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Preparation and characterization of a protein hydrolysate from an oilseed flour mixture

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Abstract

A novel protein hydrolysate was prepared from the mixture of oilseed flours (soybean, sesame and peanut) and determined physico-chemical & functional properties along with comparison of individual oilseed flour hydrolysate of soybean. Mixed flour obtained from oilseed flours viz. soybean, sesame and peanut by using calculated amounts in the ratio of 1.1:1.7:0.7, respectively was used as a starting raw material having balanced amino acid profile. Protein hydrolysates were prepared from mixed flour and soybean flour by a double enzyme treatment method to a level of 40% degree of hydrolysis. The dried protein hydrolysate prepared from the mixed flour had 72% crude protein. This protein was characterized by gel filtration chromatography and SDS-PAGE. Comparison of the amino acid profile of the protein hydrolysate from mixed flours and soyabean flours showed a significant increase in the former one with respect to amino acid contents usually deficient of single oilseed flour hydrolysate. The product is creamish yellow in colour and had a solubility of >90% over a wide pH range of 2–10. The mixed flour protein hydrolysate showed better functional attributes such as foaming, as compared to that from soybean flour alone.

Keywords: Mixed oilseed flour; Protein hydrolysate; Balanced essential amino acid contents; Functional properties; Foam capacity; Foam stability

1. Introduction

Protein hydrolysates have been used, since 1940s for the nutritional management of individuals who cannot digest protein (Cuthbertson, 1950). Food protein hydrolysates have a wide range of applications as ingredients in the areas of nutrition, food industry, health care and cosmetics and it was well reported (Bautista et al., 2000; Clemente, 2000; Frokjaer, 1994; Giese, 1994; Weir, 1986). Protein hydrolysates possess a number of functional properties, which make them more attractive as a protein source in human nutrition, both in products for general and special medical use (Sven Frokjaer, 1994). From a nutritional point of view, the demand for protein or truly for amino acids can be fulfilled equally well by an intake of free amino acid, protein hydrolysate or intact protein. For dietary use, the protein hydrolysates should be rich in low molecular weight peptides, especially di- and tripeptides, with as little as possible free amino acids, for qualifying to have a high nutritional and therapeutic values (Vijayalakshmi, Lemieux, & Amoit, 1986). On the other hand, large molecular weight peptides (more than 20 amino acid residues) are presumed to be associated with the improvement of functionality of hydrolysates. Thus, irrespective of the use of the protein hydrolysates, it is always important to characterize them on the basis of their peptide size (Gauthier, Vachon, & Savoie, 1986). Application of proteinases is often an attractive means for obtaining better functional properties of food proteins, without impairing their nutritional value. Enzymatic hydrolysis can produce hydrolysates with well defined peptide profiles and extensive review of enzymatic protein hydrolysates in human nutrition was reported in literature (Clemente, 2000).

Oilseeds are attracting increasing interest as a source of edible proteins. Physico-chemical and functional properties

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of oilseed proteins such as soybean, peanut (groundnut) and sesame have been well reviewed (Kinsella, Damodaran, & German, 1985; Prakash & Narasinga Rao, 1986). These oilseeds contain about 20–25% protein and the protein content of defatted meals from dehulled oilseeds depends on the seed and ranges between 35% and 60% and proteins from the defatted cake can be extracted in water or dilute salt solutions. The amino acid composition (expressed as milligrams/gram crude protein) of these three oil seed proteins (soybean, peanut and sesame) comprises of total amino acids (TAA) of about 936, 945 and 947, respectively and in that total essential amino acids (TEAA) constitutes about 365, 349 and 373 which essentially about 39%, 37% and 39%, respectively (Bodwell & Hopkins, 1985).

