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Evaluation of Aqueous Extraction Techniques for Isolating Proteins from White Sesame (*Sesamum indicum L.*)

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Protein extraction, Sesamum indicum, Extraction method, Food processing, Water-soluble proteins.

Introduction
Plant protein plays a significant role in human nutrition, particularly in developing countries where the average protein intake is less than required. Because of the inadequate supply of food proteins, there has been a constant search for unconventional legumes and oil seeds, as new protein sources for use as both functional food ingredients and nutritional supplements [1]. Plant protein products are gaining increased interest as ingredients in food systems in many parts of the world. However, the desirable characteristics like amino acid content, foaming properties, whipping, disperseability, and water and oil holding capacity, are all important in processing any protein isolate as these properties will increase the quality of the protein isolated [1].

Sesame seed (*Sesamum indicum L.*) is one of the world's most important and oldest oilseed crop known to man. It has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein [2]. Most of the sesame seeds are used for extraction and production of oil. The flour and meal from sesame seeds have provided a high protein source in formulated diets, particularly for children in Africa [3]. The potential functional uses of sesame protein have so far been limited by the low protein extractability from sesame flour by just varying one factor and fixing the others at a particular point to determine protein extractability.

The chemical and physiochemical studies on the nutritionally important proteins of sesame, available in the literature are mostly concerned with the extraction of the oil from the seeds, isolation of total protein by acid precipitation, its solubility at different pH and the antioxidant properties of sesame [4-7]. Information on the combination of various important factors like pH, temperature, time, and ratio using water for the extraction and characterization of sesame protein to analyze it amino acid content has been a limited area of research have been conducted. Response surface methodology (RSM) is a useful tool applied towards the optimization of several food processing operations [8]. RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables [9]. The objective of this work was to determine through the use of RSM the combined effects of pH, temperature (T), time (t), and defatted sesame flour/ water ratio (DSF: W) on protein extraction from defatted sesame flour and analyzed the amino acid content of the protein extracted, study determined the different protein content through protein fractionation, study the denaturation temperature for the protein fractions got.

Materials and Methods
Materials
White dehulled sesame seeds (Sesamum *indicum L.*) were purchased from a supermarket in Wuxi, China. The chemicals and reagents used were of food-grade quality and obtained from the chemical store of Jiangnan University Wuxi, People's Republic of China.

Methods
Protein Extraction
The sesame seeds were ground in a miller (DFY 600-20062103, Shanghai). The sesame flour was defatted using the Soxhlet method with Diethyl ether for 8 hours. The procedure was repeated three times. The flour was sieved through a 100mm mesh sieve. Moisture and ash content of the defatted flour were analyzed according to sections 2.2.2.1.2 and 2.2.2.1.3 respectively. The total crude

protein (N x 6.25) content of the defatted sample was determined according to section 2.2.2.1.1. The carbohydrate content was estimated according to section 2.2.2.1.4.

The defatted sesame flour was stored in a refrigerator at -10°C until needed for further analysis. The protein extraction was carried out by varying the four variables, that is, pH, temperature, time, and sesame flour/water ratio. The process is shown in Figure 1 as a flow chart. The defatted sesame flour was mixed according to the description of the design. The pH, temperature, time, and ratio of flour to solvent were adjusted according to the design. 1.0N NaOH and 1.0 N, HCl were used to control the pH for the experiments that were monitored by a Hanna Precision pH meter (Model pH 212). An electric steel stirrer (KIKA- WERKE KMO2 basic) was used to stir the solution of flour at a speed of 800rpm in an enzyme reactor bottle of 250 ml to the required working time from the design. After the stirring, the suspension was centrifuged in a Beckman Coulter centrifuge, USA (Avanti J-26XPI) at 4500 for 20 minutes at room temperature (23-25°C). The supernatant was decanted and washed with the precipitate with deionized water (50ml) and the solution was brought to the working pH and stirred for 15 minutes then centrifuged again as described above.

Determination of Protein Extraction (PE)

The protein recovered was determined using equation 1 after the extraction which was followed by the design generated by the model software. An aliquot of the supernatant was taken to determine the protein concentration in each of the supernatants according to section 2.2.2.1.1:

Amount of nitrogen in supernatant (mg)

$$PE\ (\%) = \frac{\text{Amount of nitrogen in supernatant (mg)}}{\text{initial sample (g)}} \times 100\% \quad \text{.....Eq. (1)}$$

N in initial sample (g)

Protein concentration was expressed as a percentage of extracted protein recovered.

Experimental Design

To establish optimal conditions for the extraction of protein from defatted sesame flour, response surface methodology (RSM) was used. Processing variables investigated were pH, temperature (T), time (t), and defatted sesame flour/ water ratio (DSF: W).

A Central Composite Rotatable Design (CCRD) with four factors and five levels was applied [9] as shown in Table 1. Five levels were adopted and coded -2, -1, 0, +1 and +2. % protein recovery (%PR) was the dependent variable. To predict the parameter of the mathematical model, a second-order multiple regression analysis using the least square regression methodology was made. The regression model between dependent variable (Y) and independent variables was according to the equation (2).

$$Y = \beta_0 + \sum_{i=1}^{i=3} \beta_i X_i + \sum_{i=1}^{i=3} \beta_{ii} X_i^2 + \sum_{i<j=2}^{i=3} \beta_{ij} X_i X_j + e \quad \text{.....Eq. (2)}$$

Y is the measured response variable;

β_0, β_i, β_{ii} and β_{ij} are the constant, linear, quadratic and cross-product regression coefficients of the model respectively. X_i and X_j represent the independent variables (extraction parameters). e is the random error.

