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Exploring Functional Changes in Sesame Flour Hydrolysates Produced by Enzymatic Hydrolysis

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Introduction

Functional properties of food protein are important in food processing and food product formulation. Some of these properties are solubility, water, oil, foam, and emulsification capacities [1-5]. Nonetheless, some of these properties are affected by the intrinsic factors of proteins such as molecular structure and size, and some environmental factors, including the method of protein isolation from the seed [6]. The importance of these properties varies with the type of food products in which the protein isolate is to be used. For instance, protein isolates with high oil and water-holding capacities are desirable for use in meat and sausages, while proteins with high emulsifying and foaming properties are good for salad dressing, confectionaries, frozen foods, and soups [7]. Attention on plant protein isolates has been focused mainly on cotton seed, peanut, rapeseed, soya protein, and sunflower seed, and in some areas, commercial preparations are available [8-9]. In contrast the functionality of sesame protein has received little attention particularly when different production parameters say more than two are combined to isolate the protein from sesame seed that could be used in food formulations. Few studies that have been made are mainly on the properties of the defatted sesame flour or meal, sesame oil, the antioxidant property of sesame, and functional properties of sesame protein as influenced by pH only [10-12]. Very limited information is available for the functional properties of sesame isolate as influenced by other factors during its protein extraction from dehulled and defatted sesame seed flour with the use of selected proteases [13]. Nilo-Rivas et al. [13]., reported that sesame protein isolated by precipitation had poor nutritional value unless hydrolyzed by enzymes. This statement necessitates or justifies the present study.

Enzymatic modification of proteins using selected proteases to split specific peptide bonds is widely used to improve their functional properties [14]. For some food applications, proteins are hydrolyzed, among others, for hypoallergenic infant nutrition, for the nutrition of patients with digestion disorders, and for sports nutrition [14]. Also, enzymatic hydrolysis of food proteins generally results in profound changes in the functional properties of proteins treated [15-16].

The critical parameters for better control of enzymatic hydrolysis are temperature, time of hydrolysis, pH, the nature of substrate, type of enzymes used, the enzyme/substrate ratio, the concentration of substrate and the degree of hydrolysis [17]. When many factors and interactions affect a desired response, response surface methodology (RSM) is an effective tool for optimizing the process. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and less time-consuming than other approaches required to optimize a process [18]. There is little information on the effect of enzyme treatment on the various functional properties of sesame seed protein. Therefore, the objectives of this study were to screen different proteases for protein extraction from defatted sesame flour, apply RSM to optimize the production of sesame protein hydrolysates with the protease that gives the highest protein recovery and study the effect on the functional properties of the resulting hydrolysates.

Materials and Methods

Dehulled white sesame seeds were obtained from a local supermarket in Wuxi, PR China. The sesame seed were ground and defatted according to the method of Kanu et al. [19]., and kept in a freezer (Haier- BC/BD-275SB, Shanghai, PR China) at -10C till when needed for the experiments. Prior to the hydrolysis process, a portion of the defatted sesame flour was properly mixed. The chemicals and reagents used in the experiment were of analytical or food-grade quality.

Methods

Protease Selection

Protamex, a *Bacillus* protease complex, Neutrase from *Bacillus subtulis* strain, Alcalase® 2.4L from a strain of *Bacillus licheniformis* and Flavourzyme from *Aspergillus oryzae* were obtained from Novo Nordisk's Enzyme Business in Wuxi, PR China. The four proteases were evaluated for ability to hydrolyze sesame seed protein. The four bacterial enzymes were screened using a pH Stat method according to Adler-Nissen [20]. In order to select one with the best properties to be used for the defatted sesame seed protein hydrolysis. The DH to which an enzyme can hydrolyze a protein is a popular parameter for protein hydrolysis experiments. DH is intricately related to the properties of the hydrolysates [21].

