JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Preparation and Characterization of Papain-Modified Sesame (*Sesamum indicum* L.) Protein Isolates

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Defatted sesame meal (∼40−50% protein content) is very important as a protein source for human consumption due to the presence of sulfur-containing amino acids, mainly methionine. Sesame protein isolate (SPI) is produced from dehulled, defatted sesame meal and used as a starting material to produce protein hydrolysate by papain. Protein solubility at different pH values, emulsifying properties in terms of emulsion activity index (EAI) and emulsion stability index (ESI), foaming properties in terms of foam capacity (FC) and foam stability (FS), and molecular weight distribution of the SPI hydrolysates were investigated. Within 10 min of hydrolysis, the maximum cleavage of peptide bonds occurred as observed from the degree of hydrolysis. Protein hydrolysates have better functional properties than the original SPI. Significant increase in protein solubility, EAI, and ESI were observed. The greatest increase in solubility was observed between pH 5.0 and 7.0. The molecular weight of the hydrolysates was also reduced significantly during hydrolysis. These improved functional properties of different protein hydrolysates would make them useful products, especially in the food, pharmaceutical, and related industries.

KEYWORDS: Functional properties; molecular weight; papain; protein hydrolysate; *Sesamum indicum*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is grown extensively in tropical and subtropical areas as an important oilseed crop (*1*). Defatted sesame meal is rich in protein (40−50%), and it may be an excellent protein source for supplementing soybean, peanut, and other vegetable proteins, which lack sufficient sulfur-containing amino acids, mainly methionine (*2*, *3*).

Dehulling of sesame seed is necessary as the hull contains a very high amount of oxalic acid (2−3%), which could complex with calcium and reduce its bioavailability (*4*). The hull also contains indigestible fiber, which reduces the digestibility of the protein and imparts a dark color to the meal. The undesirable components such as fiber, soluble sugar, phytates, and oxalates can be eliminated to a large extent by producing protein isolates or concentrates from dehulled and defatted meal (*5*, *6*).

Proteolytic enzyme modification of protein is an effective way to improve the various functional properties and to increase the field of application of the protein (*7*−*9*). The peptides that are produced by partial hydrolysis of proteins have smaller molecular size and less secondary structure than the original proteins. The protein solubility, emulsifying properties, and foaming capacities can be improved with a limited degree of hydrolysis (*10*−*12*), whereas excessive hydrolysis often causes loss of some of these functionalities (*13*). Protein hydrolysates are widely used as nutritional supplements, functional ingredients, and flavor enhancers in foods, coffee whiteners, cosmetics, personal care products, and confectionery, and in the fortification of soft drinks and juices. Protein hydrolysates are also used in soups, sauces, gravies, snacks, meat products, and other savory applications (*14*, *15*).

Extensive protein hydrolysates can be used in special medical diets such as in the production of hypoallergenic foods for patients with reduced absorption surface or reduced digestive capacity (*16*). Sunflower protein hydrolysates are utilized for dietary treatment of patients with liver failure (*17*).

In the present study, protein isolate was produced from dehulled and defatted sesame meal by alkaline extraction followed by acidic precipitation. Protein hydrolysates, with variable degrees of hydrolysis, were prepared from protein isolate by papain and characterized by their functional properties to determine the potential application of these hydrolysates in various food products.

MATERIALS AND METHODS

Authentic sesame seed (brown variety) was collected from the District of Hooghly, West Bengal, India, during the Kharif crop, which is sown in June−July with the onset of the monsoon season and is harvested in January−February. Dehulling was done in a low-speed laboratory grinder (REMI, Ato-Mix Blender, Mumbai, India) followed by air classification. The deoiled meal was prepared by extraction with commercial hexane (food grade) in a Soxhlet apparatus. After complete extraction, the deoiled meals (both whole and dehulled seed meal) were desolventized in a vacuum oven at 60 °C and at 4 mmHg for 4 h. The deoiled meals were stored in a desiccator and used for further experiment.

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10.1021/jf020320x CCC: $22.00 © 2002 American Chemical Society
Published on Web 10/11/2002

Papain from papaya latex, having an activity of 100 TU (6000 NF units) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India, and was used for protein hydrolysis.

