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Optimization of Enzymatic Hydrolysis of Defatted Sesame Flour by Different Proteases and their Effect on the Functional Properties of the Resulting Protein Hydrolysate

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Abstract: Sesame Protein Hydrolysate (SPH) was prepared from defatted sesame (*Sesamum indicum* L.) flour (DSF) after screening with different proteases. The proteases under different conditions showed varied effects on the protein recovery process. For example, Alcalase® 2.4 L produced the highest degree of protein recovery (96.68%) at 60°C and pH 8 followed by Flavourzyme (69.76%). However, at 50°C and pH 7, the highest protein recovery was noted for Flavourzyme (79.28%) followed by Alcalase 2.4 L (77.62%). The hydrolysis conditions (Temperature T, pH, Enzyme/Substrate E/S, time t) were engineered to optimize the degree of hydrolysis (DH) with the process studied using the Response Surface Methodology (RSM). The DH ranged from 1.19 to 18.8% while the solubility of the Defatted Sesame Protein Isolate (DSPI) increased with increase in pH. The SPH was observed to be a better emulsifier with significantly higher foaming properties, water and oil capacities compared to the untreated DSF. Nonetheless, the stability of the resulting foam diminished on standing over time. The Sesame protein hydrolysate obtained using Alcalase was noted to have better functional attributes compared to that obtained using Flavourzyme.

Key words: Enzymes, functional properties, protein hydrolysate, response surface methodology, sesame

INTRODUCTION

Sesame belongs to the genus *Sesamum*, one of the sixteen genera in the family *Pedaliaceae.* Its protein is known to have higher nutritional value compared to other seed proteins because of its relatively higher essential amino acids content (John Kanu *et al.*, 2007; Radha *et al.*, 2007) and its chemical composition assay establishes it as an important protein source (Chen *et al.*, 1998; Bandyopadhyay and Ghosh, 2002). Apart from the high protein content, sesame seeds have many other essential nutrients needed for the

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maintenance of human health (Coulman *et al*., 2005). Consequence on the increasing costs and limited supply of animal protein coupled with certain health concerns, tremendous efforts have been directed lately, to investigate plant proteins sources as viable options for incorporation into formulated foods blends.

In addition, they are particularly noted for imparting better functional properties to food system (Bernard-Don *et al*., 1991; Chiang *et al*., 1999; Chove *et al*., 2002). Functional properties of food proteins are crucial characteristics considered in food processing and food product formulation. Some of these properties include solubility, water holding capacity, oil holding capacity, emulsification, foaming properties, bulk density and viscosity (Bandyopadhyay and Ghosh, 2002; Jung *et al*., 2005) which are in turn affected by certain intrinsic characteristics of proteins such as molecular structure and size. They can also affected by some environmental factors, including methods of protein isolation from seeds (Fuhrmeister and Meuser, 2003).

In the past, a huge attention on plant protein isolates had mainly targeted cotton, peanut, rapeseed, soya and sunflower seeds this has resulted in the rapid commercial production of these oil seeds (Tsumura *et al*., 2005; Chove *et al*., 2002). But the functionality of sesame protein has not been exploited especially in the realm of engineering the production process at different reaction conditions in isolating its protein and the possible application in food formulations. Although, existing literature reveals investigations into the properties of the defatted sesame flour or meal, sesame oil, the antioxidant properties of sesame, functional properties of sesame protein as influenced by pH only (Khalida *et al*., 2003; Shahidi *et al*., 2006; Aly *et al*., 2000) and the extraction of protein through the use of water (John Kanu *et al*., 2007), very limited information is yet available on the functional properties of sesame protein isolates especially under varying extraction conditions from dehulled sesame seeds. Nilo-Rivas *et al*. (1981) only speculated the application of selected proteases as a possible means of obtaining sesame protein with some desirable functional properties and this speculation has been the main thrust of our present study. An enzymatic hydrolysis of food proteins generally results in profound changes in their functional properties mostly reflected in their enhanced solubility, emulsification, water and oil holding capacities (Bernard-Don *et al*., 1991; Hrckova *et al*., 2002; Ferreira *et al*., 2007).

Preliminary screening using four kinds of microbial proteases (Flavourzyme, Protamex, Neutrase and Alcalase® 2.4 L) for the enzymatic hydrolysis of the sesame seed protein was done by regulating the critical parameters that are known to control the process: temperature, hydrolysis (reaction) time, pH, substrate nature, enzyme type, enzyme/substrate ratio, substrate concentration and the degree of hydrolysis (Diniz and Martin, 1996; Tsumura *et al*., 2005).

In the circumstance where many factors and interactions affect a desired response, the Response Surface Methodology (RSM) is used as an effective tool for optimizing the process. The main advantage of RSM is it requires lesser number of experimental trials to evaluate multiple parameters and their interactions, thus, making it less laborious and done within a minimal time frame as compared to other approaches for the same purpose (Cheison *et al*., 2007).

With the limited information on the effect of enzyme treatment on various functional properties of sesame seed protein, the core aims of this study is to screen different the proteases in terms of optimizing the DH and to ascertain the proteases that give the highest protein recovery using RSM and also to determine their effect on the functional properties of the resulting hydrolysates.

