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Enzymatic Modification of White Defatted Sesame Flour: Effects of Alcalase on Amino Acid Profile, Molecular Weight Distribution, Chromatic Attributes, and Functional Properties

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Introduction

Sesame (*Sesamum indicum L.*) was reported to be a very important source of protein for human consumption because of the presence of Sulphur-containing amino acids, mainly methionine [1]. For plant protein to be useful and successful in food application, they should ideally possess several desirable characteristics, such as functional properties, as well as providing essential amino acids, molecular weight, and appealing colour of the protein hydrolysates [2]. These properties are intrinsically responsible for the physicochemical properties, which affect the behaviour of protein in food systems during processing and storage [3]. Proteins have unique surface properties due to their large molecular size and their amphiphilic properties. However, the industrial applications of food proteins are limited, because proteins are generally unstable to heating, organic solvents, and proteolytic attack [4]. Attempts have been made to modify plant proteins to improve their physical functionality, i.e. gelation, viscosity, emulsification, and foaming by using chemicals to hydrolyze the protein [4]. Several molecular parameters, such as mass, conformation, flexibility, net charge, and hydrophobicity, as well as interaction with other food components, have already been shown to play an important part, in both their emulsifying and foaming properties [5]. However, most chemical modifications are not now too desirable to the food industry [6,7].

Many studies have demonstrated the selective enzymatic hydrolysis of soy protein [8]. peanut protein [9], and whey protein [7], improved their functional properties, including solubility, water and oil holding, emulsifying, and foaming characteristics. Similarly, proteins extracted from defatted sesame flour have been modified by enzymatic hydrolysis to improve the quality and functional characteristics [1]. The choice of substrate, the protease enzyme employed, and the degree of hydrolysis (DH) can greatly affect the physicochemical properties of the resultant hydrolysate. However, limited information is available on the effect of enzymatic hydrolysis with Alcalase on the functional properties of defatted sesame protein hydrolysates (DSPH) and other parameters. The objective of this study was to study the effects of hydrolysis using Alcalase® on the colour, amino acid profile, and molecular weight of protein hydrolysate from the defatted sesame flour (DSF) and some functional properties.

Materials and Methods

Sesame seeds obtained from a local supermarket were ground and defatted as according to Kanu et al. [10], and kept in a freezer at -10ºC till needed for the experiments. Alcalase® (a declared activity of 2.4 AU/kg and a density of 1.18g/ml), a bacterial Endo-proteinase from a strain of *Bacillus licheniformis* which was provided by Novo Nordisk's Enzyme Business in Wuxi, PR China, and stored at 5ºC until use. Other chemicals and reagents were obtained from local manufacturers (Sinopharm Chemical Reagent Co., Ltd, Shanghai.

Methods

Preparation of Protein Hydrolysates

Three hydrolyzed samples with varying hydrolysis (DH) were produced as described in (Table 1). The working temperature and pH were selected depending upon the activity of the enzyme used as alcalase could work better in low alkaline environment at a particular range of temperature as recommended by the supplier. All reactions were done in triplicates in a 1 l polyethylene-jacketed glass enzyme reactor vessel in a thermostatically controlled water bath (NUOHAI- XMTD-204, Tokyo, Japan) with constant mixing (700 rpm). The vessel was covered with openings 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 alkali. During the reactions, pH was maintained at the desired level according to the experimental design of (Table 1) by the addition of 0.2 m NaOH.

The reaction vessel with 50 g of previously mixed defatted sesame flour and a measured volume (500 ml) of de-ionized water was placed in a previously heated water bath. Homogenization was carried out for 5 min with the adjustment of pH (through the addition of 0.2 m NaOH) and temperature to the desired levels. When the 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 DH.

Table 1: Characteristics of samples used in the evaluation.

	Sample characteristics				
	T	pH	t	E/S	DH
DH 1	40	7.5	70	1	5
DH 2	50	8	65	2	10.4
DH 3	55	9	60	3	17.94

T: Temperature (°C), t: Time (min), E/S: Enzyme/Substrate (%vw of DSF), DH: Degree of hydrolysis.