Preparation of Sesame Protein Isolate (SPI). Dehulled sesame meal was milled and passed through an 80-mesh screen (∼80% passed through) and mixed with water (1:10, w/v). The pH was adjusted to 9.5 with 1 N NaOH solution, stirred for 1 h at 50−55 °C, and then centrifuged at 4000*g* for 10 min (to remove the insoluble portion). The supernatant liquid was separated, the pH was adjusted to 4.9 with 1 N HCl (at isoelectric point), and the liquid was stirred for 1 h at 50−55 °C and again centrifuged at 8000*g* for 20 min. The solid residue was collected and dried in a vacuum oven at 50 °C for 10 h.

Preparation of Protein Hydrolysate by Papain. Five 10 g portions of SPI were each dispersed in 200 mL of distilled water, adjusted to pH 10 with 1.0 N NaOH, and incubated at 50 °C for 1 h with shaking. Each portion was then adjusted to pH 8.0 and hydrolyzed with 0.01 g of papain (0.1% w/w) at 37 °C for 10, 20, 30, 45, and 60 min with constant shaking. The resulting hydrolysates (PH10, PH20, PH30, PH45, and PH60, respectively) were adjusted to pH 8.0 and rapidly inactivated the enzymes by heating at 95 °C for 5 min. Each hydrolysate was then freeze-dried and stored at 4 °C. As a control an SPI was also treated similarly without added enzyme for 60 min (SPI60).

Total nitrogen was determined according to the Kjeldahl method. Crude protein content was estimated using a conversion factor of 6.25. Ash, fiber content, protein dispersability index (PDI), and nitrogen solubility index (NSI) were determined by using Official and Tentative Methods of the AOCS (*18*). Phytic acid was determined according to the method of Thompson et al. (*19*), and oxalic acid present in different test samples were determined by using the method of Deosthale (*20*).

Determination of Protein Solubility. To determine protein solubility, 20 mg of protein sample was dispersed in 20 mL of deionized water, adjusted to pH 3, 5, 7, and 9 with 0.1 N HCl or 0.1 N NaOH, magnetically stirred at ambient temperature for 30 min, and centrifuged at 12100*g* for 10 min. Protein contents were determined by using the Lowry et al. (*21*) method of protein assay with SPI60 as standard, for which the protein content was determined by using the Kjeldahl method. Protein solubility was calculated as

$$\text{solubility (\%)} = \text{protein content in supernatant/protein content in sample} \times 100$$

Emulsifying and Foaming Properties. Emulsifying properties were measured according to the method of Pearce and Kinsella (*22*). Pure corn oil (2 mL) and 6 mL of 0.1% protein solution (pH 8.0) were homogenized in a mechanical homogenizer (REMI Make) at the highest setting for 1 min. Fifty microliter portions of the emulsions were pipetted from the bottom of the container at 0 and 10 min after homogenization. Each portion was diluted with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbances of these diluted solutions were measured at 500 nm in a spectrophotometer (Shimadzu, UV-1601). The absorbances measured immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI)

$$\text{EAI (m}^2\text{/g)} = 2T[(A_0 \times \text{dilution factor})/(c \times \phi \times 10000)]$$

where $T = 2.303$, dilution factor = 100, and ϕ is the oil volume fraction of the emulsion.

$$\text{ESI (min)} = A_0 \times \Delta t/\Delta A$$

where $\Delta t = 10$ min and $\Delta A = A_0 - A_{10}$.

Foaming properties in terms of foam capacity (FC) and foam stability (FS) were determined by using the method of Okezie and Bello (*23*). It was expressed as percentage volume increase and calculated as

$$\text{FC} = (\text{vol after whipping} - \text{vol before whipping})/\text{vol before whipping} \times 100$$

FS was determined by measuring the foam height at 10, 30, 45, and 60 min. Both the FC and FS were determined at pH 8.0 and at room temperature (31 ± 2 °C).