Enzymatic Hydrolysis

Experiments to study the effects of hydrolysis variables in the range given in Table 4.1 were done in accordance with the experimental design depicted in Table 2. All reactions were done in triplicates in a 1L polyethylene-jacketed glass vessel in a thermostatically controlled water bath (NUOHAI- XMTD-204, Tokyo, Japan) with constant stirring (700 rpm). The vessel was covered with a close-fitting lid which was given an opening for an automatic temperature compensator (ATC) probe, a PH electrode (Hanna Precision pH meter (Model PH 212, SIGMA, USA), an overhead mixer shaft (KIKA- WERKE KMO2, KIKA Co. Tokyo, Japan) and for the addition of acid or base. During the reactions, pH was maintained at a desired value by the addition of 1.0N mol/L NaOH. The reaction vessel with 100g of defatted sesame flour was mixed with 500 mL of distilled water was placed in a previously heated water and placed in a previously heated water bath.

Table 1: Hydrolysis variables and levels for response surface analysis. Independent Factors

Levels	T (°C)	pH	E/S (%)	t(min)
-1	50	7	0.2	30
0	55	8	2.05	60
1	60	9	3	90

T = temperature, E/S = enzyme/substrate ratio (%v/w of Defatted Sesame flour) T = time (minutes).

Homogenization was carried out for 5 minutes in order to adjust the pH (through the addition of 1.0N mol/L NaOH) and temperature to the desired values. After equilibrium was reached, the enzyme (Alcalase®) was added and the reaction was allowed to proceed. The amount of alkali added to keep the pH constant during the hydrolysis was recorded and used to calculate the degree of hydrolysis (DH). The reactions were terminated by immersing the reaction vessel into hot water at 95°C for 15 minutes with continuous stirring to ensure the inactivation of the enzyme. The temperature of the reaction mixture at the end of the inactivation was 90 to 95°C. The resultant slurry was cooled at room temperature (23-25°C) and then centrifuged in a Beckman Coulter Centrifuge (Avanti J-26XPI, Beckman Co. USA) at 2800 x g for 20 minutes at room temperature. The supernatant was collected and freeze-dried.

Table 2: Box-Behnken experimental design matrix and the response of the dependent variable degree of hydrolysis (DH) for defatted sesame flour hydrolysis by alcalase 2.4L®.

Run	T	pH	E/S	t	DH (%)
1	-1	-1	0	0	7.12
2	-1	1	0	0	9.19
3	1	-1	0	0	9.98
4	1	1	0	0	11.82
5	0	0	-1	-1	1.19
6	0	0	-1	1	7.9
7	0	0	1	-1	6.3
8	0	0	1	1	9.12
9	-1	0	0	-1	2.07
10	-1	0	0	1	4.7
11	1	0	0	-1	4.8
12	1	0	0	1	12.2
13	0	-1	-1	0	3.6
14	0	-1	1	0	9.1
15	0	1	-1	0	6.9
16	0	1	1	0	18.8
17	-1	0	-1	0	6.4
18	-1	0	1	0	10.4
19	1	0	-1	0	7.7
20	1	0	1	0	11.3
21	0	-1	0	-1	3.6
22	0	-1	0	1	6.3
23	0	1	0	-1	6.2
24	0	1	0	1	13.72
25	0	0	0	0	10.1
26	0	0	0	0	8.9
27	0	0	0	0	9.2

Determination of Degree of Hydrolysis

The hydrolysis was carried out using the pH-stat method as described by Adler- Nissen [22]. Degree of hydrolysis (DH) is defined as the percentage ratio between the number of peptide bonds cleaved (h) and the total number of peptide bonds in the substrate studied (htot). The degree of hydrolysis was determined based on the consumption of the base necessary for controlling the pH during the batch assay as depicted in Equation 1.

$$Y = \beta_o + \sum_{i=1}^{i=3} \beta_i X_{ii} + \sum_{i=1}^{i=3} \beta X_i^2 + \sum_{i \neq j=2}^{i=3} \beta_{ij} X_i X_j + e \quad \text{.....Eq. (4)}$$

Where h_{tot} is the total number of peptide bonds in the protein substrate, in mmol/gprotein; h is the number of hydrolyzed peptide bonds; B is the base consumption in mL; N_b is base normality; a is the average degree of dissociation of the a-NH groups and MP is the mass of protein in g (N x 6.25).