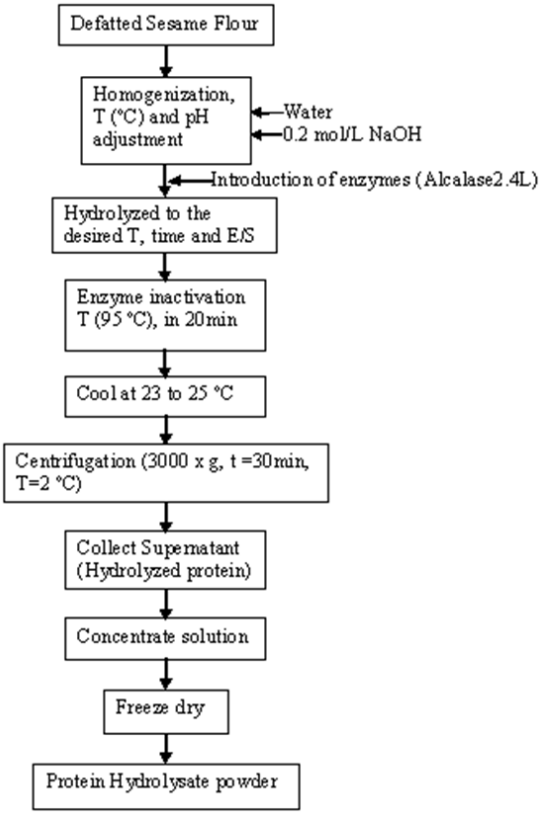

Figure 1: Flow chart for alcalase-assisted in the preparation of defatted sesame flour protein hydrolysate powder.

The reactions were terminated by immersing the reaction vessel in hot water at 95 ºC for 15 min with continuous stirring to ensure the inactivation of enzyme. The resulting slurry was cooled on ice and centrifuged at 2800 x g for 20 min at 2ºC. The supernatant was collected and freeze dried. The freeze-dried hydrolysates were stored in a desiccator until used in functional property, amino acid, molecular weight, and colour analyses. Figure 1 outlines the process used in the preparation of protein hydrolysate from DSF.

Estimation of Degree of Hydrolysis

The hydrolysis was carried out using the pH-stat method as described according to section 4.2.2.3.

Proximate Composition

Moisture content was determined as described in section 2.2.2.1.2. Ash content was estimated as described in section 2.2.2.1.3. The total protein (N x 6.25) content was determined as in section 2.2.2.1.1. The extraction and determination of fat from the samples was performed as described in section 2.2.2.1.7.

Water holding capacity (WHC)

The WHC was determined as described in section 4.2.2.8.

Oil-holding capacity (OHC)

This was measured as explained in section 4.2.2.8

Emulsification Capacity (EC)

This was measured according to the description in section 4.2.2.10

Foaming Capacity (FC)

This was evaluated according to as explained in section 4.2.2.11.

Colour Measurement

The colour of the hydrolysate powder was evaluated using the Hunter Lab colorimeter (WSC-S Colour Difference Meter, USA) and reported as L*, a*, and b* values, in which L* is a measure of lightness, a* represents the chromatic scale from green to red and b* represents the chromatic scale from blue to yellow. The instrument was standardized to measure the colour difference with an L* value of 91.32, an a* value of 0.03, and a b* value of 0.01. The three different levels of DH were investigated to see which one has appealing colour to the consumers.