Table 1. Composition and Properties of Whole and Dehulled Sesame Seed Meal[a]

property	whole seed meal	dehulled seed meal
moisture (%)	4.81 ± 0.21	5.11 ± 0.30
protein (%)	39.82 ± 0.22	53.50 ± 0.41
fiber (%)	28.32 ± 0.31	10.14 ± 0.12
ash (%)	8.63 ± 0.23	7.41 ± 0.20
phytic acid (%)	1.62 ± 0.11	1.71 ± 0.12
oxalic acid (%)	3.32 ± 0.21	0.28 ± 0.03
PDI	30.0 ± 1.0	40.2 ± 1.2
NSI	23.0 ± 1.1	33.0 ± 1.3

[a] Values are mean ± SD, $n = 3$.

Table 2. Characteristics of Sesame Protein Isolate (SPI) Prepared from Dehulled Sesame Meal[a]

property	composition	property	composition
yield (%)	47.0 ± 5.0	phytic acid (%)	0.04 ± 0.01
protein (%)	80.01 ± 0.31	oxalic acid (%)	0.11 ± 0.01
fiber (%)	2.33 ± 0.14	PDI	115.2 ± 1.3
ash (%)	3.21 ± 0.33	NSI	43.0 ± 1.5

[a] Values are mean ± SD, $n = 3$.

Degree of Hydrolysis. Degree of hydrolysis, defined as the percentage of peptide bonds cleaved, was calculated by the determination of free amino groups by reaction with 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Sigma Chemical Co., St. Louis, MO) according to the method of Alder-Nissen (*24*), where the pH of the medium was maintained at 8.2 using phosphate buffer. The total number of amino groups was determined in a sample 100% hydrolyzed at 110 °C for 24 h in 6 N HCl (10 mg sample in 4 mL of HCl).

Molecular Weight Distribution. Molecular weight distributions of SPI60 and protein hydrolysates were determined by capillary SDS gel electrophoresis (P/ACE System 5010, Beckman Instruments Inc., Fullerton, CA) (*25*) using a P/ACE UV absorbance detector.

Preparation of Test Mix and Sample. The protein test mix (SDS 14-200 test mix, containing seven proteins, ranging from 14.2 to 205 kDa of α-lactalbumin, 14.2 kDa; carbonic anhydrase, 29 kDa; ovalbumin, 45 kDa; bovine serum albumin, 66 kDa; phosphorylase *b*, 97.4 kDa; *β*-galactosidase, 116 kDa; myosin, 205 kDa) was dissolved in 750 *μ*L of sample buffer (SDS sample buffer, 0.12 M, Tris-HCl/1% SDS, pH 6.6); 750 *μ*L of deionized water was then added to it and mixed thoroughly. Two hundred microliters of test solution (∼0.1−1 mg of protein) was mixed with 100 *μ*L of sample buffer, 10 *μ*L of Orange G reference marker (0.1% solution), 5 *μ*L of 2-mercaptoethanol, and 85 *μ*L of deionized water in a 400 *μ*L vial and vortexed for 2 min. Then the mixture was boiled in a water bath at 100 °C for 10 min in a closed microfuge vial and then placed in an ice bath to cool for 3 min before injection.

Instrument conditions: capillary, 0.27 m long and 100 *μ*m i.d.; detector, UV at 214 nm; voltage, 8.1 kV; time, 20 min.

Molecular weight was calculated with reference to the standard protein (test mix) by using Beckman System Gold P/ACE control software.

RESULTS AND DISCUSSION

Composition and Properties of Defatted Sesame Seed Meal. The proximate compositions of both the defatted whole and dehulled sesame seed meal are shown in **Table 1**. Dehulling of sesame seed remarkably improved the protein content from 39.82 to 53.50%, and the fiber content was reduced from 28.32 to 10.14%. One of the most important effects of dehulling was the reduction of oxalic acid from 3.32 to 0.28%. The quality of protein in terms of PDI and NSI values was also improved

Table 3. Degree of Hydrolysis of Different Protein Hydrolysates[a]

sample	time of hydrolysis (min)	degree of hydrolysis (%)
PH10	10	18.9 ± 0.2
PH20	20	19.7 ± 0.5
PH30	30	21.0 ± 0.1
PH45	45	23.4 ± 0.3
PH60	60	24.1 ± 0.2

[a] Values are mean ± SD, $n = 3$.