Soy beans are the most abundant protein meal and the approximate protein content of whole oilseed and defatted soy flour is about 43% and 52%, respectively. The total essential amino acid composition of soy flour constitutes 41.18 g amino acid/16 g nitrogen and individually these amino acid contents (His, Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr, Try and Val) are 2.6, 4.8, 6.5, 5.7, 1.34, 1.45, 4.72, 3.4, 4.27, 1.8 and 4.6, respectively and aspartic and glutamic acids which are acidic amino acids constitutes 11.26 and 17.18, respectively (Campbell, Kraut, Yackel, & Yang, 1985).

Sesame seed contains about 22–25% protein and defatted sesame meal contains 40–50% protein content. This oilseed is very important as a protein source and the amino acid composition of the sesame seeds is unique and unusual among the oilseed proteins, due to its high content of sulphur-containing amino acids (methionine and cysteine) and low content of lysine (Johnson, Suleiman, & Lusas, 1979). The total essential amino acid composition of sesame flour constitutes 37.4 g amino acid/16 g nitrogen and individually these amino acid contents (His, Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr, Try and Val) are 2.4, 3.9, 6.7, 2.6, 2.5, 1.5 4.5, 3.7, 3.4, 1.5 and 4.7, respectively & aspartic and glutamic acids constitutes 8.2 and 16.2, respectively (Kinsella & Mohite, 1985).

Defatted peanut flour contains 47–55% high quality protein which is deficient in both lysine and methionine but with other high essential amino acid content (Basha & Pancholy, 1982; USDA-NAL, 2005). The total essential amino acid composition of peanut flour constitutes 34.1 g amino acid/16 g nitrogen and individually these amino acid contents (His, Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr, Try and Val) are 2.3, 3.2, 6.4, 3.0, 0.9, 1.0, 4.7, 3.7, 2.6, 1.0 and 5.3, respectively and aspartic and glutamic acids constitutes 11.26 and 17.18, respectively (Rhee, 1985) and lends itself being used in many food applications (Prinyawiwatkul, Beuchat, & McWatters, 1993).

In recent times, exhaustive compilation of the most interesting techno-functional properties of several products (meals, concentrates and isolates) obtained from oilseeds on various operational conditions is well reviewed (Moure, Sineiro, Dominguez & Parajo, 2006). However, these proteins could find applications in the food industry if appropriate hydrolysis processes are applied. One of the most efficient means of increasing protein solubility as well as improving the functional properties of oilseed proteins is to subject them to enzymatic hydrolysis (Delvalle, 1981; Kabirulla & Wills, 1981; Shen, 1992; Were, Hettiarachchy, & Kalapathy, 1997). By means of protease hydrolysis, functional properties of oilseed flour can be improved (Hrckova, Rusnakova, & Zemanovic, 2002; Taha & Ibrahim, 2002). It has been observed that the extent of proteolytic degradation of food proteins affects the functional properties of the hydrolysates (Babiker, 2000; Hettiarachchy & Kalapathy, 1998; Kristinsson & Rasco, 2000; Quaglia & Orban, 1987). It is possible, largely depending on enzyme specificity and the degree of hydrolysis (DH) achieved, to generate hydrolysate products with either enhanced or reduced functionality, e.g., solubility, emulsification, foaming and gelation properties. (FitzGerald & O'Cuinn, 2006). Structural modification of proteins is expected to alter their functional properties for use as food ingredients and for rendering proteins, most usable in formulating nutritional food products (Hamada, 1994).One of the important physico-chemical and functional property of protein hydrolysates is their solubility over a wide range of pH, temperature, nitrogen concentration and ionic conditions (Adler-Nissen, 1986; Kester & Richardson, 1984). The degradation of the protein into peptides generally renders the product more soluble especially at the isoelectric point (Chobert et al., 1996). Lot of work has been carried out in the field of functionality of individual oilseed protein products & enzymatic food protein hydrolysates and it has been well reviewed (Moure et al., 2006; FitzGerald & O'Cuinn, 2006; Raksakulthai & Haard, 2003). But until now, studies pertaining to mixture of oilseed flour protein hydrolysate has not been carried out and so we have taken up this study to mix the oilseed flours in specific and strategic ratio in preparing protein hydrolysate which is rich in desired amino acids and properties.