Molecular Weight Distribution of the Hydrolysates

This was determined using a WatersTM 600E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA). A TSK gel 2000SWXL (7.8 x 300 mm) column was used with 10% acetonitrile + 0.1% TFA in HPLC grade water as the mobile phase. The calibration curve was obtained by running bovine carbonic anhydrase (29,000

Da), horse heart cytochrome C (12,400 Da), bovine insulin (5800 kDa), bacitracin (1450 Da), gly-gly-tyr-arg (451 kDa), and gly-gly-gly (189 Da). The results obtained were processed with the aid of Millennium 32 Version 3.05 software (Waters Corporation, Milford, MA 01757, USA). Molecular weight distribution of defatted sesame protein, sesame protein from defatted sesame flour extracted with water, and the sesame protein hydrolysates were determined by Sodium dodecyl sulfate-polyacrylamide

gel electrophoresis (SDS-PAGE), according to the procedure of Schagger & Von-Jagow [11], as modified by Chesion et al. [7], using 15% Tricine gels. The electrophoresis was run at 50 mA in 1.00 mm thick gels. The gels were stained with Coomassie blue. The approximate molecular weight of the hydrolysate was determined using low molecular weight standards obtained from Sigma Chemical Company, St. Louis, MO, USA.

Amino Acid Analysis

The hydrolysate exhibiting the highest DH was used for the analysis of amino acid profile as described in section 2.2.2.1.9.

Determination of *in vitro* protein digestibility (IVPD)

The method of Saunders et al. [12]., was used with slight modifications to determine the in vitro protein digestibility of the hydrolysate from the highest DH, thus with the highest protein recovery. The sample (5g) were placed in a 50 mL centrifuge tube, to which 15 ml of 0.1N HCL containing 1.5 Mg pepsin was added and then the tube was incubated at 37 ºC for 3h. The suspension was then neutralized with phosphate buffer (pH 8.0), containing 0.005 M sodium azide. 1 ml of toluene was added to prevent microbial growth and the mixture was then gently shaken and incubated for additional 24 h at 37 ºC. After incubation, the sample was treated with 10 mL of 10% trichloroacetic acid (TCA) and centrifuged at 5,000 rpm for 20 min at room temperature. The protein in the supernatant was estimated using the Kjeldahl method. The percentage of protein digestibility was calculated using equation 1:

$$\text{Protein Digestibility (\%)} = \frac{\text{Protein in Supernatant}}{\text{Protein in sample}} \times 100 \quad \ldots\ldots \text{Eq. (1)}$$

Determination of *in vitro* Enzymic Protein Efficiency Ratio (E-PER)

The computed E-PER was determined using the model developed by Ihekoronye [13]. This model provides estimates of protein quality based on amino acid profiles after enzymatic proteolysis. The predictive model was defined as shown in equation 1;

$$\text{E-PER} = -3.02 + 0.14\,(\text{Asp}) + 0.15\,(\text{Glu}) - 0.18\,(\text{Pro}) + 0.14\,(\text{Ala}) + 0.52\,(\text{Met}) + 0.21\,(\text{Lys}) + 0.09\,(\text{Arg}) - 0.45\,(\text{Trp}) \quad \ldots\ldots \text{Eq. (2)}$$

Calculation of Chemical Score, Essential Amino Acid Index, Biological Value and Nutritional Index

Chemical score which is based on the amount of the most limiting amino acid present in the test protein relative to the amount of that amino acid in reference egg protein was calculated using the equation 2 given by Block & Mitchel [14] as follows:

$$\text{Chemical Score} = \frac{\text{EAA in test protein (g)}}{\text{EAA in test protein (g)}} \times \frac{\text{Total EAA in egg (g)}}{\text{EAA in egg (g)}} \times 100$$

$$\ldots\ldots \text{Eq. (3)}$$

Essential amino acid index (EAA Index) was calculated according to the procedure of Oser taking into account the ratio of EAA in the test protein relative to their respective amounts in whole egg protein following the equations 4 and 5 given below:

$$\text{EAA Index} = \sqrt[10]{\frac{100a}{a_e} \times \frac{100b}{b_e} \times \cdots \times \frac{100j}{j_e}} \quad \ldots\ldots \text{Eq (4)}$$

$$\text{Or log EAA Index} = \frac{1}{10}\left(\log\frac{100a}{a_e} + \log\frac{100b}{b_e} + \cdots + \log\frac{100j}{j_e}\right) \quad \ldots\ldots \text{Eq (5)}$$

Where a, b,…j are the % of each of the ten essential amino acids (EAA) in the food protein (NX 6.25) and a_e, b_e,…….j_e are the % of respective amino acids in whole egg protein.