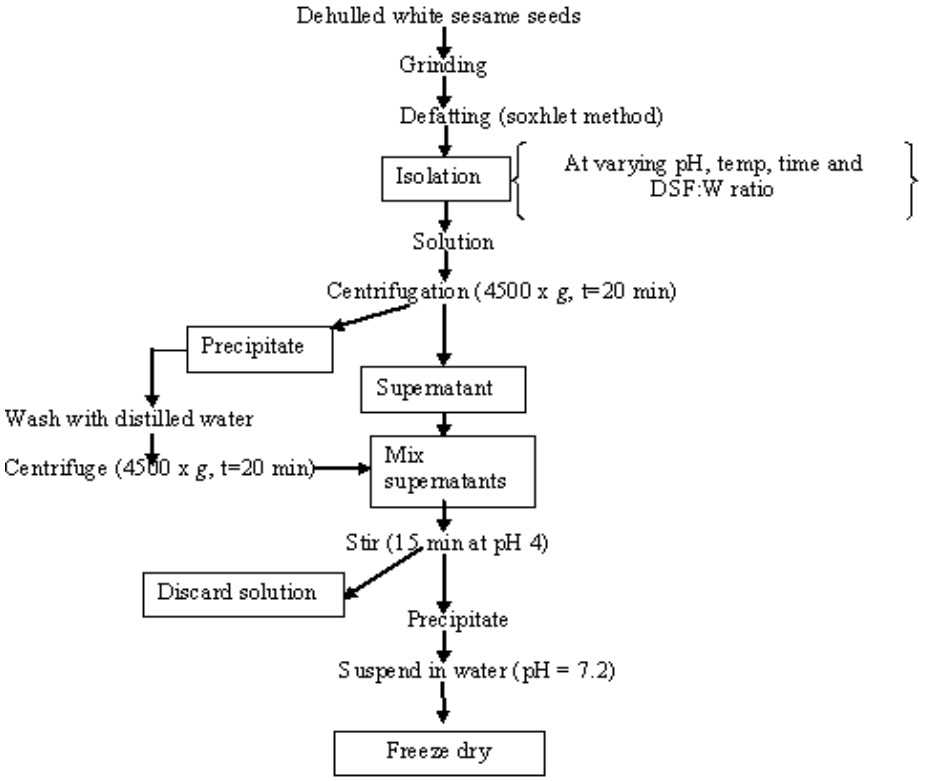


Figure 1: Schematic flow chart for the isolation and recovery of the sesame protein.

For the models calculated from the linear regression, analysis of variance (ANOVA) was performed using Design-Expert® Version 6.0.11 (State-Ease, Inc., Minneapolis, MN). The R^2 value was calculated.

Amino Acid Analysis

The protein extracted that gives the highest recovery was analyzed for amino acid content according to section 2.2.2.1.9.

Protein Fractionation

Protein fractionation was performed as described below;

Albumin (Water)

Suspensions of flour/water (1:10 w/v) stirred for 2h at room temperature and centrifuged at 8000rpm for 20 min. The supernatant was recovered and stored. The residues re-extracted with the same solvent and recovered supernatants combined and categorized as albumin fractions, the two combined supernatants were concentrated and freeze-dried.

Table 1: Experimental variables for the recovery of protein from defatted sesame flour (DSF) and experimental design levels for response surface methodology.

Code	*Actual factor levels*			
	pH	**T**	**t**	**DSF:W**
+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 (°C), t = time(minutes), DSF: W = ratio of defatted sesame flour to Water (g/ml)

Globulin (NaCl)

The pellet was re-suspended in a solution of 50 mM Tris-HCl, pH 8, containing 0.5 M NaCl, and stirred, the pellet re-extracted again with the same solvent. The resulting supernatants were combined, concentrated, and dialyzed against de-ionized water for 72h. The dialysates are freeze-dried, and the resulting protein powder is designated as globulin fraction.

Prolamin (alcohol)

The pellet was re-suspended in 70% aqueous 2-propanol (2PrOH), extracted twice by stirring for 2h, and centrifuged at 8000rpm for 20 min. The resulting supernatant was concentrated and freeze-dried, the product designated as prolamine fraction.

Glutelin (NaOH)

The pellet was re-suspended in a solution of 0.05 M NaOH, after centrifugation and concentration, the supernatants dialyzed against de-ionized water for 72h, the dialysates freeze-dried, and the resulting protein powder was designated as glutelin fraction. In all the above experiments a micro-Kjeldahl method was used to determine protein content in the protein fractions (N × 6.25).

Differential Scanning Calorimetry (DSC)

The thermal denaturation of the protein fractions was examined with a Perkin Elmer Pyris-1 DSC. The lyophilized and pulverized samples (1mg each) were directly measured into the aluminum pans and 5µL of phosphate buffer (7.5 pH) was added. Scanning was carried out at a temperature between 30-120ºC at a heating rate of 10ºC/min. High-purity indium standards were used for temperature and energy calibrations. Thermal denaturation temperature (T_d) and denaturation enthalphy (H) were calculated from thermograms.

Scanning electron microscopy (SEM)

SEM studies of the flour were carried out using a scanning electron microscope (LEO 355 VP, Cambridge UK). The samples were coated before being loaded to the scanning electron microscopy. The coated samples were loaded into the system and the image was viewed under 10 KV potential using a secondary electron image. The image was captured using 9.0 mm Ricoh Camera of 160x mga.

Statistical Analysis

The results were subjected to statistical analysis of variance (ANOVA) as described in section 2.3.

Results and Discussion

PE

The chemical composition of the dehulled sesame flour before defatting was determined, results obtained were in accordance with previous results [10]. they studied the modeling of moisture, color, and texture changes in sesame seeds during the conventional roasting the results are shown in Table 2.

Table 2: Chemical composition (%) of dehulled white sesame flour.