The degree of dissociation was calculated as in equation 2.

$$a = \frac{10^{pH-pK}}{1+10^{pH-pK}} \quad \text{.....Eq. (2)}$$

Where pK is the average value of the a-amino groups liberated during the hydrolysis and varies significantly with temperature but is relatively independent of the substrate as such. The pK at different temperatures (T in °C) was calculated according to equation 3.

$$pK = 7.8 + \frac{298-T}{298T} \times 240 \text{ ......Eq (3).}$$

Determination of Free Amino Acid

The number of free amino groups was determined by using TNBS following the method of Mutilangi et al. [23]., with some modifications. TNBS (1 mL of 1% solution) was added to 1 mL of protein solution (0.15 mg/mL) containing 1% sodium dodecyl sulfate (SDS) and 4% NaHCO3, pH 9.5. After rapid mixing, the mixture was held at 40 °C for 2hr in a water bath and the reaction was stopped by adding 0.5 mL of 1mol HCL and 1 mL of 10% SDS. The absorbance of the sample was read at 335nm against a blank and the readings were converted to free amino acids by preparing a standard curve using glycine.

Experimental Design

To establish optimal conditions for hydrolysis of defatted sesame flour, RSM was used. The processing variables investigated were T, pH, E/S, and t. A Box-Behnken factorial design with four factors and three levels was applied according to Box & Behnken [24] as shown in Table 1. Three levels were adopted and coded as -1, 0, and +1. Degree of hydrolysis (DH) was the dependent variable (Table 1). To predict the optimal point, experimental data were fitted to a second-order polynomial parameter of the model, according to equation (4). The regression model between a dependent variable (Y) and independent variables was according to equation 4.

$$Y = \beta_o + \sum_{i=1}^{i=3} \beta_i X_{ii} + \sum_{i=1}^{i=3} \beta X_i^2 + \sum_{i,j=2}^{i=3} \beta_{ij} X_i X_j + e \text{ ......Eq. (4)}$$

Where Y is the measured response variable; *ßo, ßi, ßii* and *ßij* are the constant, linear, quadratic, and cross-product regression coefficients of the model respectively. *Xi* and *Xj* represent the independent variables (hydrolysis parameters). *e* is the random error. Regression coefficients of the model were obtained and tested by analysis of variance (ANOVA), performed by SAS according to SAS/STAT [25]. The R^2 value, residual error, pure error, and lack of fit were calculated.

Proximate Composition

Moisture and ash contents were determined as described in section 2.2.2.1.2 and 2.2.2.1.3 respectively. The total crude protein was determined according to section 2.2.2.1.1 The extraction and determination of fat from the samples was performed using methanol according to the method of Bligh & Dyer [26].

Scanning electron microscopy (SEM)

SEM was done according to the method described in section 3.2.2.6.

Nitrogen solubility index

The nitrogen solubility index (NSI) procedure was used to measure the solubility of the sample according to Panya & Kilara [27]. Samples were dispersed in water (10g/L) and PH of the solution was adjusted to the desired values (1-13 pH) with either 0.5N HCL or 0.5N NaOH while continually stirring for 45 minutes. At the end of this period, a 25ml aliquot was centrifuged at 2800 x g for 30 minutes. A 15ml aliquot of the supernatant was analyzed for nitrogen content (N) by the Kjeldahl method according to AOAC [28] and NSI calculated as in equation 5.

$$NSI\,(\%) = \frac{Supernatant\,(N)}{Sample} \times 100 \text{ ......Eq. (5)}$$

Water-Holding Capacity (WHC)

WHC was determined using the centrifugation method according to Booma & Prakash [29]. Triplicate samples (5g) of hydrolysate and native protein which is the defatted sesame flour were dissolved with 20 mL of water in centrifuge tubes and dispersed with a vortex mixer for 30 seconds. The dispersion was allowed to stand at room temperature for 6 hours, and it was then centrifuged at 2800 x g for 30 min. The supernatant was filtered with Whatman No. 1 filter paper and the volume recovered was accurately measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined, and the results were reported as mL of water absorbed per gram of protein sample.

Oil-holding capacity (OHC)

OHC was measured as the volume of edible oil held by 5g of material as described by Xiangzhen et al. [30]. 0.5g of samples were added to 10 mL of soybean oil in a centrifuge tube, and mixed for 30 seconds in a vortex mixer in triplicates. The oil dispersion was centrifuged at 2800 x g for 30 minutes. The volume of oil separated from the hydrolysate was measured and OHC was calculated as the mL of oil absorbed.