MATERIALS AND METHODS

Dehulled white sesame seeds of *Sesamum indicum* variety were obtained from a local supermarket in Wuxi, Jiangsu Province, People's Republic of China. The sesame seeds were ground and defatted using the method of John Kanu *et al*. (2007) and kept in a freezer (Haier-BC/BD-275SB, Shanghai, People's Republic of China) at -10°C until required for further experiments. Prior to the hydrolysis process, a portion of the defatted sesame flour was mixed properly for the purpose of homogeneousness.

Alcalase® (in solution form with declared activity of 2.4 Activity Units (AU) kg^{-1} and density of 1.18 g mL^{-1}) a bacterial endoproteinase from a strain of *Bacillus licheniformis* was provided by Novo Nordisk's Enzyme Business in Wuxi, Jiangsu Province, PR China and stored at 5°C until required for the hydrolysis experiments. All chemicals and reagents used in the experiment were of analytical or food grade standard. This research was conducted in the State Key Lab of Food Science and Technology between the periods of 25th July 2008 to 30 June 2009.

Protease Selection

Protamex, a *Bacillus* protease complex, Neutrase from *Bacillus subtulis* strain, Alcalase® 2.4 L from a strain of *Bacillus licheniformis* and Flavourzyme from *Aspergillus oryzae* were obtained from Novo Nordisk's Enzyme Business in Wuxi, Jiangsu Province, People's Republic of China. The four proteases were evaluated for their ability to hydrolyze sesame seed protein. The four bacterial enzymes were screened using the pH Stat method as described by Adler-Nissen (1986), for the purpose of identifying those with better capabilities of hydrolyzing the defatted sesame seed protein. The DH is intricately related to the properties of the hydrolysates (Mahmoud *et al*., 1992) and was used as a factor to determine the effectiveness of the enzymes in hydrolyzing the protein.

Enzymatic Hydrolysis

Experiments to study the effect of hydrolysis variables in the range given in Table 1 were carried out in accordance with the experimental design depicted in Table 2. All reactions were carried out in triplicates in a 1 L polyethylene-jacketed glass vessel in a thermostatically controlled water bath (NUOHAI-XMTD-204, Tokyo, Japan) with constant stirring (700 rpm). The experimental vessel was covered with a tight fitting lid with bore holes to accommodate

Table 1: Hydrolysis variables and experimental design levels for response surface analysis

	Independent factors			
Levels	T (°C)	pH	E/S (%)	t (min)
-1	50	7	0.20	30
0	55	8	2.05	60
1	60	9	3.00	90

T: Temperature, pH, E/S: Enzyme/Substrate ratio (% v/w of Defatted Sesame flour), t: Time (min)

Table 2: Protein yield of enzymatically hydrolyzed defatted sesame flour using different proteases

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

an Automatic Temperature Compensator (ATC) probe, a pH electrode Hanna Precision pH meter (Model pH 212, SIGMA, USA), an over head mixer shaft (KIKA-WERKE KMO2, KIKA Co. Tokyo, Japan) and for pouring required reagents during the course of the hydrolysis process. In the reaction vessel, 50 g of defatted sesame flour was mixed in 500 mL of distilled water and placed in a water bath at the required temperature. The pH of the reaction content was maintained at desired values by adding required amounts of 0.2 mol L^{-1} NaOH.

Homogenization was carried out for 5 min in order to adjust the pH (through addition of 0.2 mol L^{-1} NaOH) and temperature to the desired values. After equilibrium was reached, the enzyme (Alcalase®, which gave the highest protein recovery from the preliminary tests) 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 DH. The reaction was terminated by immersing the reaction vessel into hot water at 95°C for 15 min with continuous stirring to ensure enzyme inactivity. The temperature of the reaction mixture at the end of the inactivation was 90 to 95°C. The resulting slurry was cooled to room temperature (23-25°C) and then centrifuged using a Beckman Coulter Centrifuge (Avanti J-26XPI, Beckman Co., USA) at 2800x g for 20 min at room temperature. The supernatant was collected and freeze dried.

Determination of Degree of Hydrolysis

The hydrolysis was carried out using the pH-stat method as described by Adler-Nissen (1986). Degree of hydrolysis is the ratio (expressed in percent) of the number of peptide bonds cleaved (h) and the total number of peptide bonds in the substrate studied (h_{tot}). The degree of hydrolysis was determined (Eq. 1) based on the quantity of base required to maintain the pH during the hydrolysis process in the batch assay.

$$\text{DH}\ (\%) = \frac{h}{h_{tot}} \times 100 = B \times N_b \times \frac{1}{\alpha} \times \frac{1}{MP} \times \frac{1}{h_{tot}} \times 100 \quad (1)$$

where, h_{tot} is the total number of peptide bonds in the protein substrate in mol $g^{-1}{}_{protein}$, h is the number of hydrolyzed peptide bonds, B is the base consumed in mL, N_b is base normality and α is the average degree of dissociation of the α-NH groups and MP is the mass of protein in g (N × 6.25).