Protein Ash	**Fat**		**Carbohydrate**	**Moisture**
21.72±1.30a	52.30±0.91a	15.60±1.09b	4.04±0.63a	4.24±0.76a

aValues are mean ± SEM (n = 3), different letters in the same column are not significant at level ($p< 0.05$) but significant at $p< 0.01$.

The effect of pH, temperature, time, and flour-to-water ratio on protein extraction is shown in Table 3. The protein recovery varied from the range of 41 to 79% at design points 4 and 26 respectively (Table 3). The coefficient regression model estimated by multiple regressions was analyzed as shown in Table 4. The coefficients of the RSM as provided by equation 1 were evaluated. Statistical analysis indicated that all four extraction factors [pH, temperature (T), time (t), and defatted sesame flour/ water ratio (DSF: W)] had a strong influence on protein extraction (%PE). The regression coefficients showed that the pH, temperature, time, and sesame flour/ water ratio are very significant at a 5% level (Table 4). The results were in agreement with those reported by Sefa-Dedeh & Stanley [11]. on cowpea protein extraction using RSM. Similar work has been reported also by Jiamyangyuen, et al. [12]., using rice bran protein concentrate.

Regression coefficients in their linear form (pH, T, t and DSF:

W) as well as their quadratic and cross product interaction terms were significantly different at 5% probability level. Only one Cross product interaction (t*DSF: W) was not significant at 5% probability level. The final response model equation to estimate the protein extraction from defatted sesame seed is shown by an equation in Table 5 where %PR is the response factor of protein recovery (%). pH, T, t, and DSF: W are the values of the independent factors, reaction pH, reaction temperature (°C), reaction time (min) and defatted sesame flour/ water ratio (g/ml) respectively. The equation, in terms of coded factors, was generated using regression coefficients with statistical significance of up to 5% probability level. The equation indicates that the variables had almost equal effects on the extraction of protein from defatted sesame flour since they had almost similar slope values.

The second-order model showed a good fit with the experimental data since the adjusted coefficient of determination (R^2adj) of 0.9669 indicated that 96.69% of the variability in behavior within the range of values studied could be explained by the model (Table 5). The suitability of the model equation for predicting the optimum response values was tested using the recommended optimum conditions.

Table 3: Central composite rotatable design (CCRD) and the responses of the independent variable on protein recovery for defatted sesame flour during the extraction process.

Run no.	pH	T	t	DSF:W	Protein recovery a(%)
1	-1	-1	1	1	60
2	0	0	2	0	70
3	1	1	1	0	60
4	0	0	0	0	41
5	0	0	0	0	42
6	1	-1	1	0	74
7	-1	1	-1	1	55
8	1	1	-1	-1	68
9	0	-2	0	0	63
10	1	-1	-1	-1	57
11	-1	-1	-1	1	59
12	0	2	0	0	58
13	0	0	0	0	71
14	1	1	-1	1	60
15	1	-1	1	1	56
16	0	0	0	-2	43
17	-1	1	-1	-1	62
18	1	-1	-1	1	52
19	0	0	-2	0	66
20	0	0	0	2	56
21	-2	0	0	0	61
22	-1	1	1	1	57
23	1	1	1	1	67
24	-1	-1	1	-1	75
25	-1	-1	-1	-1	69
26	2	0	0	0	79
27	-1	1	1	-1	73

[a]Average of triplicate determinations from different experiments
T = temperature, t = time, DSF:W = ratio of defatted sesame flour to water.

Table 4: Coefficient regression model estimated by multiple regression analysis.

Factor	Coeffiecient	Significance level
pH	0.082	0.0204 Significant
T	0.071	0.0131
t	0.06	0.022
DSF:W	0.0817	0.0122
pH2	-13724	0.0061
T 2	-11001	0.0043
t 2	-36668	0.0075
DSF:W 2	-490	0.006
pH*T	-32400	0.0104
pH*t	0	0.01
pH*DSF:W	-1600	0.0206
T*t	-1600	0.0261
T*DSF:W	-1600	0.0068
t*DSF:W	900	0.1005 Not Significant

T = temperature, t = time, DSF:W = ratio of defatted sesame flour to water
* = Sign for the combination of the variables studied.

This set of conditions was determined to be optimum by the RSM optimization approach, which was also used to experimentally validate and predict the value of the responses using model equations generated by the software. The experimental values were found to be in agreement with the predicted ones.

Table 5: The equation for independent variable on protein recovery (%PR) of deffated sesame flour.

Best explanatory equation	%PR=51.33+2.75pH+1.25T+2.00t-3.58DSF:W+8.02pH2+2.27T2+4.15t2-0.48DSF :W2+4.50pH*T+0.00pH*t-1.00pH*DSF :W-1.00T*t-1.00T*DSF :W-0.75t*DSF :W

P level 0.05
R-Squared 0.9847
Adj R-Squared 0.9669
* = Sign for the combination of the variables studied.

Canonical analysis showed a maximum predicted %PE of 80.4% had the following critical values for the recovery factors: pH =11.6, T 45.9°C, t = 46.4 minutes, and DSF: W = 6.9. The experimental %PE was 79.8%, which was not significantly different statistically from the predicted %PE.

Contour plots were generated graphically from predicted data to illustrate the effect of each pair of independent variables (pH, T, t and DSF: W) on %PE as shown in Figure 2. As shown from the plots, an increase in %PE during the extraction of defatted sesame flour protein is generally achieved by increasing pH, temperature, time and defatted sesame flour/ water ratio. As shown in the plots, %PE during the extraction process of the defatted sesame flour protein was achieved by increasing, temperature and pH, a sharp slop was observed on the graph (Figure 2a). This implies that both response and the two factors increased concomitantly. Intuitively, this is attributable to the high protein extraction depending on the increase of those two factors to a particular point for the temperature. Conversely, reducing the pH and increasing the temperature will reduce the recovery of the protein.