The aim of the research work was to prepare a protein hydrolysate which contains all the essential amino acids. This report describes the composition, amino acid profile, molecular weight distribution and functional properties of a protein hydrolysate prepared from the enzymatic hydrolysis of a mixed flour that consisted of soybean, sesame and peanut flours.

2. Materials and methods

2.1. Materials

Soybean, peanut and sesame seeds were purchased from the local market. Food grade enzyme protease P "Amano" 6 (having not less than 60,000 u/g proteolytic activity) was purchased from M/s. Amano Pharmaceutical Co. Ltd., Nagoya, Japan. Papain having not less than 20,000 tyrosine units (TU)/mg was purchased from

EnzoChem Laboratories. Sodium dodecyl sulphate (SDS), 2,4,6-trinitrobenzene sulphonic acid (TNBS), L-leucine, tricine, and acrylamide were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other reagents used were of Analar (AR) grade from Qualigens and E. Merck, Mumbai, India.

2.2. Methods

2.2.1. Preparation of defatted flours

Soybean, peanut and sesame seeds were cleaned, graded in a grading machine to remove stones and impurities. Water was sprayed on the seeds to raise the moisture level by 2%. The conditioned seeds were dried in an electrically heated roaster at 50–55 °C. The dehulling was done by passing through a plate type mill (Model A 453, Chandra Manufacturing Co., Chennai, India) with an attached air blower. The dehulled seeds were equilibrated at 20% moisture and passed through a flaking machine (Model J #6725, Kvarnmaskiner, Malmo, Sweden) maintaining a drum clearance of 0.3–0.5 mm to obtain flakes of 0.3mm thickness and dried to 5% moisture level. The dried flakes were defatted by repeated extractions with *n*-hexane, vacuum dried to remove solvent and ground, passed through 60 mesh sieve. The fat content was determined by Soxhlet extraction method (AOAC, 2000). The defatted flakes were dried and powdered in a Quadrumat mill (Brabender, Quadrumat Senior, Duisburg, Germany). The fractions obtained using standard sieves which have pore size below 100 μ were used as defatted flours in this study.

2.2.2. Preparation of the raw material

Amino acid composition of the three defatted flours (soybean, sesame and peanut) were determined using HPLC (Shimadzu- LC-10A, Japan). The flours were mixed at a ratio of 1.1:1.7:0.7 for soybean, sesame and peanut, respectively, to get a mixture having a balanced amino acid profile. This mixed flour was used for the preparation of protein hydrolysate.

2.2.3. Preparation of protein hydrolysate

To prepare protein hydrolysate, 10 g of mixed flour was suspended in 100 mL of distilled water and pH was adjusted to 7.6 using 1 N sodium hydroxide (NaOH) and stirred for 1 h for the extraction of total protein at room temperature. The slurry was hydrolysed using 0.3–1% w/w of fungal protease at 40–45 °C for 2 h. After incubation at 45 °C for 2 h, the temperature was raised to 50–55 °C and the hydrolysis was continued with the addition of 0.3–1% w/w of papain for another 90 min. The enzyme was inactivated by keeping the mixture in boiling water bath for 10 min. The slurry was cooled to room temperature and the insoluble carbohydrate rich fraction was removed by centrifugation at 6000*g* for 30 min. The clarified protein hydrolysate was freeze-dried. Soy protein hydrolysate was also prepared under identical conditions. One set of mixed flour was subjected to similar treatment, but without the addition of enzyme (untreated flour) in order to determine the extent modification due to proteolysis/processing conditions. The degree of hydrolysis was determined spectrophotometrically by the trinitrobenzene sulphonic acid method, as described by Adler-Nissen (1979).

