful tool in predicting the extractability of defatted sesame flour protein within the limits considered in the experimental design; it indicated that the extraction was quite successful.

The amino acid profile: The content of amino acids of the highest extracted sesame protein is shown in Table 4. The difference in the content of amino acids when compared with other results of previous reports was not significantly different ($p< 0.05$). The amino acids profile of the extracted protein was compared with other reports else

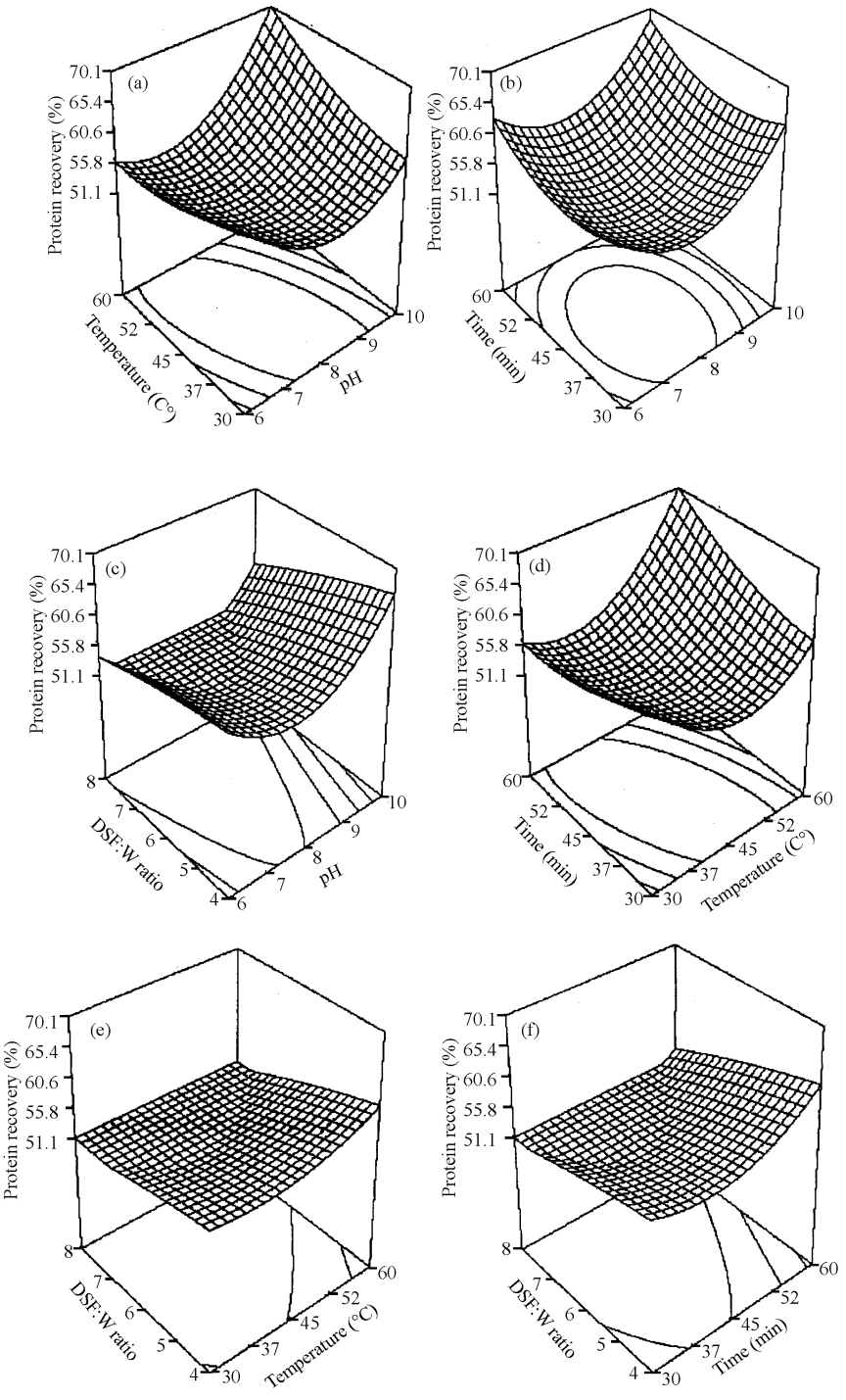

Fig. 2: The combined effect of (a) pH and temperature, (b) time and pH, (c) defatted sesame flour to water ratio and pH, (d) time and temperature, (e) defatted sesame flour to water ratio and temperature and (f) defatted sesame flour to water ratio and time the on extraction of protein from defatted sesame seed

where when they studied various legumes and some oil seeds (Godfrey *et al*., 1976; Joseph *et al*., 1962; Krishnamurthy *et al*., 1959; Nath and Giri, 1957; Prakash and Nandi, 1978). It was observed that the extracted sesame protein showed a high profile of the essential amino acids. In our results lysine, histidine, threonine,