The biological value (BV) was calculated using the formula of Oser [15] given below in equation 6:

$$\text{BV} = 1.09\,(\text{EAA Index}) - 11.7 \quad \ldots\ldots \text{Eq. (6)}$$

The nutritional index (NI was calculated using the equation given by Crisan & Sands [16]. Given below in equation 7:

$$\text{NI} = \frac{\textit{EAA Index} \times \textit{\% protein Content}}{100} \quad \ldots\ldots \text{Eq. (7)}$$

Statistical Analysis

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

Results and Discussion

Protein and ash contents of the hydrolysates were significantly higher ($p < 0.05$) than those of the native defatted sesame flour but the fat content was significantly ($p < 0.05$) lower for all 3 hydrolysates (Table 2). The protein contents for the three DH were almost the same and no significant differences ($p < 0.05$) were observed. The increase in protein content of the hydrolysates could be attributed to the fact that the oil-trapped protein was released when the oil was removed in the hydrolysis process. The ash is due to the addition of the NaOH for the control of the working pH as all the working pH (7.5, 8, 9) were brought down when the enzymes were added. During the addition of NaOH to keep the pH at the working level, salt was accumulated in the process. It was observed that DH3 had the highest ash content accumulated and the amount was significantly different ($p < 0.05$), followed by DH2 and DH1 (Table 2). This could be due to the fact that DH3 has the highest working pH and needs lots of NaOH during the hydrolysis process. The reduction of oil in all 3 DH was achieved because when the sample was subjected to heat and centrifugation, the oil content was removed further from the sample.

Table 2: Proximate composition (%) of DSFP and its hydrolysate.

	Moisture	Protein	Fat	Ash
Native DSFP	3.83 ± 0.05a	54.07 ± 1.75b	3.94 ± 0.02a	6.89 ± 1.56 b
DH1	2.0 ± 1.09a	89.8 ± 1.84a	0.5 ± 0.46a	9.6 ± 0.74a
DH2	1.8 ± 0.51a	90.4 ± 1.84a	0.9 ± 1.09a	10.3 ± 0.62a
DH3	2.48 ± 1.07a	93.89 ± 1.84a	0.3 ± 0.34a	12.4 ± 1.45b

[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 centrifugation, the various components in the slurry separated, during which process the oil floats while the other components that are not protein was separated. The floated oil was skimmed from the sample. Our results corroborated those of Chabanon et al. [17].

The DSF protein hydrolysates exhibited good WHC for all 3 levels of degree of hydrolysis (Table 3). Several studies have shown that soy protein hydrolysates [18]. and rapeseed protein hydrolysates [17], have excellent WHC and can increase the cooking yield when added to foods. WHC increased with increased hydrolysis and the values were statistically different ($p< 0.05$) for all the samples (Table 3). A similar trend in WHC was observed for whey protein hydrolysate with increased degree of hydrolysis from 75-87 % [19]. Our results also corroborated those of Dench et al. [20], for sesame flour and two protein isolates. The presence of polar groups such as COOH and NH_2 that increased during enzymatic hydrolysis had a substantial effect on the amount of adsorbed water [21]. Nonetheless, the native protein had lower WHC than the DH1, DH2 and DH3. This signifies that the functional properties of the three will have a better attribute than the native protein from defatted sesame flour.

Table 3: Functional properties of hydrolyzed DSF.