2.2.4. Analysis

Moisture content was determined by using a Sartorius MA-30 Moisture meter (Fischer General Scientific (SEA) Pvt. Ltd, Singapore) at 130 °C to reach a constant weight. Total nitrogen content was determined according to the Kjeldahl procedure (AOAC, 1990).

2.2.5. Molecular weight distribution

Molecular weight distribution in the protein hydrolysates was determined by SDS-PAGE, according to the procedure of Laemmli (1970), as modified by Schagger and Von Jagow (1987), 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.

The molecular weight distribution of the samples was determined by gel filtration using a Fast Protein Liquid Chromatography system, equipped with a Superdex-75 HR 10/30 column from Amersham Pharmacia, Uppasala, Sweden. A sample volume of 100 μl was loaded and the eluent used was 20 mM phosphate buffer, (pH 7.6 containing 0.2 M sodium chloride) at a flow rate of 0.5 ml/min. Elution was monitored at 280 nm.

2.2.6. Amino acid analysis

Amino acid composition was determined by HPLC(Shimadzu Model LC-10A, Japan) using the method of Bidlingmeyer, Cohen, and Tarvin (1984). Samples were hydrolysed using 6 N HCl (1% w/v) phenol vapour at 110 °C for 24 h under vacuum. Protein hydrolysates were treated with phenyl isothiocyanate to form phenylthiocarbamyl derivatives of the amino acids, which were then analyzed by C_{18} reverse phase column.

2.2.7. Scanning electron microscopy

Scanning electron microscopic (SEM) studies of the flour and hydrolysates were carried out using Scanning Electron Microscope (LEO 435 VP, Cambridge, UK). Before loading the sample into the system, it was coated with gold using Poloron SEM coating system E-5000. Average coating time was 2–3 min. Thickness of the coating was 200–300 nm, which was calculated using the formula: $T = 7.5$ It, where $I =$ current in mA, $t =$ time in minutes, $T =$ thickness in Å. The coated sample was loaded on the system and the image was viewed under 20 kV potential using secondary electron image. The image was captured using 35 mm Ricoh Camera.

2.2.8. Nitrogen solubility

Solubilities of the untreated sample, soy protein hydrolysate and mixed flour hydrolysate were determined according to the method of Chobert, Sitohy, and Whitaker (1988). Samples were dispersed in distilled water (1% w/v) and the pH of the solution was adjusted to 10 with HCl or NaOH of high normality to limit dilution. After a 30 min equilibrium period at room temperature and readjustment of the pH, if necessary, the samples were centrifuged at 10,000*g* using a Hitachi 55P-2 Automatic Preparative Ultracentrifuge. The solubility was determined by measuring the amount of Kjeldahl nitrogen in the supernatant. The nitrogen solubility (NS) was calculated according to the formula:

$$\mathrm{NS}(\%) = \frac{\text{Nitrogen content in the supernatant}}{\text{Total Nitrogen content in the sample}} \times 100$$

2.2.9. Foaming properties

Foam capacity (FC) and foam stability (FS) were determined according to the methods described by Lawhon, Cater, and Mattil (1972) and Kinsella (1976) as simplified by Booma and Prakash (1990). A 2% aqueous dispersion of the protein sample was mixed thoroughly in a blender and whipped for 3 min at high speed. The contents were immediately transferred into a 250 mL graduated measuring cylinder, along with foam. The volume of foam was recorded after 30 s and FC was calculated as the increase in volume (mL) of the protein dispersion upon mixing and expressed as percentage increase in volume. FS was determined by measuring the fall in volume of foam after 30 min of standing.

2.2.10. Statistical analysis

Data and Statistical analysis were performed using Scientific Graphic and analysis Computer software OriginPro (version 7) supplied by Origin Lab Corporation, Northampton, MA, USA and data was expressed as Mean ± standard deviation of three experiments.