Table 4: Total amino acid composition of sesame protein isolate compared with other reported values and FAO/WHO standard

	Composition in g/100 g of protein		
Amino acid	Present study	Reported values[a]	EAA[b]
Lysine	2.6	2.8-3.4	1.6
Histidine	2.2	1.7-2.5	1.6
Arginine	12.5	8.4-13.4	
Aspartic acid	8.4	4.3-8.6	
Threonine	3.6	2.2-3.9	0.9
Serine	4.1	2.2-4.7	
Glutamic acid	19.6	3.5-20.1	
Proline	6.3	1.0-3.0	
Glycine	5.1	2.9-5.4	
Alanine	5.0	3.3-5.0	
Valine	4.5	3.3-5.0	1.3
Methionine	3.6	1.6-3.8	1.7[c]
Isoleucine	3.4	2.8-4.2	1.3
Leucine	7.1	6.9-7.4	1.9
Tyrosine	3.7	3.5-3.8	
Phenylalanine	4.6	4.3-6.4	2.7
Tryptophan	2.1	2.0-2.5	1.4

[a]:*Source*: (Godfrey *et al*., 1976; Joseph *et al*., 1962; Krishnamurthy *et al*., 1959; Nath and Giri, 1957; Prakash and Nandi, 1978); [b]: Suggested profile of essential amino aid requirements for adults (FAO/WHO, 1990); [c]: Methionine + Cystine

Valine, methionine + cystine, isoleucine, leucine, phenylalanine and tryptophan were 2.6, 2.2, 3.6, 4.5, 3.6, 3.4, 7.1, 4.6 and 2.1, respectively in g/100 g of protein. The results showed that the amino acid profile of the sesame protein was generally higher with particular reference to the essential amino acid profile compared with the suggested pattern of requirement by FAO/WHO for human beings particularly infants (FAO/WHO, 1990) and the difference was significant ($p<0.05$). It shows that the method employed in the process to extract the protein from sesame did not affect the protein content since the protein extracted exhibited good amino acid content which is one of the indices to show the quality of the protein extracted particularly the high essential amino acids content, as one of the quality of any protein extract can be known on the amount of amino acids content present especially the essential amino acids.

CONCLUSIONS

RSM was successfully used in present research to study the combined effect of pH, temperature, extraction time and flour/water ratio. The critical extraction conditions were found to be pH (12), temperature (45°C), time (45 min) and ratio (6/100 w/v). The results indicated that RSM is a suitable tool for optima hunting in order to establish the conditions suitable for the extraction of sesame protein from defatted sesame flour. RSM provided immense savings on time and resources as the method assisted in the selection of specific combinations of the independent protein recovery variables in order to attain a desired level of % protein extraction. The model showed good fit for the experiment, as it was able to identify when combined all the parameters studied, the influence of those parameters in the extraction process thus made it possible for the extraction of sesame protein from defatted sesame flour. Sesame seed protein extracted could find potential use as supplements in various food formulations particularly in countries with limited protein supply for the people.

ACKNOWLEDGMENTS

The authors wish to thank the Governments of Sierra Leone and People's Republic of China for financially supporting this study.

REFERENCES

Akpapunam, M.A. and P. Markakis, 1981. Protein supplementation of cowpeas with sesame and watermelon seeds on the influence of flour meal to solution ratio. J. Food Sci., 46: 960-961.

AOAC, 1995. Association of Official Analytical Chemists. Official Methods of Analysis. 16th Edn., Washington, DC.

Bas, D. and I.H. Boyac, 2007. Modeling and optimization I: Usability of response surface methodology. J. Food Eng., 78: 836-845.

Basha, S.M. and S.K. Pancholy, 1982. Composition and characteristics of basic proteins from peanut (*Arachis hypogaea* L.). J. Agric. Food Chem., 30: 1176-1179.

Benjakul, S. and T. Morrissey, 1997. Protein hydrolysates from pacific whiting solid wastes. J. Agric. Food Chem., 45: 3423-3430.

Cheison, S.C., Z. Wang and S. Xu, 2007. Use of response surface methodology to optimize the hydrolysis of whey protein isolate in a tangential flow filter membrane reactor. J. Food Eng., 80: 1134-1145.

De-Wit, J.N. and G. Hontelez-Backx, 1988. Effects of various heat treatments on structure and solubility of whey protein. J. Dairy Sci., 67: 2701-2710.

FAO/WHO, 1990. Protein quality evaluation. Report of the Joint FAO/WHO Expert Consultation, Food and Agriculture Organization of the United Nations, Rome.