Emulsification Capacity (EC)

EC was measured by an oil titration method according to Chove et al. [9], with slight modification. 5 g of freeze-dried sample and 30 mL of soybean oil were added to 60 mL of 0.5N NaCl solution and mixed using an Ultra-Turrax T18 homogenizer (Shanghai, China) at 3000 rpm for 10 minutes. After this period, another 30 mL of soybean oil was added over 1.5 minutes and mixed for 30 seconds again. The mixture was transferred to centrifuge tubes, held in a water bath at 85°C for 15 minutes, and then centrifuged at 2800 x g for 30 minutes. Emulsification capacity was calculated as in equation 6.

$$EC = \frac{O_A - O_R}{W_s} \text{ ......Eq. (6)}$$

Where OA is the volume of oil added to form an emulsion, OR is the volume of oil released after centrifugation and WS is the weight of the sample.

Foaming Capacity and Stability

Foaming capacity and stability was evaluated according to the method of Darwicz et al. [31]. A 5g of the samples were dissolved in a 30 mL quantity of water; the aqueous dispersion was mixed thoroughly using an Ultra-Turrax T18 homogenizer at 3000 rpm for 3 minutes in a 250 mL graduated cylinder. Foaming capacity was calculated as percentage increase in volume upon mixing.

Foam stability was estimated as the percent of foam remaining after 5, 10, 20, 40, and 60 minutes.

Statistical Analysis

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

Results and Discussion

Protease selection

The results for degree of hydrolysis (DH) are presented in Figure 1(a) and (b). Among all proteinases, Alcalase 2.4L at pH 7 and 8 determined at 50°C and 60°C, respectively, showed the highest values in terms of DH. An initial rapid increase in DH against time was observed during the initial stages of hydrolysis related to the amount of NaOH used to maintain pH.

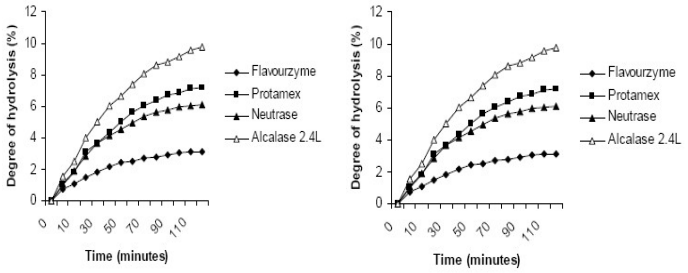


Figure 1: (a) Enzymatic hydrolysis of defatted sesame flour by different proteases at 50°C and pH 7, (b) enzymatic hydrolysis of defatted sesame flour by different proteases at 60°C and pH8.

This is because the enzymes were completely saturated with the substrate leading to a large number of peptide bonds being broken. The increase in rate of degree of hydrolysis till a stationary phase with hydrolysis time. This phenomenon may be accounted for by many factors such as the reduction in E/S ratio because of a reduction in substrate concentration (substrate limitation), reduction of enzyme activity or change in the nature of substrate for hydrolysis, and reduction in peptide bonds to be broken in the substrate. Protein yield (Table 3) revealed that Alcalase 2.4L produced the highest value (96.68%) at 60°C and pH 8 followed by Flavourzyme (69.76%). However, at pH 7 and 50°C, Flavourzyme showed a higher protein yield value (79.28%) followed by Alcalase 2.4L (77.62%). This difference may be due to the hydrolysis conditions used whereby Alcalase 2.4L was used during defatted sesame flour hydrolysis at 50°C and pH 7 which is lower than its reported optimum conditions [20]. As Alcalase has been reported to work more effectively above pH 7 [20]. Of the four enzymes screened in this study, Alcalase 2.4L displayed superior defatted sesame flour hydrolysis properties. Alcalase 2.4L was selected, for the current study because of its high yield under optimum conditions, readily available, cost-effectiveness, ease of handling, and low amount of free amino groups observed compared to the other enzymes.