The degree of dissociation was calculated as in Eq. 2.

$$\alpha = \frac{10^{pH-pK}}{1+10^{pH-pK}} \quad (2)$$

where, pK is the average value of the α-amino groups liberated during the hydrolysis which varies significantly with temperature but relatively independent of the substrate. The pK at different temperatures (T in °C) was calculated according to Eq. 3.

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

Determination of Free Amino Groups

The number of free amino groups was determined by using Trinitrobenzene sulfonate (TNBS) as described by Mutilangi *et al*. (1995) with slight modifications. TNBS (1 mL of 1% solution) was added to 1 mL of protein solution (0.15 mg mL^{-1}) containing 1% Sodium

Dodecyl Sulfate (SDS) and 4% $NaHCO_3$, pH 9.5. After rapid mixing, the mixture was kept at 40°C for 2 h in a water bath and after which, the reaction was stopped by adding 0.5 mL of 1 mol HCl and 1 mL of 10% SDS. The absorbance of the sample was read at 335 nm against a blank and the readings were converted to free amino acids by preparing a standard curve.

Experimental Design

To establish optimal conditions for hydrolysis of defatted sesame flour, RSM was used. The processing variables investigated were temperature (T), pH, Enzymes/Substrate (E/S) and time (t). A Box-Behnken factorial design with four factors and three levels was applied according to Box and Behnken (1960), as shown in Table 1. Three levels were adopted and coded as -1, 0 and +1 with DH considered the dependent variable. To predict the optimal point, experimental data were fitted into a second order polynomial parameter model, according to Eq. 4. The regression model between dependent variable (Y) and independent variable (X) was in accordance with Eq. 4.

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

where, Y is the measured response variable, β_o, 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 (hydrolysis parameters), e is the random error.

Regression coefficients of the model were obtained and tested by analysis of variance (ANOVA), performed by SAS method according to SAS (2002). The R^2 value, residual error, pure error and lack of fit were calculated by the model.

Proximate Composition

Moisture content was determined by placing 2 g of SPH sample into a preweighed aluminum dish. The sample was then dried in a forced-air convection oven at 105°C until a constant weight was reached. Ash content was estimated by charring in a crucible at 600°C until the ash had a white appearance. The total crude protein (N × 6.25) content of the samples was determined using the Kjeldahl method. The extraction and determination of fat from the samples were performed using the soxhlet method described by James (1995).

Nitrogen Solubility Index

The Nitrogen Solubility Index (NSI) procedure was used to measure the solubility of the SPH according to Hrckova *et al*. (2002) with slight modifications. SPH (10 g) were dispersed in water (100 mL) and the solution's pH was adjusted to the desired values (3.0, 5.0, 7.0 and 9.0) with either 0.5 N HCl or 0.5 N NaOH while continually stirring for 45 min. At the end of this period, the mixture was centrifuged at 2800x g for 30 min.

A 15 mL aliquot of the supernatant was analyzed for nitrogen content (N) by the Kjeldahl method according to AOAC and NSI calculated using Eq. 5.

$$NSI\ (\%) = \frac{Superna\tan t(N)}{Sample} \times 100 \quad (5)$$

Foaming Capacity and Stability

Foaming capacity and stability were evaluated according to the method of Darwicz *et al*. (2000). A 0.5 g of the SPH was dissolved in a 30 mL distilled water; the aqueous dispersion was mixed thoroughly using an Ultra-Turrax T18 homogenizer (Shanghai, People's

Republic of China) at 3000 rpm for 3 min in a 250 mL graduated cylinder. The same was done for the DSF. 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 min.

Emulsification Capacity

Emulsification Capacity (EC) was measured by an oil titration method described by Chove *et al*. (2002) with slight modification. 0.5 g of freeze-dried SPI and 30 mL of soybean oil were added to 60 mL of 0.5 N NaCl solution and mixed using an Ultra-Turrax T18 homogenizer (Shanghai, People's Republic of China) at 3000 rpm for 10 min. Thereafter, another 20 mL of soybean oil were added and mixed for 5 min again. The mixture was transferred to centrifuge tubes, held in a water-bath at 85°C for 15 min and then centrifuged at 2800x g for 30 min. Emulsification capacity was calculated as given in Eq. 6.

$$EC = \frac{O_A - O_R}{W_s} \tag{6}$$

where, O_A is the volume of oil added to form an emulsion, O_R is the volume of oil released after centrifugation and W_S is the weight of the SPH. The same was done for the DSF.

Water-Holding Capacity

Water-Holding Capacity (WHC) was determined using the centrifugation method as described by Jung *et al*. (2005). Triplicate of the SPH (0.5 g each) was dissolved in 20 mL of water in centrifuge tubes and dispersed with a vortex mixer for 10 min. The dispersion was allowed to stand at room temperature for 2 h and it was then centrifuged at 2800x 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

Oil-Holding Capacity (OHC) was measured as the volume of edible oil held by 0.5 g of the SPH and DSF as described by Xiangzhen *et al*. (2007). About 0.5 g of the SPI was added to 10 mL of soybean oil in a centrifuge tube and mixed for 10 min in a vortex mixer in triplicates. The oil dispersion was centrifuged at 2800x g for 30 min.