A similar observation was reported when developing protein mixtures and evaluation of their sensory properties using the statistical response surface methodology [13]. When time and pH were combined (Figure 2b) an increase was also observed but on the axis of time, it was observed that when the time went up to more than 60 min the increase was not as sharp as when it was low. The increase is seen to be in a linear. These two factors revealed that to increase the recovery of protein extracted from defatted white sesame flour, the increase of those factors has to be at a particular point of which the reverse holds true. Similar observations have been reported when isolating protein from defatted Gevuina avellana nuts [14]. For ratio and pH (Figure 2c), for that relationship, it was observed that the increase of pH gives a positive increase in protein recovery but increasing the ratio did not reveal a significant protein recovery. The graph on the axis of the ratio shows a drop in the recovery as the ratio increases. Increasing the two concomitantly will not give a significant increase in the response monitored (PE). So, the increase of the pH has to be done while the ratio is fixed to a particular rate thinking of the amount of solvent to be used in the experiment. Our findings supported the report of Krishna-Murti [15]. who studied the extraction of protein from sesame cake after an industrial extraction of the oil. But we observed a contradiction on the results of Dave-Oomah et al., [16] they studied the optimization of protein extraction from flaxseed meal they observed that temperature was not significant but the ratio of meal to solvent was very significant to their study. The combined effect of time and temperature was shown (Figure 2d) from which it is clear that an increase in time and temperature led to a decrease in the protein recovery of sesame flour during extraction. This could be explained by the fact that when the extraction time is prolonged the probability of the mixture to form foam due to the denaturation and coagulation of the protein is a possible phenomenon. For the temperature is also the same; when the temperature is high and the extraction is carried out for a long period the above for time will be true. So these two factors have to be fixed at a low point when fixing them for the extraction of sesame protein. Our results corroborated the findings of Rustom et al., [17]. When ratio and temperature were combined for their effect (Figure 2e) an increase of the two gives an increase in protein recovery during the extraction period which is in a linear shape. That is to say, the increase of the two will not significantly increase the protein extraction/recovery from the defatted sesame flour. This could be explained by the fact that high temperature will cause protein inactivation which will lead to low extractability of the protein. Our results confirmed previous reports when extracting cowpea and pigeon pea proteins respectively [13,18]. The combined effect of ratio and time (Figure 2f) showed an increase of protein extraction when ratio was held

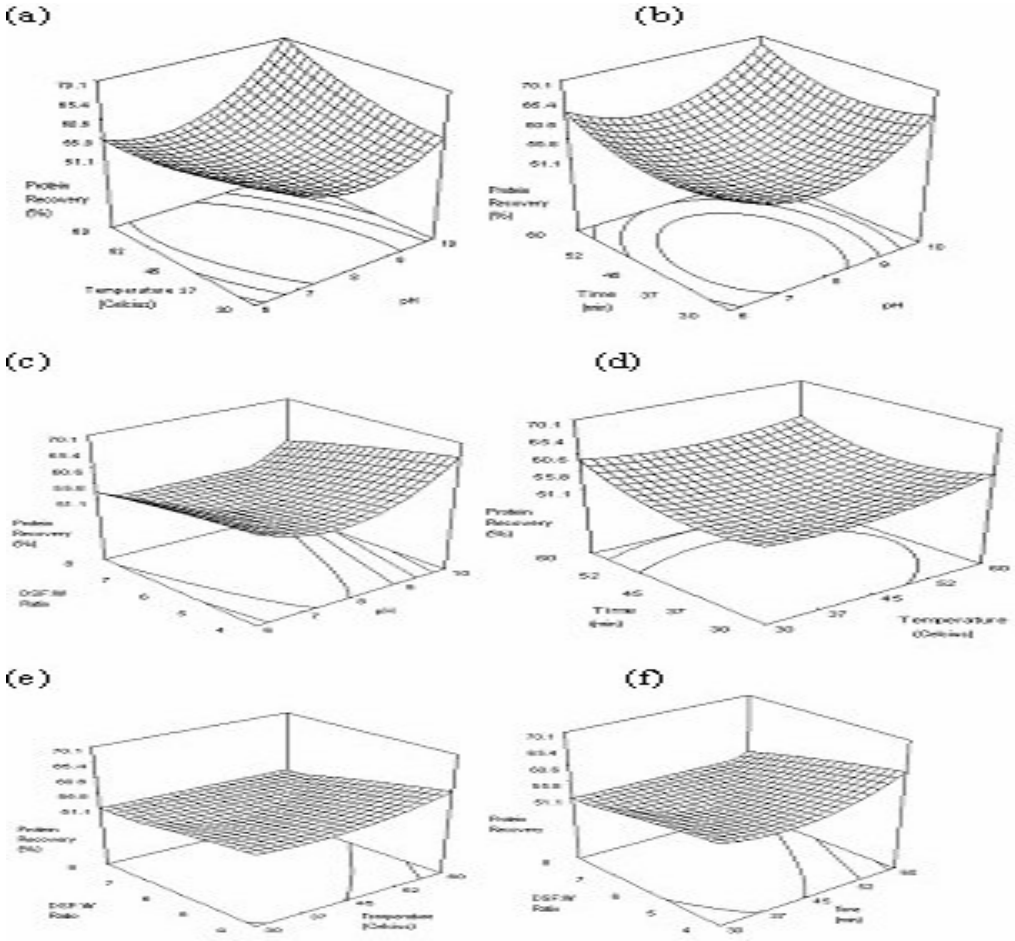


Figure 2: Surface plots to show the combined effect of (a) pH and temperature, (b) time and pH, (c) DSF to water ratio and pH (d) time and temperature, (e) DSF to water ratio and temperature, and (f) DSF to water ratio and time the on extraction of protein from defatted sesame seed.

constant for up to 60 min then the increase was not significant again. The ratio was the same when time was held constant. After 6g it was observed that the increase of the ratio was not too sharp.