Free amino groups increased through the course of hydrolysis and the increase was related to DH. The number of generated free amino acid groups increases as the DH increases for all the enzymes but Alcalase remains to produce the lowest free amino groups than the three enzymes throughout the experiments as shown in (Figure 2). The presence of free amino acids produces shorter peptides as they generate higher free amino groups than longer peptides. Enzyme specificity is a key factor controlling the generation of free amino groups [23]. Alcalase produced little free amino groups compared to Flavouzyme, Protamex, and Neutrase as observed in this study.

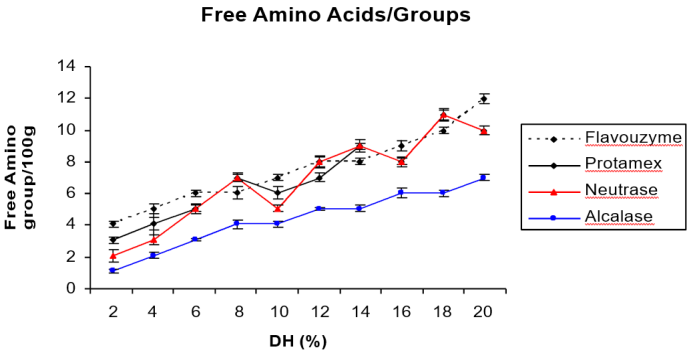


Figure 2: Free amino acid for the four enzymes.

Optimization of Hydrolysis Conditions

The effect of the independent variables on the dependent variable for the hydrolysis of defatted sesame flour protein using Alcalase® is shown in Table 2. The degree of hydrolysis (DH) ranged from 1.19 to 18.8 % at design points 5 and 16 respectively (Table 2).

The ANOVA for response surface is shown in Table 4. The coefficients of the response surface model as provided by equation (4) were evaluated. Statistical analysis indicated that all four hydrolysis factors (temperature, pH, enzyme/substrate ratio, and time) had a significant influence on DH.

Table 3: Protein yield of enzymatically hydrolyzed defatted sesame flour using different proteases.

Protein	Composition	(%)
Enzyme	50 oC pH-7	60 oC pH-8
Flavourzyme	79.28	69.76
Protamex	34.59	45.18
Neutrase	21.33	50.12
Alcalase 2.4L	77.62	96.68

Table 4: Analysis of variance (ANOVA) for the response of degree of hydrolysis.

Source	df*	Sum of Squares	Mean Squares	F-ratio
Regression Linear	4	267.15	66.788	108.77a
Quadratic	1	42.6	11.26	17.35a
Cross Product	1	11.66	3.183	4.25b
Residual Lack of Fit	10	34.34	3.434	326.1
Pure Error	4	0.04	0.004	-
Total error	14	34.38	2.863	
Temperature (oC)	1	53.09	53.881	21.62a
pH	1	48.44	48.441	19.72a
Time (Min)	1	69.12	69.12	39.299a
E/S ratio (%)	1	96.5	96.503	28.14

The combined effect of pH, temperature and enzyme/substrate ratio during the hydrolysis of some oil seeds [32] and soy protein [20]. have been shown to markedly influence the peptide bond cleavage in the protein substrate. The same effect was observed for whey protein concentrates [33]. Regression coefficients in their linear form (T, pH, E/S, and t) as well as one quadratic term (t2) were significantly different $p< 0.05$. One cross-product interaction (T t) was not significant at $p< 0.05$. The final response model equation to estimate the enzymatic hydrolysis of defatted sesame flour is shown in equation (7).

$$DH = 9.30 + 2.10T + 2.01pH + 2.40t + 2.84E / S + 1.71T \times t - 2.56t^2 \quad \text{..... Eq. (7)}$$

Where DH is the response factor degree of hydrolysis, (%). T, pH, E/S, and t are the values of the independent factors, reaction temperature (°C), reaction pH, enzyme/substrate ratio (%v/w of defatted sesame flour), and reaction time (min) respectively. The equation, in terms of coded factors, was generated using regression coefficients with statistical significance of up to $p< 0.05$. The equation indicates that the variables had almost equal effects on the hydrolysis of defatted sesame flour protein since they had similar slope values. The second-order model showed a good fit, the adjusted coefficient of determination (R^2 adj), which can check the appropriateness of a model, was 0.9870. This implies that 98.70% of the variation of the DH and DSF could be explained by Eq. (7) in terms of the independent variables, within the range of values studied. The data proved that the developed model could adequately represent the real relationship among the parameters chosen to be studied. Canonical analysis showed a maximum predicted DH of16.34% and had the following critical values for the hydrolysis factors: T = 58.79°C, pH = 8.12, E/S = 1.80% (%v/w of defatted sesame flour) and t = 51.85 min. The experimental DH was 15.78%.