The volume of oil separated from the hydrolysate was measured and OHC was calculated as mL of oil absorbed per gram of protein sample.

Statistical Analysis

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

RESULTS AND DISCUSSION

Protease Selection

The results for DH are presented in Fig. 1a and b. Among all proteinases, Alcalase 2.4 L at pH 7 and 8 determined at 50 and 60°C, respectively, showed the highest values DH. A rapid increase in DH with time was observed during the initial stages of hydrolysis as more

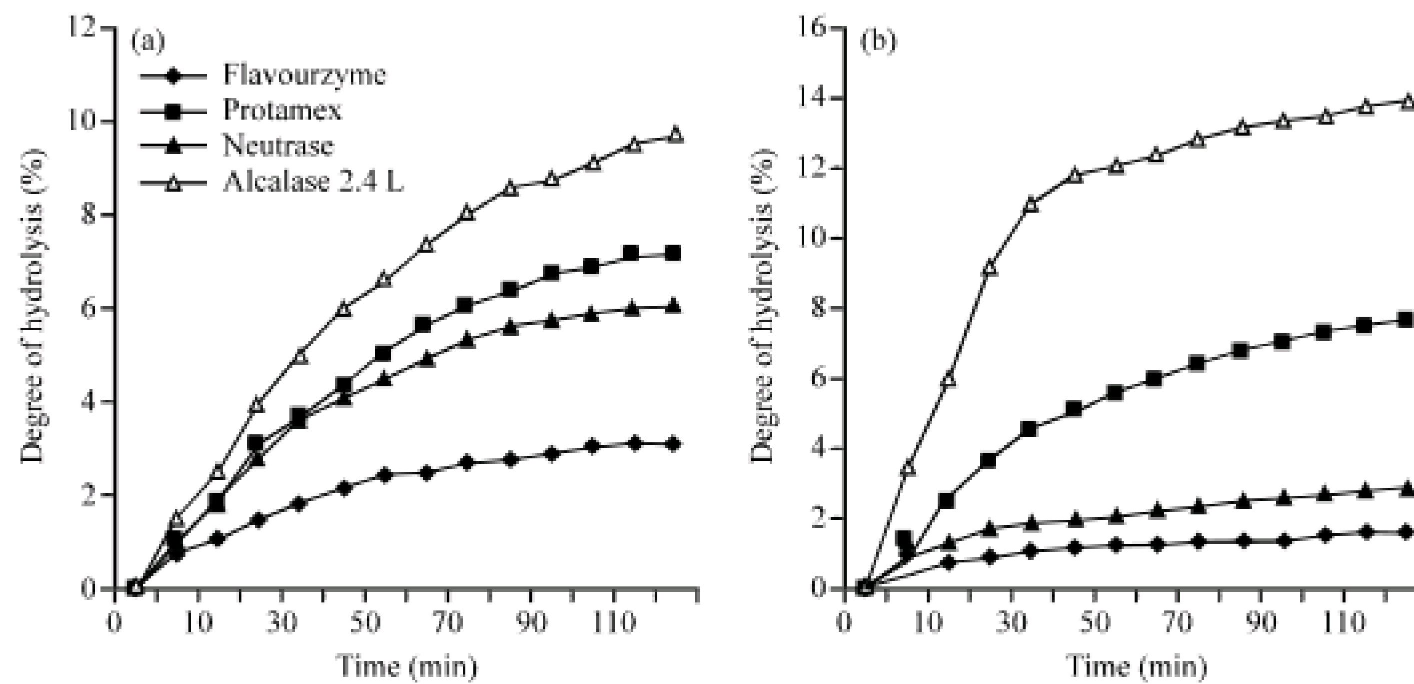

Fig. 1: Enzymatic hydrolysis of defatted sesame flour by different proteases (a) at 55°C and pH 7, (b) at 60°C and pH 8

NaOH was required to maintain the pH during this period. This is because the enzymes were largely saturated within the substrate resulting to the cleavage of large numbers of peptide bonds. The hydrolysis rate increased in an exponential manner with time until a stationary phase was attained with lesser number of peptide bonds to be broken in the substrate. The screening results for the four proteases are shown in Table 2. Alcalase 2.4 L produced the highest value (96.68%) of protein recovery at 60°C with pH 8 followed by Flavourzyme (69.76%). However, at pH 7 and 50°C, Flavourzyme showed the highest protein yield value (79.28%) followed by Alcalase 2.4 L (77.62%). This observation could be attributed to the fact that different enzymes require specific reaction conditions for optimal activity. The conditions for optimal activity for Alcalase 2.4 L, 50°C and pH 7 (for defatted sesame flour hydrolysis) differ from those reported by Adler-Nissen (1986) but the results nonetheless corroborates the fact that the enzyme works more effectively above pH 7. The difference in protein recovery between Flourzyme and Alcalase was not significant at $p<0.05$. At a temperature of 60°C with pH-8, Alcalase 2.4 L gave a 96.68% protein recovery while Flourzyme, Neutrase and Protamex gave a protein recovery of 69.76, 50.12 and 45.18%, respectively. These results reinforces observations made by Hrckova *et al*. (2002), in their investigation of enzymatic hydrolysis of defatted soy flour by three different proteases and their effect on the functional properties of the resulting protein hydrolysates.