It was possible that the model showed it to be not significant as the interaction of the two did not show remarkable increase according to the graph when an increase was made on both of the parameters (ratio and time). However, this result conflicts previous report on the effect of extraction time and ratio of flour to solvent (water) on the percentage of protein recovered [19]. Nath & Giri [19] for instance reported an optimum time of 2hr at a ratio of 6:100. However similar results have been reported when they studied the application of response surface methodology for extraction optimization of germinant pumpkin seeds protein**.** Our results corroborated their findings.

Amino Acid

The content of amino acids in the extracted sesame protein is shown in Table 6. There was a remarkable difference in the content of amino acids when compared with other.

Table 6: Total amino acid composition of sesame protein isolate compared with other reported values and FAO/WHO standard.

Amino acid	Composition in g/100g of protein		EAAb	
	Present study	Reported values	Infant	Adult
EAA				
Lysine	2.6	2.8-3.4	5.8	1.6
Histidine	3.02	1.7-2.5	1.9	1.6
Threonine	3.45	2.2-3.9	3.4	0.9
Valine	5.05	3.3-5.0	3.5	1.3
Methionine	3.06	1.6-3.8	2.5	1.70c
Isoleucine	3.72	2.8-4.2	2.8	1.3
Leucine	3.1	6.9-7.4	6.6	1.9
Tryptophan	1.87	2.0-2.5	1.1	0.5
nEAA				
Serine	2.95	2.2-4.7		
Arginine	3.59	8.4-13.4		
Proline	1.3	1.0-3.0		
Glycine	3.12	2.9-5.4		
Tyrosine	3.72	3.5-3.8		
Phenylalanine	4.06	4.3-6.4		
Alanine	2.68	3.3-5.0		
Aspartic acid	7.89	4.3-8.6		
Glutamic acid	16.65	3.5-20.1		

Source: [19-23]

[a]Suggested profile of essential amino acid requirements for adults [24].

[b]Methionine + Cysteine. EAA= Essential amino acid.

[c]nEAA= Non-essential amino acid

Results of previously investigated and reported elsewhere [20-23] of sesame seed though some of those reports in their experiments used sesame of the same variety (*Sesamum indicum L*) but from different origins of production of the seeds and different analysis methods. Our results were lower than those reported results we compared with our results and the difference was significant ($p< 0.05$). The results showed that the amino acid profile of the sesame protein extracted was generally lower in essential amino acid profile with the exception of tryptophan when compared with the suggested pattern of requirement by FAO/WHO for both infants and adults [24]. Even though from the literatures reviewed it was reported to have good amino acids but this could not be achieved in our present study. Thus making our method not too useful for the extraction of the protein from defatted sesame flour on a pH, temperature, time, and ratio of sesame flour to water at 12, 45°C, 45 minutes, and 6/100g/mL respectively. So, another method has to be employed since one of the focuses of this study is to have a higher amino acid content of the protein to be extracted for it to be utilized as protein supplements for different food systems.

Fractionation of Defatted Sesame Flour Protein (DSFP)

The distribution of protein fractions in defatted sesame is presented in Table 7. The albumin fraction had the highest protein content followed by globulin, glutelin, and prolamine. A large amount of carbohydrates and pigments were observed during the extraction process of the albumin and prolamine fractions. The globulin which is water soluble was observed to have the highest amount of protein fraction. The percentage of the fractions was observed to be reducing in percentage as the extraction of the protein fraction water to the different chemical solvents. The highest percentage of water-soluble protein could be confirmed by the amount of protein extraction/recovery observed when RSM was utilized to optimize protein recovery using water. This confirms that a higher percentage of the protein from the defatted sesame flour could be extracted with water but the extracted protein could be more of albumin. This could not give the protein extracted to have the right attributed to be incorporated in a food formulation that could be designated for infants and even adults as the protein to be incorporated should possess all the other protein fractions in higher amounts.

Table 7: Protein fractions of defatted sesame flour.