	WHC (%)	OHC (%)	EC (%)	FC (%)
NP-DSF	40.2 ± 0.02a	46.6 ± 0.5b	45.8 ± 2.3a	39.8 ± 1.3a
DH 1	78 ± 0.3a	65.2 ± 0.73b	62.4 ± 0.04b	70 ± 43 a
DH 2	80 ± 0.05b	67 ± 0.50a	65 ± 0.61b	76 ± 05 a
DH 3	82.5 ± 0.45a	73.2 ± 0.09a	74.4 ± 1.06b	85.7 ± 17 b

NP: Native Protein, WHC: Water-holding capacity, OHC: Oil-holding capacity, EC: Emulsion capacity, FC: Foam capacity

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

Table 4: Hunter colour parameter values of hydrolyzed defatted sesame flour.

	L*	a*	b*
DH 1	69.9 ± 0.06a	-5.7 ± 1.3a	20.1 ± 0.03a
DH 2	56.5 ± 0.24a	-3.6 ± 0.52b	25.1 ± 0.04a
DH 3	53.8 ± 0.56a	-1.4 ± 0.07a	29.7 ± 0.08a

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

Defatted sesame flour hydrolysates at different DH showed excellent OHC (Table 3). OHC expresses the quantity of oil directly bound by the protein and is of great interest as it is an important functional characteristic, especially in meat and confectionary industry. The hydrolysates at 5.0% DH had a significantly higher ($p< 0.05$) OHC followed by

10.4% DH and 17.92% DH than the native protein. An increase in OHC with DH increase has also been reported for rapeseed protein hydrolysis [17,22], and soy protein hydrolysates [18]. The increase in the OHC with increase in DH might have been due to the hydrolytic degradation of the protein structure. Protein is in the form of a network which is degraded by hydrolysis with consequent attracting of the sample's oil absorption capacity [23]. Other studies indicated that hydrophobic interactions are primarily responsible for that [24].

Table 5: Molecular weight distribution (% of total area) of defatted sesame flour hydrolysate.

Molecular Weight	Area		
(Da)	DH 1	DH 2	DH 3
>2500	25.4	-	1
1500-2500	17.1	13.4	7.4
1000-1500	18.5	19.7	16.2
500-1000	21.2	34.7	37.7
<500	14.8	32.3	38.7

The EC of the hydrolysates increased with increased protein hydrolysis (Table 3). A similar finding on the relationship of DH to emulsifying capacity has been reported by Darwicz et al. [25]. This may be as a result of the presence of smaller peptides, which are very effective in stabilizing emulsions [26]. Similar results on EC of whey protein hydrolysate showed an increasing trend with increasing DH [19]. An increase in EC of casein with increased DH in the range of 25-65% using porcine pancreatin has been reported by Mahmoud [27]. The higher EC value obtained at 17.92% hydrolysis can be attributed to the high level of degradation of the protein molecules by Alcalase which brought about the increase in the availability of smaller peptide units at the oil-water interface thereby providing a surface area for the interaction of water and oil [28]. Our results were compared with those of Chove et al. [29]. They studied the emulsifying properties of soy protein isolates and they are not significantly different ($p< 0.05$). The significant decrease in EC at 5.0 % hydrolysis was presumably due to the effect of pH during the hydrolysis, but when the pH increased during the process it reduced peptide chain length and increased hydrophobicity as more hydrophobic amino acids (alanine, isoleucine, leucine, methionine, phenylalanine, proline, tyrosine and valine) might have been released to increase the hydrophobic properties of the hydrolysates [24]. Generally, the pH of protein solution during emulsification affects the emulsifying properties via charge effects [30]. Addition of salts improves the emulsion properties of peptide fractions [21]. Several factors such as blending speed, protein source, temperature, pH, type of oil added and water content influence EC [31]. The native protein had lower EC as compared to all DH. This might be because in native protein most of the nonpolar amino acid side chains are located in the interior of the molecules. As the protein molecule approaches the interface, there is less opportunity for the charged groups to interact with the solvent. In the extreme case, charged groups are removed from the aqueous phase and enter the lipid phase [32]. This is energetically unfavorable and these groups are repelled from the interfacial area but if the groups nearing the interface are in a region of the protein molecule that contains some flexibility, then the molecule may begin to unfold. This unfolding cause the exposure of hydrophobic groups to the surface [32] as also observed in our present study. FC increased gradually from DH1 to DH3 (Table 5.3) agreeing with the findings of Martin et al. [33]. To possess high foamability, a protein needs to adsorb rapidly during the transient stage of foam formation [33,34]. These results suggest an increase in surface activity, probably due to the initially

greater number of polypeptide chains [35]. which were formed from partial proteolysis, allowing more air to be incorporated.