Determination of Free Amino Groups

Free amino acid groups increased through the course of hydrolysis and the increase was related to increase in DH. The number of generated free amino acid groups increases as the DH increases for all the enzymes except Alcalase which produced the lowest free amino acid groups throughout the experiments as shown in Fig. 2. Enzyme specificity is a key factor controlling the generation of free amino groups (Mutilangi *et al*., 1995) which may be attributed to the above observation. Alcalase produced little free amino groups compared to Flavouzyme, Protamex and Neutrase as observed in this study.

Alcalase 2.4 L was selected for the current study because of its high protein yield under optimum conditions, low free amino groups generated, ready availability, less expensive and ease of handling.

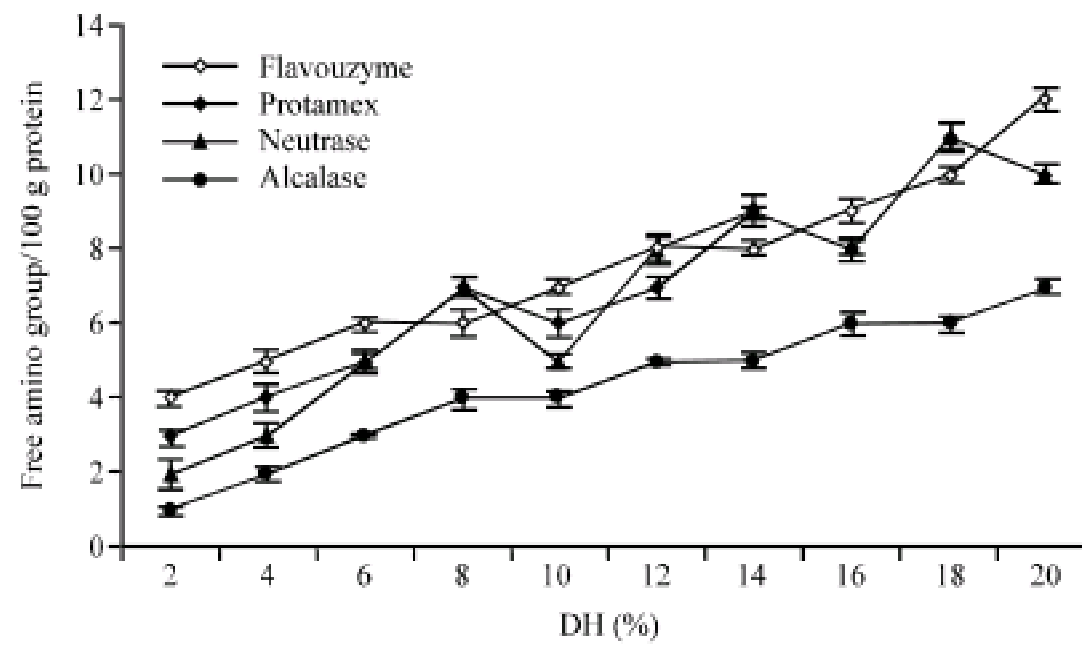

Fig. 2: Free amino groups for the four enzymes

Table 3: Box-Behnken design matrix and the response of the dependent variable degree of hydrolysis (DH) for defatted Sesame flour hydrolysis by Alcalase 2.4 L®

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	7.90
7	0	0	1	-1	6.30
8	0	0	1	1	9.12
9	-1	0	0	-1	2.07
10	-1	0	0	1	4.70
11	1	0	0	-1	4.80
12	1	0	0	1	12.20
13	0	-1	-1	0	3.60
14	0	-1	1	0	9.10
15	0	1	-1	0	6.90
16	0	1	1	0	18.80
17	-1	0	-1	0	6.40
18	-1	0	1	0	10.40
19	1	0	-1	0	7.70
20	1	0	1	0	11.30
21	0	-1	0	-1	3.60
22	0	-1	0	1	6.30
23	0	1	0	-1	6.20
24	0	1	0	1	13.72
25	0	0	0	0	10.10
26	0	0	0	0	8.90
27	0	0	0	0	9.20

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 3. The DH ranged from 1.19 to 18.8% at design points 5 and 16, respectively.

The ANOVA of the coefficients of the response surface model as provided by Eq. 4 were evaluated. Statistical analysis indicated that all the four hydrolysis factors (temperature, pH, enzyme/substrate ratio and time) had significant influence on DH. Similar observations have been reported in previous studies that show the combined effect of pH, temperature and enzyme/substrate ratio on the hydrolysis of some oil seeds (Taha and Ibrahim, 2002) and soy

protein (Adler-Nissen, 1986) and also having markedly influence on peptide bond cleavage in the protein substrate. The same effect was observed for whey protein concentrates (Mota *et al*., 2004).