3. Results and discussion

3.1. Preparation of protein hydrolysates

The double enzyme method of protein hydrolysis yielded 40% degree of hydrolysis. For obtaining an extensive hydrolysate, a mixture of exo and endo peptidases is often used. In general, single enzymes may not provide an extensive hydrolysate in a reasonable period of time (Lahl & Barun, 1994). Here the oilseed proteins were hydrolysed using fungal protease and papain sequentially. Fungal protease is a well known non-specific endoprotease broadly used in food research for the generation of protein hydrolysates (Babiker, 2000). Starting with this enzyme pre-digestion of the protein is achieved increasing the number of C-terminal sites for the action of papain which is an endoproteinase.

3.2. Chemical composition

Proximate analysis of the mixed flour hydrolysate demonstrated a high content of crude protein (72.2%), which is comparable with the protein content of soy protein hydrolysate (69%). The moisture contents were 11.6% and 9.4% for mixed flour hydrolysate and soy protein hydrolysate respectively. The degree of hydrolysis of the hydrolysate was 40% and for soy protein hydrolysate was 32–35%.

3.3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of untreated flour, soy protein hydrolysate and mixed flour hydrolysate are shown in Fig. 1. The results show that hydrolysates with >35% degree of hydrolysis are mainly composed of low molecular weight proteins and peptides. 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 larger number of lower molecular weight protein bands in the mixed flour hydrolysate (lane 2), compared to the soy protein hydrolysate alone (lane 3) and this may be due to the presence of other proteins (peanut and sesame) in the mixed flour.

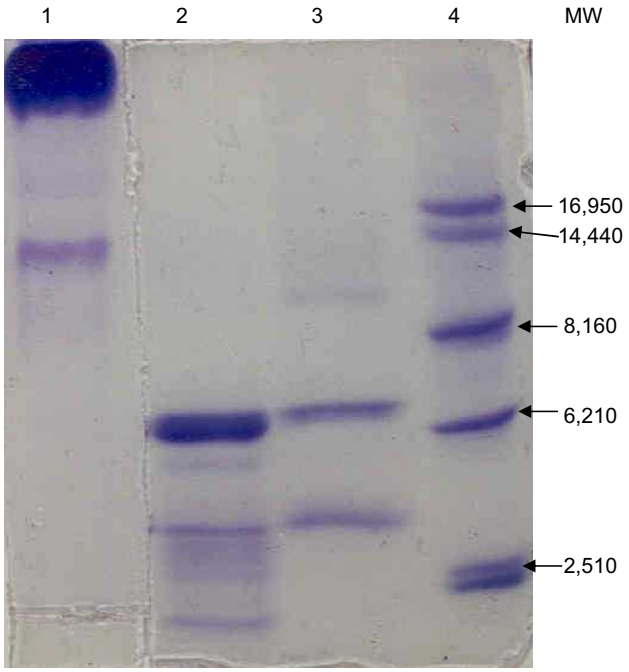

Fig. 1. SDS-PAGE of untreated mixed flour (lane 1), mixed flour hydrolysate (lane 2), soy protein hydrolysate (lane 3) and molecular weight standards (lane 4 – Molecular weight ranges from 2510 to 16,950 Da-peptide molecular weight marker (MWSDS17S) from Sigma Chemical Company, USA). The positions and molecular masses of standards are indicated on the right.