Colour influences the overall acceptability of food products [36]. Hydrolysis of DSF produced protein powders that were light yellow in colour (Table 4). DH3 sample was the darkest (L* = 53.8) and most yellowish (b* = 29.7) whereas DH1 sample was the lightest (L* = 69.9) and least yellowish (b* = 20.1). Increased time of hydrolysis resulted in increased enzymatic browning reactions. Enzymatic browning reactions are assumed to have contributed to reduce the luminosity, giving a darker appearance at high DH [9]. The b* value was significantly ($p< 0.05$) different for all samples.

Chain length of peptides, depending on DH, is of special interest because properties such as EC and bitterness depend at least in part on molecular size [37]. Figure 2 shows the size exclusion chromatography profiles of DSF hydrolysates. The major components in the hydrolysates had a molecular weight of 150–2500 Da. The molecular weight distributions of the 3 hydrolysates were different as the relative proportions of the peaks varied with DH. The average molecular weight of the peptides was below 3500 Da and that the peptide range repartition depends on DH (Table 5). Hydrolysates with lower DH (5.0%) were characterized by a higher % of high molecular weight peptides ranging from 2500 to 3500 Da compared with hydrolysates with higher DH (17.92%) that were characterized by low molecular weight peptides ranging from 150 to 500 Da. The proportion of the high molecular weight peptides decreased as the DH increased as evidenced with sunflower products [38]. This could be viewed as holding potential in the area of food protein from sesame as small molecular mass distribution of hydrolysates have the potential ability to reduce the allergencity of sesame protein [39]. It could also be the responsible factor of hydrolysates to display the superior functional attributes observed in this work as compared to the native protein of DSF.

SDS-PAGE of defatted sesame flour, native protein isolated with water from defatted sesame flour, and the sesame protein hydrolysates is shown in Figure 3. It was observed that lane 1 shows one main band this shows less degradation in the protein matrix was done in it the same could be seen in lane 2, it shows very weak bands but lane 3 shows

Different band indicating many polypeptides have been developed during hydrolysis which shows that the hydrolysates composed of low molecular weight proteins and different peptides with many bands as shown in (Figure 3). In the absence of enzyme treatment, in untreated flour the protein had a low mobility because of the high molecular size of protein (lane 1). The protein has a high mobility after the enzyme treatment because of the proteolytic degradation, and the presence of a larger number of lower molecular weight protein bands in the sesame protein hydrolysates (lane 3), compared to the other samples. The amino acid composition did differ significantly between the native DSF protein sample before hydrolysis and the resultant hydrolysate (Table 6). This could be