Regression coefficients in their linear form (T, pH, E/S and t) as well as one quadratic term (t^2) were significant at $p< 0.05$. One crossproduct interaction (T t) was not significant at $p<0.05$.

The final response model equation to estimate the enzymatic hydrolysis in terms of DH of the defatted sesame flour is shown in Eq. 7.

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

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 effect on the DH 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 DH of 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 for study.

Canonical analysis showed a maximum predicted DH of 16.34% and had the following critical values for the hydrolysis factors: T = 58.79°C, pH = 8.32, E/S = 1.80% (% v/w of defatted sesame flour) and t = 82.85 min. The experimental DH was 15.78%.

Contour plots generated from the predicted data illustrated the effect of each pair of independent variables (T, pH, E/S and t) on DH as shown in Fig. 3a-f. As observed from the plots, 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*. (2007), when they optimized the hydrolysis of whey protein. An increase in DH was also achieved as the time increased up to a certain level beyond which DH slightly decreases. The decrease in percentage hydrolysis can be explained by the increasing denaturation of the protein molecules hence reducing its biological activity (Claver and Huiming, 2005). It could also result from the exhaustion of the substrate or reduction of substrate concentration if all other reaction conditions remained the same. Similar observations have been reported for hydrolytic response of food proteins using three different kind proteases (Hrckova *et al*., 2002).

The non-linear relation between time and DH as noticed in Fig. 3b, d and f implies that the hydrolytic reaction depends on the availability of susceptible peptide bonds on which the primary enzymatic attack is based and also the physical structure of the protein molecule.

The DH was optimized using the freeze dried hydrolysate following the critical values for maximizing DH for the hydrolysis factors. This result was not significantly different ($p<0.05$) compared to the optimal value predicted by the design model. It further confirms that the process could be used to optimize the DH on DSF protein hydrolysates using RSM. A high protein recovery by Alcalase and its low cost may provide an incentive for using it in commercial operations.