Table 4. Solubility of Sesame Protein Isolate (SPI60) and Different Protein Hydrolysates Prepared by Papain at Different pH Values[a]

sample	pH 9.0	pH 7.0	pH 5.0	pH 3.0
SPI60	86.99 ± 0.07	55.97 ± 0.11	4.42 ± 0.23	33.05 ± 0.07
PH10	92.87 ± 0.14	88.82 ± 0.18	14.93 ± 0.08	38.05 ± 0.08
PH20	92.84 ± 0.15	89.73 ± 0.20	14.92 ± 0.09	38.68 ± 0.10
PH30	95.52 ± 0.14	92.38 ± 0.13	15.03 ± 0.07	40.78 ± 0.08
PH45	97.74 ± 0.09	92.55 ± 0.12	15.74 ± 0.07	43.43 ± 0.06
PH60	98.52 ± 0.07	93.36 ± 0.07	16.84 ± 0.06	44.82 ± 0.06

[a] Values are mean ± SD, $n = 3$.

significantly, so dehulling of sesame seed was essential to improve its quality in terms of protein content and solubility.

Recovery and Analysis of Sesame Protein Isolate. SPI was produced from the dehulled and defatted sesame seed meal. The chemical analysis of the protein isolate is presented in **Table 2**. The yield of protein isolate was 47%, and the protein content was 80%. Therefore, ∼70% of the total protein was recovered. Globulin is the predominant protein fraction in sesame seed (*26*), so extractability of protein increases with the increase of salt concentration (*27*, *28*). In the present study, protein was extracted without salt with a view to produce protein hydrolysate from the salt-free protein isolate. The fiber content was reduced to ∼2.33%, and phytic acid and oxalic acid contents were also very low. PDI and NSI values were remarkably improved.

Protein Solubility and Degree of Hydrolysis of Isolated Sesame Protein Hydrolysates. The degree of hydrolysis and solubility of protein hydrolysates at pH 3.0, 5.0, 7.0, and 9.0 are shown in **Tables 3** and **4**, respectively. After hydrolysis, protein solubility at pH 7.0 increased from 55.97% for control SPI60 to 88.82, 89.73, 92.38, 92.55, and 93.36% for PH10, PH20, PH30, PH45, and PH60, respectively. The greatest increase in solubility was observed between pH 5.0 and 7.0. Protein solubility at other pH values was also increased significantly for different protein hydrolysates. The increased protein solubility could be due to smaller molecular peptides being produced by papain hydrolysis. Unfolding of protein molecules due to hydrolysis was one of the reasons for its improved solubility. Improvement of protein solubility due to enzymatic hydrolysis was also reported by several workers (*10*, *11*, *29*). The increase in solubility with the time of hydrolysis was also supported by the increase in the degree of hydrolysis. After 10 min, the degree of hydrolysis and also the increase in solubilities were observed to be maximum. Therefore, within 10 min of hydrolysis, maximum cleavage of peptides occurred.

Table 5. Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI) of Sesame Protein Isolate (SPI60) and Different Protein Hydrolysates at pH 8.0[a]

sample	EAI (m^2/g)	ESI (min)
SPI60	114.33 ± 0.07	35.51 ± 0.14
PH10	179.52 ± 0.13	47.07 ± 0.09
PH20	182.65 ± 0.12	47.13 ± 0.12
PH30	208.93 ± 0.05	46.12 ± 0.07
PH45	188.46 ± 0.15	46.54 ± 0.07
PH60	189.38 ± 0.14	46.68 ± 0.10

[a] Values are mean ± SD, $n = 3$.

Emulsifying Properties of Sesame Protein Hydrolysates. From **Table 5** it was found that papain modification significantly improved the EAI of SPI60 from 114.33 to 179.50, 182.65, 208.93, 188.46, and 189.38 for PH10, PH20, PH30, PH45, and PH60, respectively. The lower EAI of PH60 compared to that of PH30 could be due to lower surface hydrophobicity. A similar observation was reported by Wu et al. (*8*) in the case of soybean protein peptides. The EAI is mainly dependent on the diffusion of peptides at oil−water interfaces. The hydrolysates with higher solubilities and smaller molecular size should facilitate that diffusion and enhance the interaction between proteins and lipids.