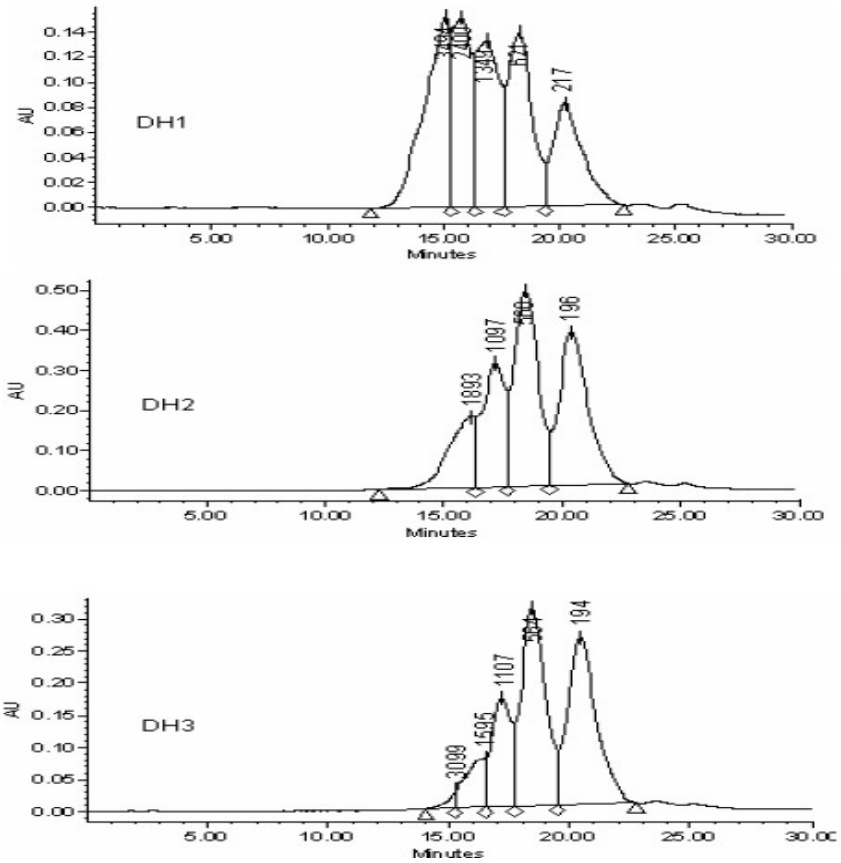

Figure 2: Size exclusion chromatography profiles of defatted sesame flour hydrolysates.

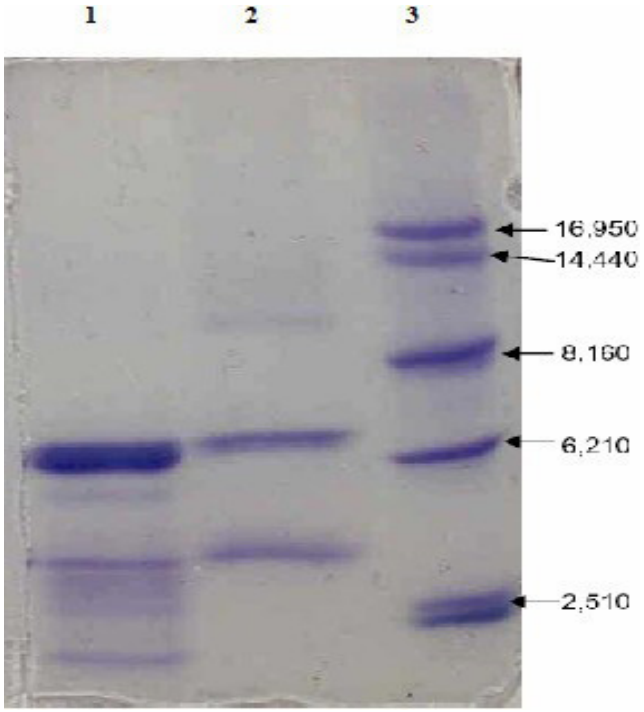

Figure 3: SDS-PAGE lane (1) defatted sesame protein, lane (2) sesame protein from defatted sesame flour extracted with water, lane (3) sesame hydrolysate. The positions and molecular masses of standards are indicated on the right.

attributed to the fact that, in the process of hydrolysis, more amino acids were released during the protein degradation.

Table 6: Total amino acid composition of native defatted sesame protein and its hydrolysate (g 100 g protein).