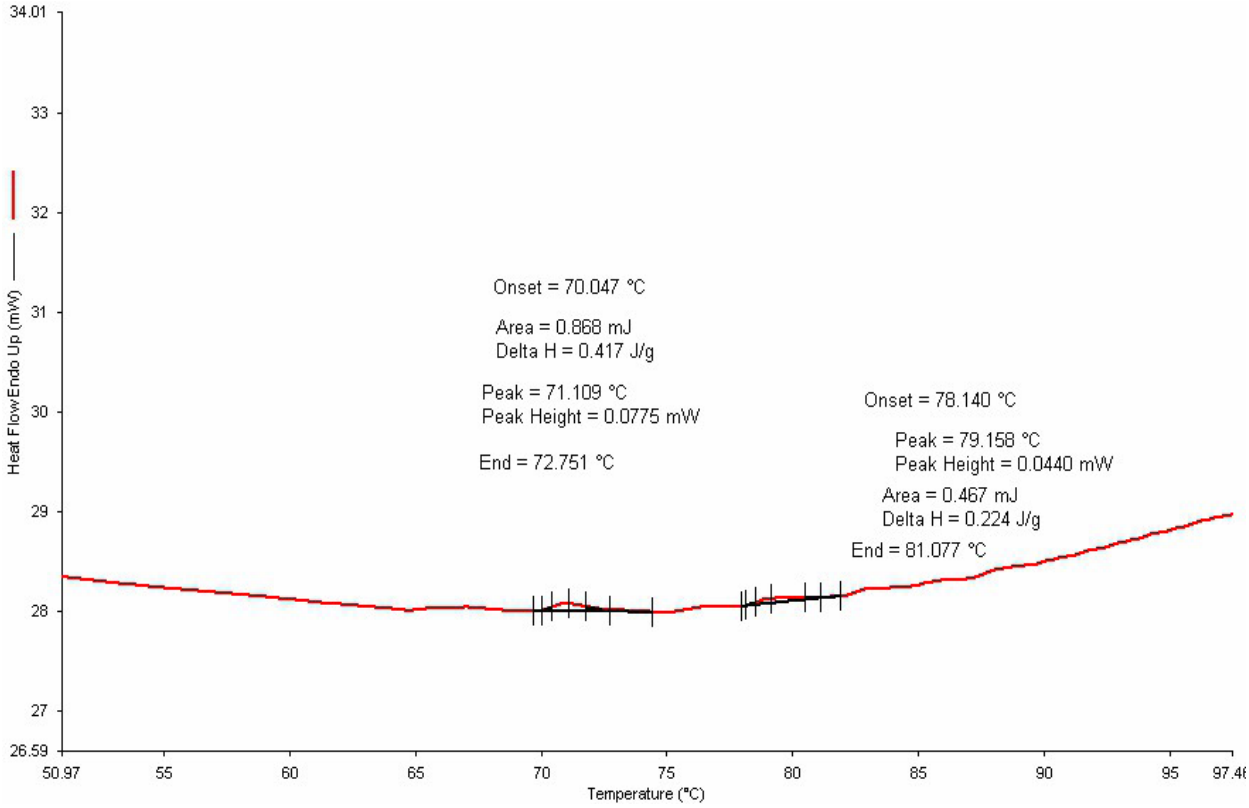
Protein Fractions	a% Protein Content
Albumin	48±1.07b
Globulin	26±0.52a
Prolamin	11±1.21a
Glutelin	15±0.81a

[a]Values are mean ± SEM (n = 3), different letters in the same column are not significant at level ($p< 0.05$) but significant at $p< 0.01$.

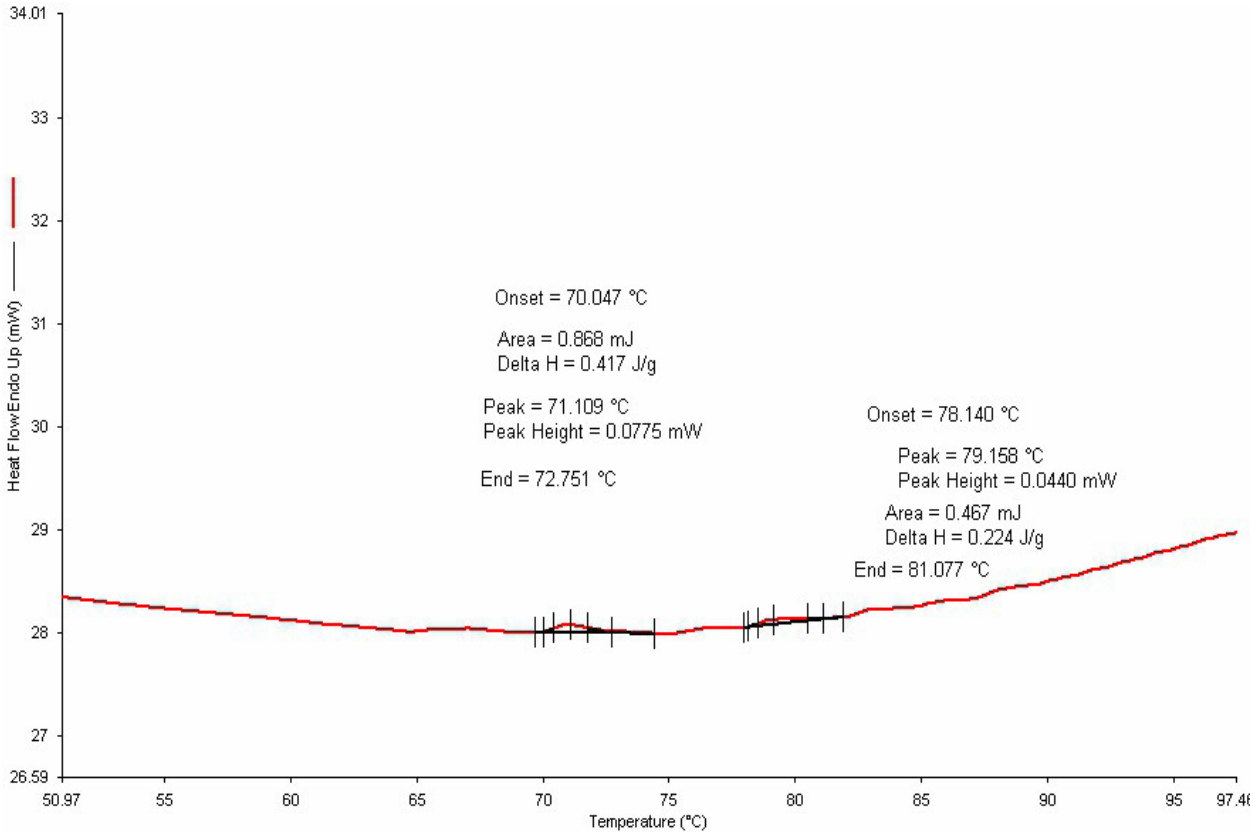
Our results corroborated those of Nath & Giri [19] but are different from the one of Mizubuti, et al. [18]. The difference observed could not be unconnected with the fact that they studied another oil seed. Therefore, the preparation of protein isolate would be an effective method for recovering proteins if the target is only the protein fraction that could be isolated with water. But that protein will not carry all the required amino acids particularly those that are not water soluble during the extraction process.