Contour plots were generated from predicted data to illustrate the effect of each pair of independent variables (T, pH, E/S, and t) on DH as shown in Figure 3 (a)-(f). As shown from the plots,

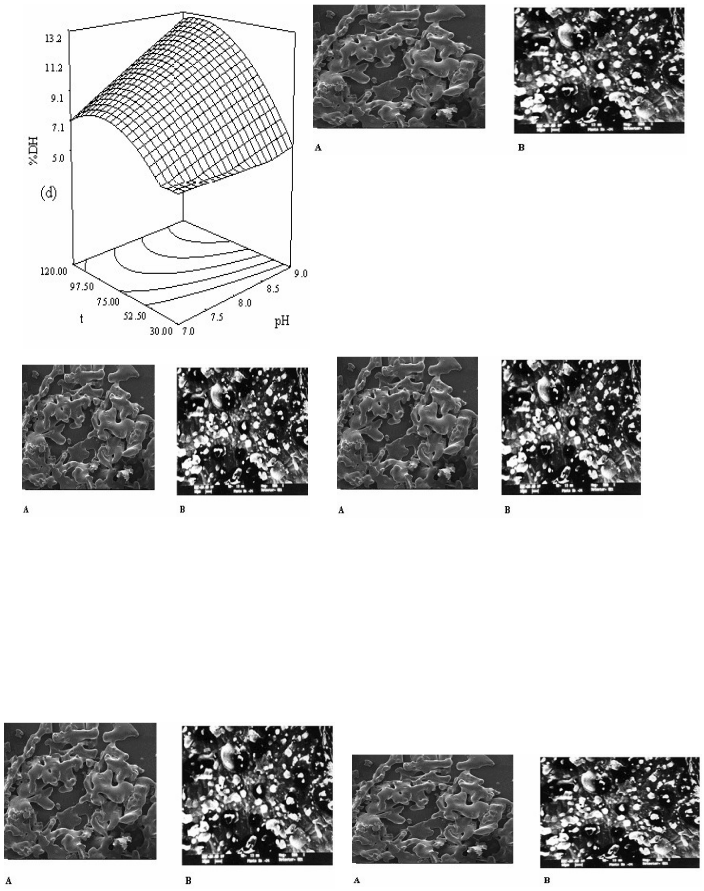


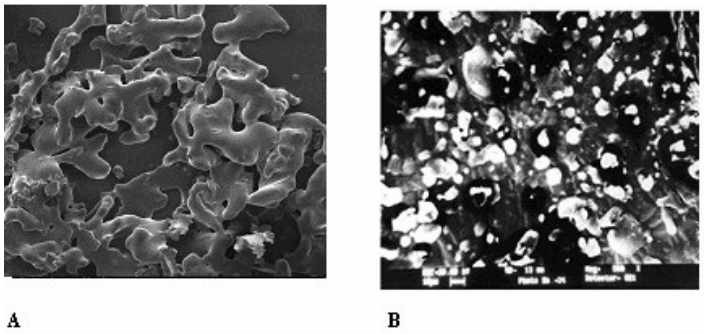
Figure 3: (a)-(f) Surface plots to show the combined effect of (a) pH and temperature, (b) time and temperature, (c) enzyme/substrate ratio and temperature, (d) time and pH, (e) enzyme/substrate ratio and pH and (f) enzyme/substrate ratio and time the on hydrolysis of defatted sesame flour by Alcalase®.

an increase in DH during the hydrolysis of defatted sesame flour is achieved by increasing temperature, pH, and enzyme/substrate ratio. Similar results have been reported by Cheison et al. [18], when they optimized the hydrolysis of whey protein.