Amino acid	NP- DSF	Hydrolysate	EAAa	
EAA			**Infant**	**Adult**
Valine	5.18	6.72	3.5	1.3
Isoleucine	4.29	5.64	2.8	1.3
Leucine	7.5	8.95	6.6	1.9
Methionine	3.46	6.52	2.5b	1.7b
Lysine	3.3	4.04	5.8	1.6
Histidine	3.09	4.7	1.9	1.6
Threonine	4.29	6.18	3.4	0.9
Tryptophan	1.87	2.5	1.1	0.05
nEAA				
Alanine	3.37	4.46		
Glycine	3.33	5.17		
Proline	1.31	2.33		
Phenylalanine	4.58	5.52		
Tyrosine	3.84	5.78		
Serine	3.14	4.61		
Arginine	4.39	6.21		
Aspartic acid	8.95	11.55		
Glutamic acid	17.68	19.74		

[a]Suggested profile of essential amino acid requirement for infant and adult, FAO/WHO[40]. NP = Native protein. EAA = Essential amino acids, nEAA = Non Essential amino acids. [b]Methionine + Cysteine

The results show that the amino acid profiles of the DSF hydrolysates were generally higher in essential amino acids than required by the FAO/WHO for infants and adults [40]. Therefore, hydrolysates from DSF could be used as supplements in various foods.

The IVPD along with some nutritional parameters are shown in Table 7. The results indicated that enzymatic hydrolysis of DSF by Alcalase 2.4L improved the *in vitro* protein digestibility of their hydrolysates. The improvement was due to the increase in solubility and the denaturation of these protein molecules making them more accessible to proteolytic enzymes. These results are in agreement with those of Clementes [7]. *In vitro* studies showed that DSF hydrolysates had higher digestibility compared to the unhydrolyzed DSF. Obviously, this will facilitate the availability of the amino acids. The IVPD values of DSF hydrolysates were higher than those of the DSF only. This difference could be explained by the fact that protein matrix of DSF was still intact while those of the DSF hydrolysates have been denatured by the enzymes during hydrolysis. It is postulated that protein structure played an important role in increasing IVPD.

Table 7: IVPD and some nutritional indices of DSF and DSF Hydrolysates.

Parameters	DSF (Native Protein) (%)	DSF Hydrolysates (%) EAA
Index	34.43	80.26
Predicted BV	30.56	79.47
NI	48.75	85.36
E-PER	55.42	82.54
IVPD	51.48	87.75

DSF = Defatted sesame flour; EAA Index = Essential amino acid index; BV = Biological value; NI = Nutritional index; E-PER = Enzymic protein efficiency ratio; IVPD = *In vitro* protein digestibility

The computed E-PER values were lower for the DSF as compared to the DSF hydrolysates and the difference was significant. This result corroborated the findings of Bandyopadhyay & Ghosh [1]. These results suggested also that the differences in E-PER were

related to their nutritional quality and protein content. The NI value of the hydrolysates of DSF is higher than the DSF also. The DSF hydrolysates also showed higher EAA Index and BV than the DSF. All of the above scenarios will not be unconnected to the fact that during the enzymatic hydrolysis with Alcalase the protein was successful denatured releasing more amino acids than the DSF which protein remains intact which makes the release of the amino acid very difficult. These results gave the DSF hydrolysates good biological attributes for human consumption.

Conclusion

Protein hydrolysates were obtained from DSF using a commercial enzyme (Alcalase®) preparation. Functional properties of 3 hydrolysates (DH1, DH2, and DH3) were significantly different ($p< 0.05$) to those of the native protein of defatted sesame flour. We observed an increase in the WHC, EC, and FC from DH1 to DH3 while OHC decreased as the DH increased from DH1 to DH3 as compared to the native protein of the defatted sesame flour. The colour and molecular weight varied according to the various levels of DH, while the amino acid content of the defatted sesame protein hydrolysate differed significantly ($p< 0.05$) from that of the native defatted sesame protein and the essential amino acids were higher than FAO/WHO (1990) reports for both infants and adults. The IVPD and some nutritional indices were observed to be higher in the hydrolysate than in the DSF. Thus, the hydrolysates could be incorporated into food for human consumption because the small molecular mass distribution of hydrolysates has the potential ability to reduce the allergencity of sesame. Superior functional properties make the protein hydrolysates a potential competitor with dairy-based protein hydrolysates currently in use.

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