Gheyassuddin, S., C.M. Cater and K.F. Mattil, 1970. Effect of several variables on the extractability of sunflower seeds. J. Food Sci., 35: 453-456.

Godfrey, S.A.W., B.J. Francis and C.S. Kamara, 1976. The protein evaluation of cowpea (*Vigna unguiculata*) and benniseed (*Sesamum indicum*) from Sierra Leone. Trop. Sci., 18: 147-154.

Hermansson, A.M., 1972. The effect of temperature on soybean protein isolate. Wissenschart Technol., 5: 24-28.

Joseph, A.A., P.K. Tasker, K. Joseph, M.N. Rao, M. Swaminathan, A.N. Sankaran, A. Sreenivasan and V. Subrahmanyan, 1962. The net protein utilization and the protein efficiency ratio of sesame protein supplemented with Lysine to levels present in FAO reference protein pattern and milk. Ann. Biochem. Exp. Med., 22: 133-136.

Kahyaoglu, T. and S. Kaya, 2006. Modeling of moisture, color and texture changes in sesame seeds during the conventional roasting. J. Food Eng., 75: 167-177.

Khalida, E.K., E.E. Babiker and A.H. EL Tinay, 2003. Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. Food Chem., 82: 361-366.

Krishna-Murti, C.R., 1965. Sesame oil cake meal for the preparation of protein hydrolysate. Biotechnol. Bioeng., 3: 285-293.

Krishnamurthy, K., T.N. Ramakrishnan, R. Rajagopalan, M. Swaminathan and V. Subrahmanyan, 1959. The chemical composition and nutritive value of sesame seed (*Sesamum indicum* L.) and its products. J. Food Sci., 22: 316-320.

Lawal, O.S., 2004. Functionality of African locust bean (*Parkia biglobssa*) protein isolate: Effects of pH, ionic strength and various protein concentrations. Food Chem., 86: 345-355.

Lee, W.C., J.Y. Wen, C.H. Shiow and D.D. Pin, 2002. Antioxidant activity of sesame coat. Food Chem., 78: 347-354.

Makri, E., E. Papalamprou and G. Doxastakis, 2005. Study of functional properties of seed storage proteins from indigenous European legume crops (Lupin, pea, broad bean) in admixture with polysaccharides. Food Hydrocolloids, 19: 583-594.

Matheson, N.A., 1974. The determination of tryptophan in purified proteins and in feeding-stuffs. Br. J. Nutr., 8: 393-400.

Myers, R.H. and D.C. Montgomery, 2002. Response surface methodology: Process and product optimization using designed experiments. 2nd Edn., John Wiley and Sons, Inc. New York, pp: 798-803.

Nath, R. and K.V. Giri, 1957. Physicochemical investigations on indigenous seed proteins, Part III. Amino acid composition of sesame seed globulins. J. Sci. Ind. Res., 16C: 228-230.

Nilo-Rivas, R., E. Jane, P. Dench and C. Caygill, 1981. Nitrogen extractability of Sesame (*Sesame indicum* L.) seed and the preparation of two proteins isolate. J. Sci. Food Agric., 32: 565-571.

Onweluzo, J.C., Z.A. Obanu and K.C. Onuoha, 1994. Functional properties of some lesser known tropical legumes. J. Food Sci. Technol., 31: 307-310.

Prakash, V. and P.K. Nandi, 1978. Isolation and characterization of globulin of sesame seed (*Sesamum indicum* L.). J. Agric. Food Chem., 26: 320-323.

Salunkhe, D.K., J.K. Chavan, R.N. Adsule and S.S. Kadam, 1991. Sesame. In: World Oilseeds: History, Technology and Utilization. Van Nostrand Reinhold, New York, pp: 371-402.

Sathe, S.K. and D.K. Salunkhe, 1981. Functional properties of great Northern bean (*Phaseolus Vulgaris* L.) protein: Emulsion, foaming viscosity and gelation properties. J. Food Sci., 46: 71-81.

Sefa-Dedeh, S. and D. Stanley, 1979. Cowpea protein 1. Use of response surface methodology in predicting cowpea (*Vigna unguiculata*) protein extractability. J. Agric. Food Chem., 27: 1238-1243.

Shahidi, F., P.C.M. Liyana and D.S. Wall, 2006. Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chem., 99: 478-483.

Suja, K.P., J.T. Abraham, S.N. Thamizh, A. Jayalekshmy and C. Arumughan, 2004. Antioxidant efficacy of sesame cake extract in vegetable oil protection. Food Chem., 84: 393-400.

Wilde, P.J. and D.C. Clark, 1996. Foam Formation and Stability. In: Methods in Testing Protein Functionality. Hall, G.M. (Ed.), Blackie Academic and Professional, London, pp: 110-152.