An increase in DH was also achieved as the time was increased up to a certain level beyond which DH slightly decreased. The decrease in percentage hydrolysis can be explained by the increasing denaturation of the enzyme hence reducing its biological activity [34]. Similar observations have been reported for a hydrolytic response of food proteins using three kinds of proteases [15]. The non-linear relation between time and DH as noticed in Figure 3(b), (d), and (f) implies that the hydrolytic reaction depends on the availability of susceptible peptide bonds on which the primary enzymatic attack is concentrated and on the physical structure of the protein molecule.

Scanning Electron Microscopy

The SEM was used to examine the micro-structural changes of protein after the enzymatic hydrolysis. It was done to compare the particle sizes of the protein isolated by water and the one that was subjected to enzymatic hydrolysis as shown in Figure 4. The SEM images of the two protein hydrolysates were observed to be totally different in their particle sizes as could be seen in Figure 4(A & B). In Figure 4(A), which was water isolated protein, it was seen the particles clustered together, while Figure 4(B), which was enzymatic hydrolyzed, the picture shows that the protein has degraded into small fragments. This was seen as there is a reduction in the particle sizes in Figure 4(B). This will surely improve the amount of amino acid contents of the hydrolysates which was manifested by our results when the amino acids content and some functional properties were investigated.



A B

Figure 4: SEM images of, (A) water-isolated protein particles, (B) Enzymatic protein hydrolysate particles.

Composition and Functional Properties of Defatted Sesame Flour and Its hydrolysate

When the composition of defatted sesame flour and its hydrolysate were compared, the hydrolysates exhibited a higher percentage of protein recovery of 94.68% (Table 5) when the values (T= 55, pH= 9, E/S (%)=2.00, t= 60), which gave the highest DH was used as compared to the native protein which was 54.07% [19] after defatting the sesame flour. The moisture for the hydrolysate was 2.87%, ash was 0.46, fat was not detected the same for the carbohydrate. A Significant increase of protein was observed when the DSF was hydrolyzed as compared to the native protein (Table 5). This phenomenon was observed because degrading process of the enzymes allowed the release of more protein molecules trapped within the defatted sesame flour. Ash content contradicted the result of Bandyopadhyay & Ghost [5]. they reported higher ash content in their protein hydrolysates. The protein content of defatted sesame flour hydrolysate was similar to previous reports on hydrolysates ranging from 93% to 98% protein [35]. When they prepared and characterized a protein hydrolysate from oilseed flour mixture.

The solubility was observed to increase upwards in a gradual way from pH 3 up to between pH 7 and pH 8 and was maintained in that trend (Figure 5). The native protein was lower in solubility right through the experiment and was observed to drop between pH 4 -5. The solubility of the hydrolysate differs from the point that solubility always fall within the isoelectric points (pI) of most protein isolates [10]. An increase was observed around the pI though the difference was not significant to other reports [10,35]. We observed an increase in solubility as the pH increases. It has been suggested that an increase in solubility of protein hydrolysate is due to the reduction of its secondary structure, and also due to the enzymatic release of smaller polypeptide units from the protein [14]. Related studies have demonstrated the enzymatic hydrolysis of soy protein [3,4,8] and whey protein [36]. produced good functional properties, particularly solubility as nitrogen solubility of the hydrolysate was pH dependent over the range studied. Such behaviour is explained by the fact that smaller, more hydrophilic, and more solvated polypeptide units are produced as a consequence of enzymatic hydrolysis. Hence protein aggregates are no longer formed even at isoelectric pH [37]. The surface of protein has a net charge that depends on the number and identities of the charged amino acids, and on pH. These charges make the hydrolysates more soluble as without a net charge, protein-protein interaction and precipitation are more likely to happen that will reduce the solubility of protein hydrolysates as could be seed in the case of the native protein. The results of other functional properties of defatted sesame flour hydrolysates prepared from Alcalase treatment are presented in Table 6. The hydrolysate produced was highly water-soluble with good water water-holding, oil-holding capacities, and emulsifying properties than the DSF.

Foaming capacity was observed to be higher than the native protein from defatted sesame flour but its stability over a period of time (5-60 min) was however not good. The high foam capacity could be attributed to the protein in dispersion causing a decrease of the surface tension at the water-air interface thus creating higher foaming capacity [31,38].

Table 5: Proximate composition (%) of DSF and its hydrolysate.