3.4. Size exclusion chromatography

Size exclusion chromatography was used to obtain quantitative data on the size distribution after the enzyme treatment using standard kit for the column in the range of molecular weight of 3000 to 70,000 Da and the results are shown in Fig. 2. In Fig. 2a is shown elution profile of standard proteins. The standard proteins are Ovalbumin (43,000 Da); Myoglobin (17,600); Ribonuclease (13,700) and Aprotinin (6500 Da) which are represented by peak 2, 3, 4 and 5, respectively and peak 1 represents undefined aggregates. From Fig. 2b, it is clear that the untreated flour is composed of high molecular weight proteins that eluted with the void volume. This corresponds to a material totally excluded from the gel and hence a molecular weight of larger than 70,000. Fig. 2c shows elution profile of soy protein hydrolysate and in Fig. 2d elution profile of mixed flour hydrolysate is shown. During the enzyme digestion, both in the case of soy protein hydrolysate and in mixed flour hydrolysate, increasing proportions of peaks of lower molecular weight peptides and proteins were found (Fig. 2c and d). As we can seen from the Fig. 2d, which represents mixed flour hydrolysate, the second and third peaks fall in the range of 17.6–13.7 kDa and all other peaks are 6.5 kDa and lower in molecular weight. These results suggest that proteins and peptides of lower molecular weights are formed in mixed flour hydrolysate. The peak 1 represent undefined aggregates and before ovalbumin standard peak this peak emerges out and this data suggest that undefined aggregates are eluting earlier in mixed flour hydrolysate. These undefined aggregates are formed during enzymatic protein hydrolysis process which are similar to plastein type reaction but not necessarily plastein. In mixed flour hydrolysate, the peak is a very heterogeneous one showing both the unhydrolyzed protein probably and also the hydrolysed fractions (Fig. 2d).

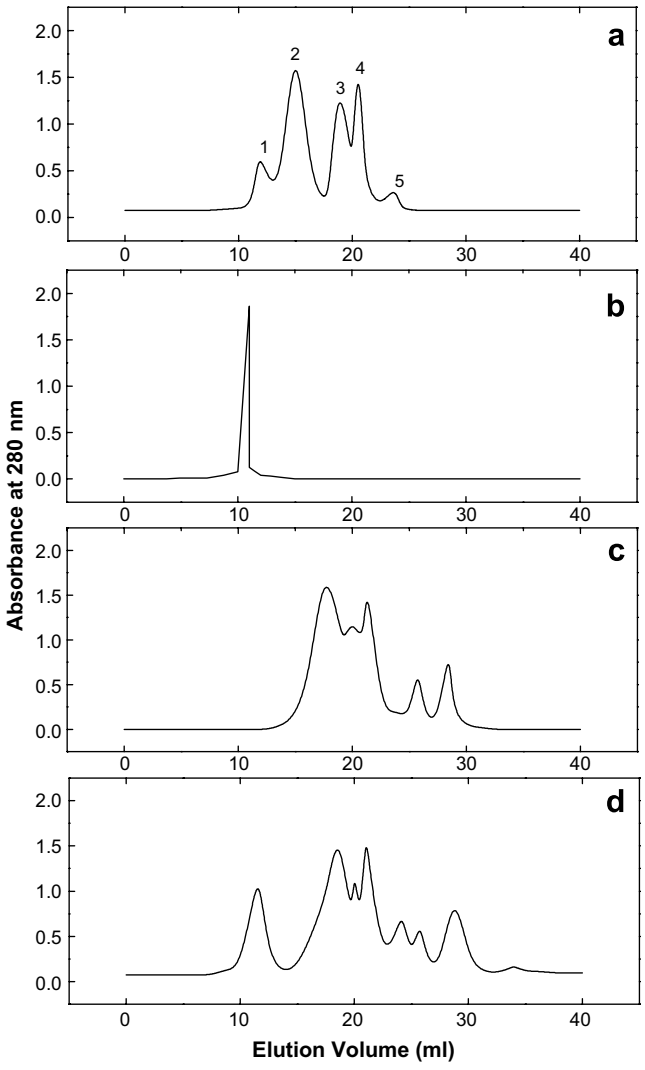

Fig. 2. Size exclusion chromatogram of FPLC Elution profile of (a) standard proteins (The standard proteins are Ovalbumin (43,000 Da-peak 2); Myoglobin (17,600-peak 3); Ribonuclease (13,700-peak 3); Aprotinin (6500 Da-peak 4)). The peak 1 represents undefined aggregates. (b) untreated mixed flour (c) soy protein hydrolysate and (d) mixed flour hydrolysate in 20 mM phosphate buffer (pH 7.6) containing 0.2 M sodium chloride. Superdex 75 HR 10/30 column (optimal separation range 3000–70,000 Da) was used.