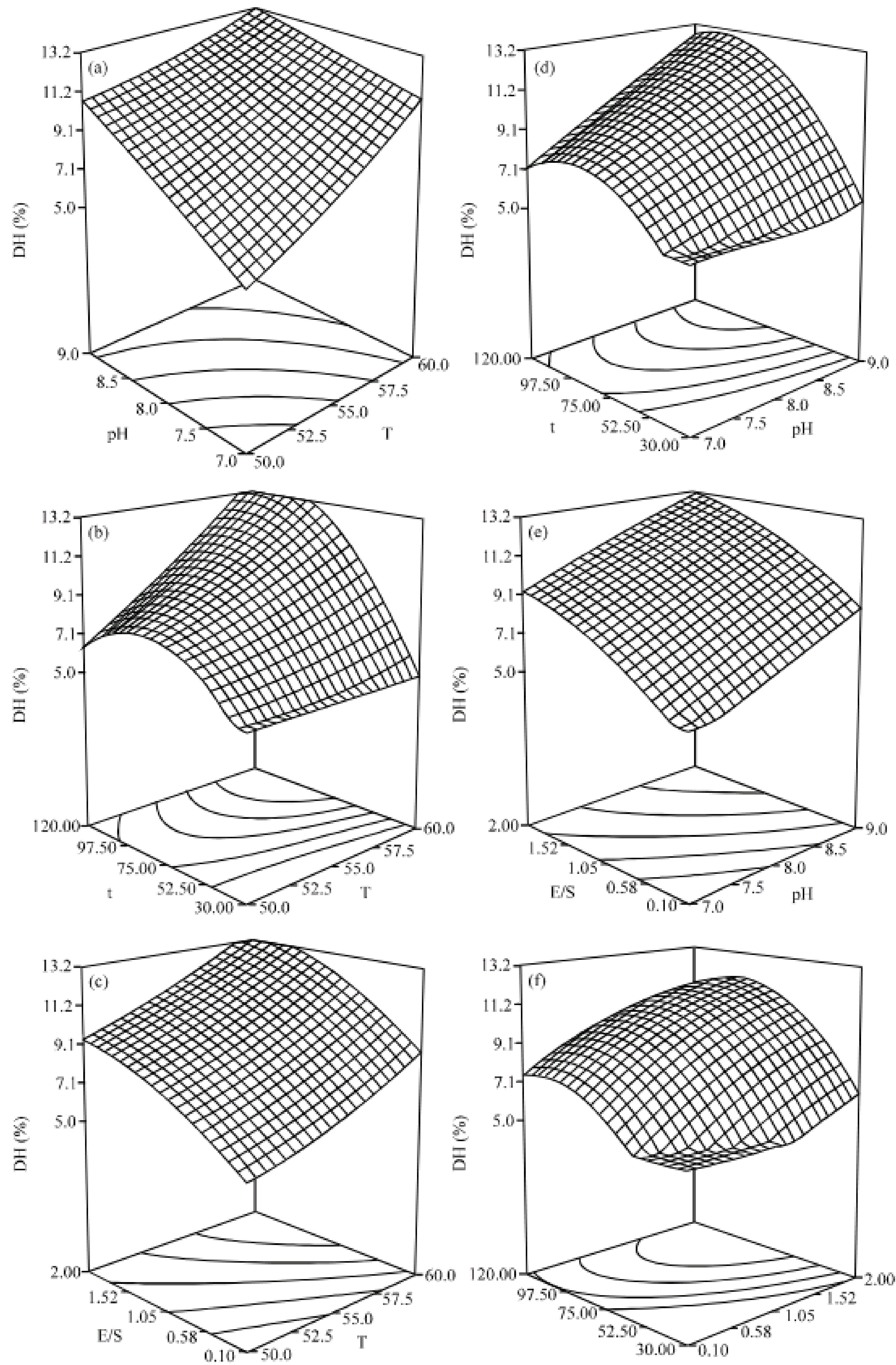

Fig. 3: Surface plots to show the combined effect of (a) pH and temperature, (b) time and temperature, (c) enzyme/substrate ratio and temperature, (d) time and pH, (e) enzyme/substrate ratio and pH and (f) enzyme/substrate ratio and time the on hydrolysis of defatted sesame flour by Alcalase®

Composition and Functional Properties of Defatted Sesame Flour and its Hydrolysate

The composition of defatted sesame flour and its hydrolysate is shown in Table 4. The hydrolysates contained a higher percentage protein (96.68%) compared to the native protein estimated at 52.04% as reported by John Kanu *et al*. (2007) after defatting the sesame flour. The moisture and ash content of the hydrolysate were estimated at 2.87 and 6.87%, respectively with insignificant amounts of fat and carbohydrate. A Significant increase of protein was observed when the DSF was hydrolyzed and this observation may be attributed to the degrading process by the enzymes that would have resulted to the release of more protein molecules trapped within the flour. Ash content is normally high in defatted sesame protein hydrolysates (Bandyopadhyay and Ghosh, 2002). The insignificant amounts of fat and carbohydrate could be attributed to the fact that the two components most times become diminished during protein extraction. The protein content of defatted sesame flour hydrolysate ranging between 93 to 98% is similar to that previously reported by Bandyopadhyay and Ghosh (2002).

The solubility was observed to increase gradually from pH 2 to 7 with a slight decrease at pH 8 but maintained afterwards as shown in Fig. 4. The native protein was lower in solubility right through the experiment and was observed to decrease between pH 4-5. The solubility of the hydrolysate obtained in this work differs on the grounds that solubility always fall within the isoelectric points (pI) of most protein isolates (Khalida *et al*., 2003). 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 (Mahmoud, 1994). Related studies have demonstrated that enzymatic hydrolysis of soy protein (Bernard-Don *et al*., 1991; Tsumura *et al*., 2005; Jung *et al*., 2005) and whey protein (Sinha *et al*., 2007) produced good functional properties, particularly solubility, as nitrogen

Table 4: Proximate analysis (%) of defatted sesame flour protein hydrolysates

Sample	DSF	Hydrolysate
Moisture	3.83±0.05[a]	2.87±0.05[a]
Protein	54.07±1.75[b]	96.68±1.56[b]
Fat	3.94±0.02[a]	ND
Ash	6.89±0.60[a]	6.87±0.60[a]
Carbohydrate	16.75±1.56[b]	ND

Values are Mean±SEM (n = 3), different letter(s) in the same column are not significant at level ($p<0.05$) but significant at $p<0.01$, DSF: Defatted Sesame Flour, ND: Not Detected

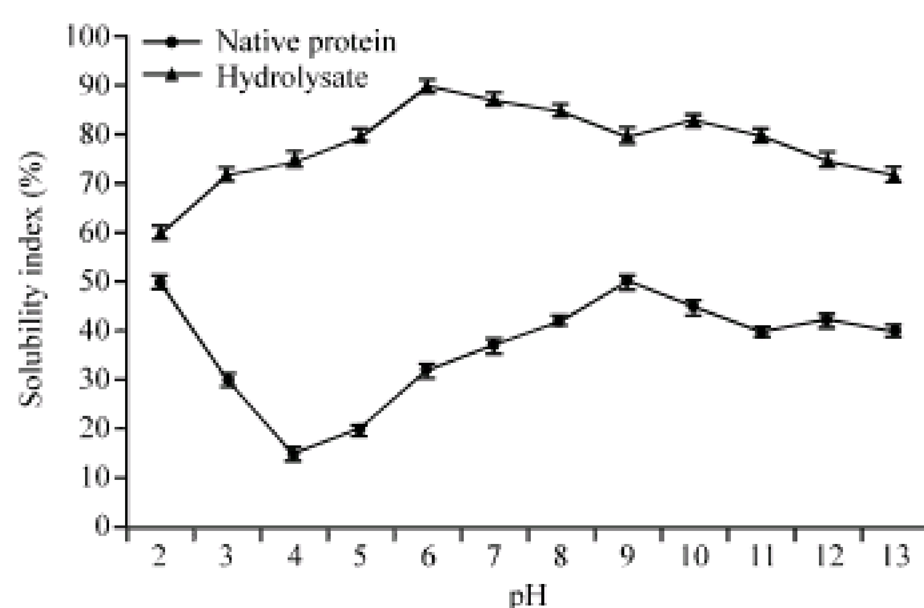

Fig. 4: Solubility of the hydrolysate and NP in water as affected by pH using Alcalase assisted hydrolysis process