Sample	*DSF*	*Hydrolysate*
Moisture	3.83 ±0.05a	2.87
Protein	54.07±1.75b	94.68
Fat	3.94±0.02a	ND
Ash	6.89±0.60a	0.46
Carbohydrate	16.75±1.56b	ND

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

High foaming capacity is also determined by molecular flexibility and physicochemical properties (hydrophobicity, net charge distribution, hydrodynamic properties) of the proteins. To form efficiently (to develop high foamability), a protein needs to adsorb more air rapidly during the transient stage of foam formation [39-,40]. which could have happened in the present study. Our results suggested an increase in surface activity, probably due to the initially greater number of polypeptide chains which were formed from the proteolysis activities, allowing more air to be incorporated into the hydrolysates [41].

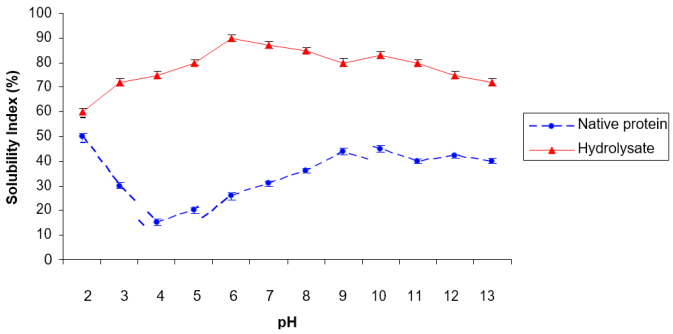


Figure 5: Solubility of the hydrolysate and native protein (NP).

Regarding the emulsifying capacity, it was observed that the hydrolysate was a good emulsifier than the native protein too, however, according to (Table 6) the difference observed was significant. This might be because proteins are composed of charged amino acids, non-charged polar amino acids and nonpolar amino acids which make protein a possible emulsifier particularly when hydrolyzed [42]. The surfactant possessing both hydrophilic and hydrophobic properties and can interact with both water and oil in the food system [39]. Also it was possible that the protein molecules reach the surface to unfold enough to expose hydrophobic groups. This phenomenon is necessary for the protein to function as a good emulsifier [42]. Water holding capacity was observed to also be significantly higher in the hydrolysate than the native protein. The interaction of water and oil with protein is very important in food systems because of their effects on the flavour and texture. Intrinsic factors affecting water holding capacity of food proteins include amino acid composition, protein conformation, and surface polarity/hydrophilicity [43]. However, the method of protein extraction also has an important impact on the protein conformation and hydrophobicity/hydrophilicity which could help to increase the water holding capacity of the protein.

Table 6: Functional properties of defatted sesame flour protein (native protein) and its hydrolysate.

Functional Properties	**Results**	
	Native Protein	**Hydrolysate**
Water holding Capacity (%)	40.2±0.2a	82.2±0.7b
Oil Holding Capacity (%)	46.6±0.5b	74.5±1.3b
Emulsification Capacity (%)	45.8±2.3a	73.1±3.1a
Foaming Capacity (%)	39.8±1.3a	85.1±2.1a
Foam stability (%)		
5 min	25.9	58.25
10 min	20.3	40.42
20 min	12.5	25.21
40 min	7.97	15.4
60 min	4.44	9.14

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

During hydrolysis the enzymes denaturated the protein molecules into smaller peptides, exposing more area for the water to interact with the protein molecules [43]. Oil holding capacity was also observed to be significantly high than the DSF. This might be the protease degraded the defatted sesame flour protein from its original protein shape to various peptides, thus exposing more hydrophobic groups to oil interface, resulting in increase in oil holding capacity [43]. As denaturation could improve the oil holding capacity of proteins, due to increased hydrophobic surface and flexibility of the protein molecules.

Conclusion

Protein hydrolysates was produced from defatted sesame flour by using Alcalase for higher protein recovery through the use of the critical values of the different conditions studied to have an optimum degree of hydrolysis. The production of hydrolysates by the use of Alcalase 2.4L should be encouraged as it has higher protein recovery and the hydrolysates produced have good functional attributes coupled with low free amino acid groups. Defatted sesame flour protein hydrolysates could be used in different food formulations for the ones needing protein supplementation because of its high protein content and good functional properties.

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