Papain modification also significantly improved the ESI of SPI60 from 35.51 to 47.07, 47.13, 46.12, 46.54, and 46.68 min for PH10, PH20, PH30, PH45, and PH60, respectively (**Table 5**). ESI remained more or less constant after 10 min of hydrolysis.

Foaming Properties of Sesame Protein Hydrolysates. Differences in foaming capacities among the samples are readily apparent (**Table 6**). The protein hydrolysates exhibited larger foam capacities, ranging from 111.3 to 136.9% volume increase, than the control SPI60 (61.9%). Hydrolyzed samples, although exhibiting increased foam capacities over the control SPI60, showed a decreased foam stability. The trends of increased FC and decreased FS caused by enzymatic modification have also been reported by Bobalic and Taranto (*30*).

Molecular Weight Distribution. Molecular weight distributions of protein isolate and protein hydrolysates were reported in **Table 7** by capillary SDS gel electrophoresis. The decrease in molecular weight with time of hydrolysis was very much prominent. It was found that the SPI60 contains ∼41% of 35 kDa protein. After 10 min of hydrolysis, it contains ∼40% of 30.4 kDa, and after 60 min of hydrolysis, it contains ∼33% of

Table 6. Foaming Properties of Sesame Protein Isolate (SPI60) and Different Protein Hydrolysates at pH 8.0[a]

	vol (mL)			foam stability (FS) vol (mL) at room temperature after			
sample	before whipping	after whipping	foam capacity (FC) (%)	10 min	30 min	45 min	60 min
SPI60	50.0	80.7 ± 0.82	61.9 ± 2.00	68.1 ± 0.55	60.1 ± 0.31	56.3 ± 0.22	55.3 ± 0.15
PH10	50.0	105.6 ± 0.56	111.3 ± 1.13	62.2 ± 0.68	57.1 ± 0.22	55.1 ± 0.51	53.6 ± 0.24
PH20	50.0	106.6 ± 0.32	113.3 ± 0.64	62.1 ± 0.45	56.3 ± 0.27	54.2 ± 0.45	53.5 ± 0.42
PH30	50.0	108.7 ± 0.35	117.5 ± 0.70	60.1 ± 0.39	56.2 ± 0.25	54.1 ± 0.23	53.4 ± 0.36
PH45	50.0	115.7 ± 0.21	131.6 ± 0.45	60.0 ± 0.22	55.3 ± 0.18	54.1 ± 0.31	52.5 ± 0.27
PH60	50.0	118.5 ± 0.45	136.9 ± 0.90	60.0 ± 0.24	55.2 ± 0.26	53.2 ± 0.31	52.1 ± 0.18

[a] Values are mean ± SD, $n = 3$.

Table 7. Molecular Weight Distribution of Sesame Protein Isolate (SPI60) and Two Different Protein Hydrolysates by Capillary Gel Electrophoresis Size Separation

SPI60		PH10		PH60	
MW (kDa)	%	MW (kDa)	%	MW (kDa)	%
4.0	15.1	6.3	37.5	4.7	22.4
20.7	18.0	27.2	22.7	14.8	21.8
27.6	9.9	30.4	39.8	25.8	22.4
31.0	16.2			28.6	33.4
35.0	40.8				

28.6 kDa protein. Small peptides of 4−6 kDa were also increased >2-fold after 10 min of hydrolysis.

Sesame protein peptides produced by papain modification have high protein solubility and emulsifying property. As papain is the least expensive of the FDA-approved vegetable-derived enzymes, the high-value protein hydrolysates prepared by papain can be used as value-added ingredients in many food formulations for medicinal purpose due to the presence of sulfur-containing amino acids. They are also suitable for a broad range of industrial food applications and also for cosmetic and personal care products.

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Received for review March 26, 2002. Revised manuscript received August 16, 2002. Accepted August 26, 2002. This research work was supported by a grant from the Council of Scientific and Industrial Research (CSIR), Government of India.

JF020320X