DSC

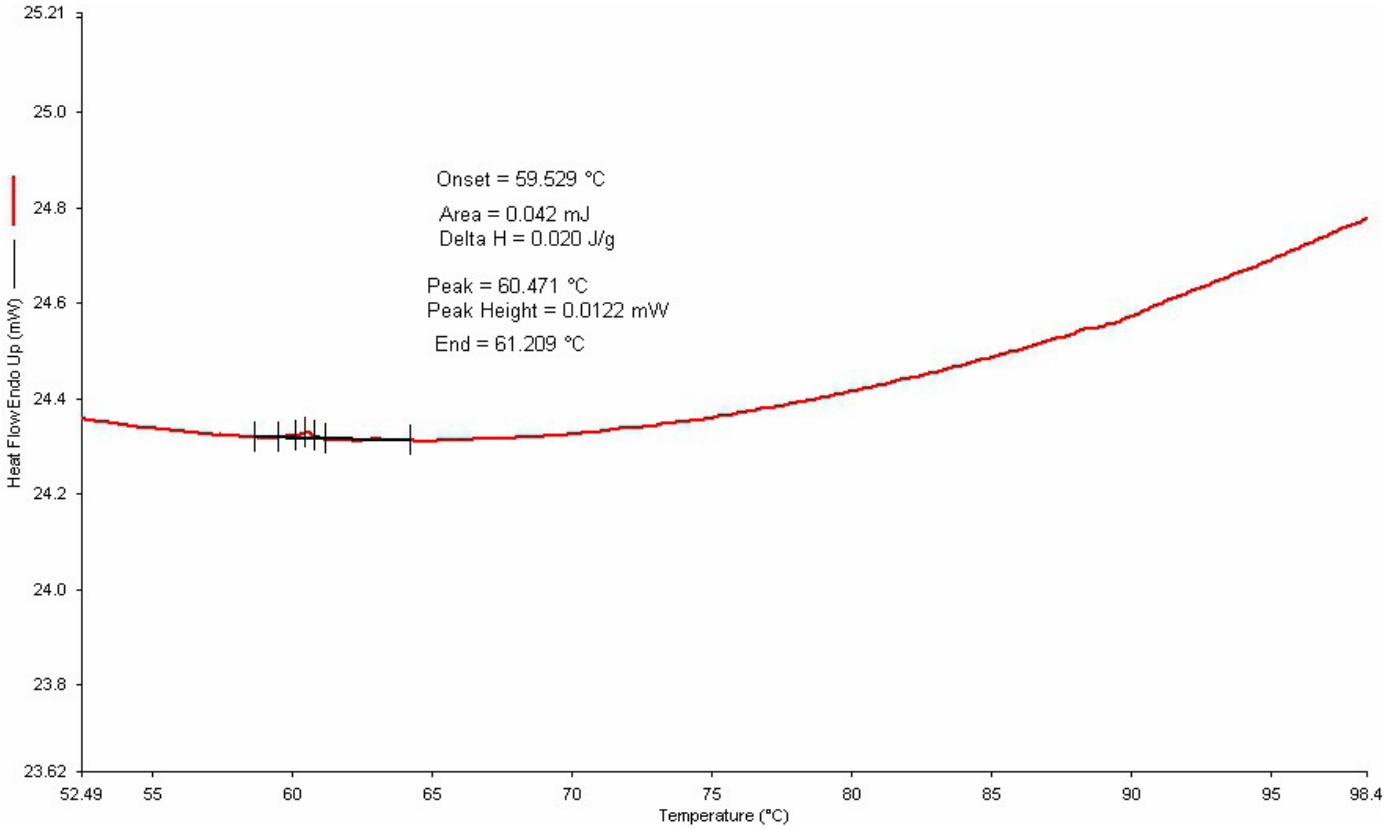
The thermal property measurements were carried out for DSF fractions (Figure 3 and Table 8). The albumin thermograph was sharper than the other three fractions. Globulin had two