Table 5: Functional properties of defatted sesame flour protein non-hydrolyzed and hydrolyzed

	Results	
Functional properties	Native protein	Hydrolysate
Water holding capacity (%)	40.20±0.2[a]	82.20±0.7[b]
Oil holding capacity (%)	46.60±0.5[b]	74.50±1.3[b]
Emulsification capacity (%)	45.80±2.3[a]	73.10±3.1[a]
Foaming capacity (%)	39.80±1.3[a]	85.10±2.1[a]
Foam stability (%)		
5 min	25.90	58.25
10 min	20.30	40.42
20 min	12.50	25.21
40 min	7.97	15.40
60 min	4.44	9.14

Values are Mean±SEM (n = 3), different letter(s) in the same column are not significant at level ($p<0.05$) but significant at ($p<0.01$)

solubility of the hydrolysate was pH dependent over the range studied. Such behavior 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 usually formed even at isoelectric pH (Cheison *et al*., 2007). The surface of protein has a net charge that depends on the number and identities of the charged amino acids and on pH. There could have been different charges generated at the protein surface after the hydrolysis process. These charges make the hydrolysates more soluble as without a net charge, protein-protein interaction and precipitation are more likely to occur which could have been reflected in a decrease in the solubility of the protein hydrolysates. The results of other functional properties of defatted sesame flour hydrolysates prepared from Alcalase treatment are presented in Table 5. The hydrolysate produced was highly water-soluble with good water and oil holding capacities and an enhanced emulsifying property than the DSF. Foaming capacity was observed to be higher than the DSF but its stability over a period of time (5-60 min) was however not good. The high foaming capacity could be attributed to the protein in dispersion causing a decrease in surface tension at the water air interface thus leading to a higher foam forming tendency (Taha and Ibrahim, 2002). High foaming capacity is also determined by molecular flexibility and physicochemical properties (hydrophobicity, net charge distribution, hydrodynamic properties) of the proteins. To foam effectively (to develop high foamability), a protein needs to adsorb air rapidly during the transient stage of foam formation (Martin *et al*., 2002; Gonzalez-Perez *et al*., 2005) and it is envisaged that this phenomenum could have influenced the above result. Other results indicate an increase in surface activity, probably due to the initially greater number of polypeptide chains which may have been formed from the proteolytic activities, thus, allowing more air to be incorporated into the hydrolysates (Khalida *et al*., 2003). For the emulsifying capacity, it was observed that the hydrolysate was a good emulsifier than the DSF and according to the result in Table 5 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-the surfactant possessing both hydrophilic and hydrophobic properties that can interact with both water and oil in the food system, thus, making the protein a good emulsifier. (Martin *et al*., 2002). It is also possible that the protein molecules on the surface unfolded enough thus exposing the hydrophobic groups. This phenomenon could also enhance the emulsifying capability of the protein. Water holding capacity was also observed to be significantly higher in the hydrolysate compared to the native protein. The interaction of water and oil with protein is very important in food systems because of its effect on flavor and texture. Intrinsic factors affecting water holding capacity of food proteins include amino

acid composition, protein conformation and surface polarity/hydrophilicity (Barbut, 1996). However, the method of protein extraction also had an important impact on the protein conformation and hydrophobicity/hydrophilicity which could lead to an enhanced water holding capacity of the protein. During hydrolysis the enzymes denaturated the protein molecules into smaller peptides, exposing more sites for water interaction with the protein molecules (Barbut, 1996). Oil holding capacity of the hydrolysate was also observed to be significantly higher compared to DSF. This could be attributed to the fact that the protease degraded the defatted sesame flour protein from its original protein structure to various peptides, thus exposing more hydrophobic groups to the oil interface resulting to an increased oil holding capacity (Barbut, 1996), since denaturation can improve the oil holding capacity of proteins due to increased hydrophobic surface and flexibility of the protein molecules.

CONCLUSION

Protein hydrolysates were produced from defatted sesame flour using different kinds of proteases to enhance higher protein recovery carried out by imposing engineered reaction conditions to optimize the degree of hydrolysis. The results show Alcalase 2.4 L to be a better enzyme in the preparation of protein hydrosylates from defatted sesame flour compared to the other proteases used in this work. Its effect produced the highest protein recovery and the hydrolysates obtained showed good functional properties. The study reveals a better alternative to protein enrichment procedures from plant materials and could serve as a blue print for the commercial production of protein supplements from plant materials. The findings prove the designed technique could be useful in the food industry especially in the realm of producing food formulations requiring high protein supplements. It could be applied in production of infant food formulations or poultry feeds etc with possible remarkable outcomes. The success of this technique further stresses the central role biotechnology plays in the food industry as efforts are stepped up to move away from conventional chemical food treatment procedures.

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