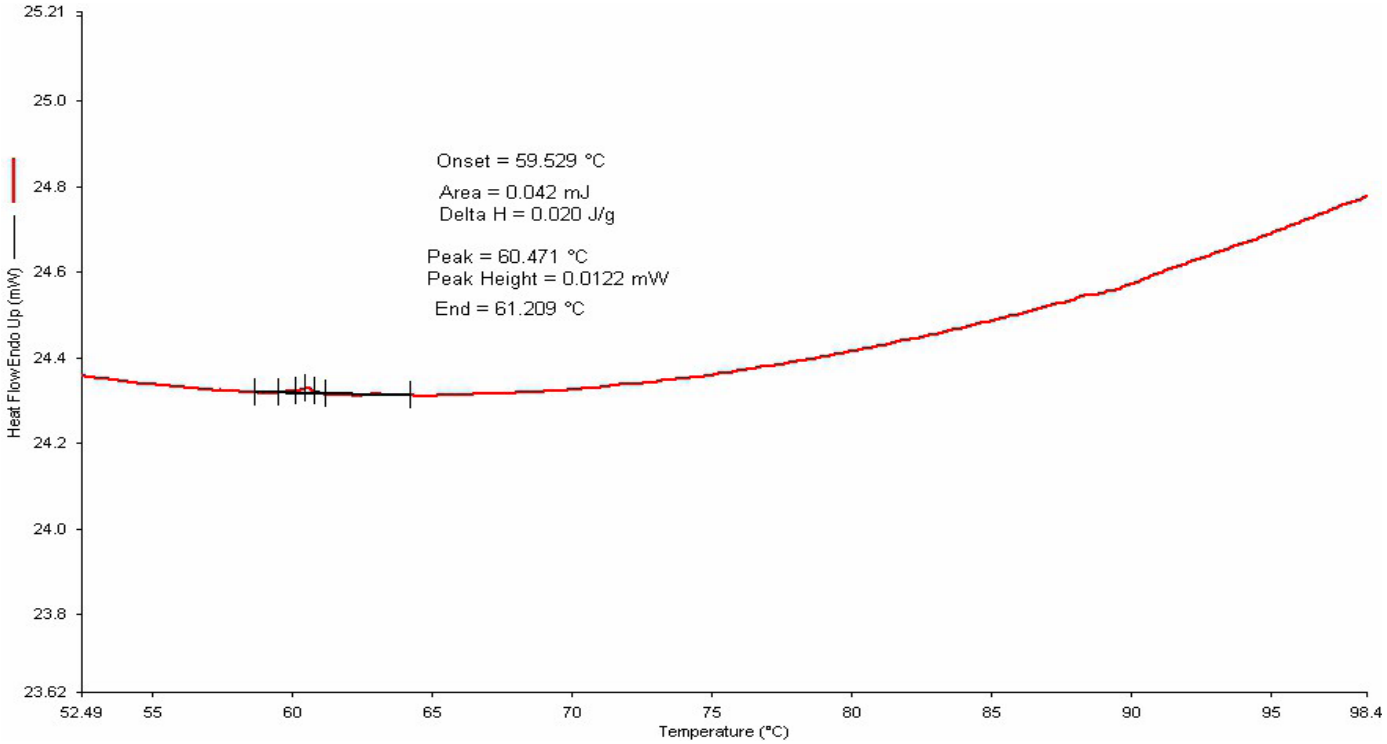
Albumin



Globulin



Glutelin



Prolamin

Figure 3: DSC thermographs of DSF protein fractions (Albumin, Globulin, Glutelin, and Prolamin.

denaturation temperatures. The occurrence of two enthalpy peaks in the globulin could be attributed to many factors that were not investigated in this work but the second denaturation temperature (79.16ºC) was observed to be significantly ($p< 0.05$) higher than the first denaturation temperature and also for the three fractions, Single enthalpy peak was obtained for glutelin and prolamin. In the Albumin, globulin, glutelin, and prolamin fractions the denaturation temperatures were significantly different ($p< 0.05$) to each other 74.86, 71.11(1st peak) 79.16 (2nd peak), 68.90, and 60.47 ºC respectively. Thus, the structural properties of DSFP fractions will be significantly affected if during their preparation the temperature is above 80ºC the protein will denature. DSC is a rapid, easy, and capable technique for supplying both thermodynamic (heat capacity, enthalpy and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation, and has been used extensively in various food systems [10].

SEM

The SEM results are shown in Figure 3. Four of the runs were scanned to see the particle of the protein isolated the three highest samples of protein recovered and the lowest. Experimental runs 26, 6, 27, and 4 of 79, 74, 73, and 41 respectively. The results showed the particles were clustered together; a scenario that showed that little or no degradation of the protein particles took place during the isolation process. That is why little protein recovery was observed and subsequently, low amino acid contents, which could not be a very good protein isolate to be incorporated in food as protein supplements.

Table 8: Denaturation temperatures of the fractionated protein from DSF.

DFSP Fractions	Denaturation Temp. (Td) (ºC)	Denaturation Enthalpy (H) (J/g)
Albumin	74.86	1505.906
Globulin	71.11 (1st)	0.417
	79.16 (2nd)*	0.224
Glutelin	68.9	0.919
Prolamin	60.47	0.02

*Additional peak observed in the thermograph of globulin.

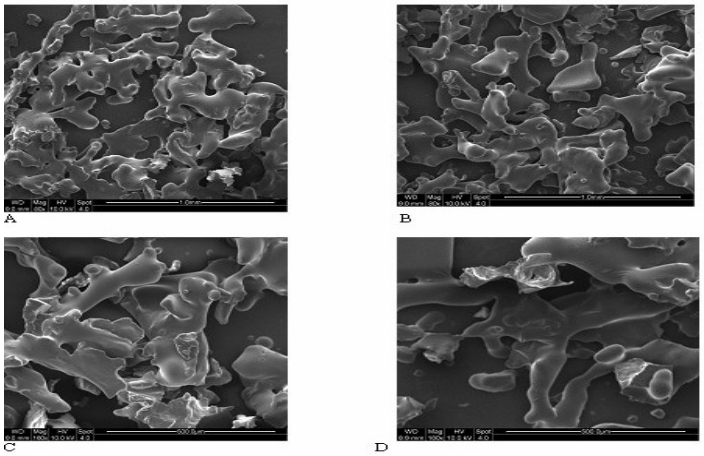


Figure 4: Scanning electron microscopy images of four samples of different experimental runs (A) 26, (B) 6, (C) 27, (D) 4.

Conclusion

Protein can be produced from the sesame seed using the critical values of the different conditions studied to have an optimum protein recovery. The surface plots assist in the selection of specific combinations of the independent recovery variables to attain the desired level of recovery of sesame protein. The application of RSM may, therefore, provide useful information in the development of economic and efficient processes in food a protein extraction system that is meant only for the protein fraction that could carry only those amino acids that are water-soluble. As the defatted sesame flour was fractionated to investigate why the protein was low during the RSM process, the results observed supported the RSM results. Moreover, protein treated at pH 10-12 is likely to form Lysinoalanine which has long been known to cause kidney damage. Albumin, globulin, glutelin, and prolamin fractions in the two preparations have similar denaturation temperatures. Since the attributes displayed were not too good other methods of protein isolation were investigated to know which method could be utilized to extract the highest protein content with higher amino acid contents from defatted sesame flour that could be utilized as safe protein supplement for different food systems.

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