Since in a typical hydrolysate, the range is very large including the unhydrolysed protein and the small peptides and perhaps the unhydrolysed protein has not penetrated the gel as there are many times polymers can form depending upon the conditions of the hydrolysis such as undefined aggregates or plastein type reactions. This is not uncommon in protein hydrolysates. Thus the most evident change observed in the hydrolysate with respect to the untreated protein is the reduction in the molecular weight of the protein, as a consequence of protease action. Doucet, Gauthier, Otter, and Foegeding (2003) studied enzyme – induced gelation of extensively hydrolyzed whey proteins by Alcalase and compared with plastein reaction and characterized the interactions and their results suggest that gelation seems to be caused by physical aggregation, mainly via hydrophobic interactions with hydrogen bonding and electrostatic interactions playing a minor role. Their data on SDS-PAGE also indicated electrophoretic gels showed aggregates not entering the gel and suggests that some aggregates survived heating in the presence of SDS even though it is considered as one of the most vigorous treatments to disrupt physical aggregation and concluded that the presence of a small amount of high molecular weight material is in accordance with other investigations that established physical aggregation as the main mechanism involved. The results of Andrews and Alichanidis (1990) suggests that the enzyme was acting as a template for hydrophobic aggregation of peptides. Lozano and Combes (1991) in their study also observed small changes in size-exclusion chromatography profiles during the plastein type reaction. In the course of hydrolysis of sodium caseinate with pancreatic proteinases aggregates were formed and the aggregates had properties almost identical to plasteins (Lorenzen & Schlimme, 1991). Our data also suggests that formation of undefined aggregates in mixed flour hydrolysate.

These results may be due to the splitting of peptide bonds as a result of series of simultaneous reactions. In the initial stage, the enzyme cleaves the soluble protein and is adsorbed on to the insoluble protein aggregates. The soluble proteins are hydrolysed more quickly than the compact insoluble protein aggregates. The later gets solubilized slowly by the action or the adsorbed enzyme in the subsequent stages of the reaction (Paraado, Millan, Hernandez-Pinzon, Bautista, & Machado, 1993).

3.5. Amino acid composition

The essential amino acid composition of mixed flour, soy protein hydrolysate and mixed flour hydrolysate are shown in Table 1, along with the essential amino acid composition according to the FAO requirement. It is clear that the mixed flour hydrolysate contains all the essential amino acids in good proportion as compared to the soy protein hydrolysate. It is also comparable with the FAO requirement of amino acids. The results in Table 1 indicate that the amino acid composition of the mixed flour hydrolysate closely resembles that of the protein from which it is prepared, except for threonine and leucine, which probably reflects the low solubility of these amino acids. If a large number of insoluble peptides had been generated as product, and lost during the clarification step, the amino acid composition of the hydrolysate would not have coincided with that of the mixed flour. The results show that the mixed flour hydrolysate is well balanced in its essential amino acid contents.

3.6. Scanning electron microscopy

Scanning Electron Microscopy was used to examine the micro structural changes of proteins after the enzymatic hydrolysis. Fig. 3a and b shows the SEM pictures of untreated mixed flour and mixed flour hydrolysate, respectively. The data shows that the protein has degraded into small fragments after the enzyme treatment. Also there is a reduction in the particle size of the flour after hydrolysis (Fig. 3b) compared to untreated mixed flour (Fig. 3a). Average particle size of the flour is in the range of 18.06–15.24 μ whereas that of the hydrolysate is 7.35–5.33 μ. Since normally SEM results are empirical what we see here is the decrease in protein aggregates.

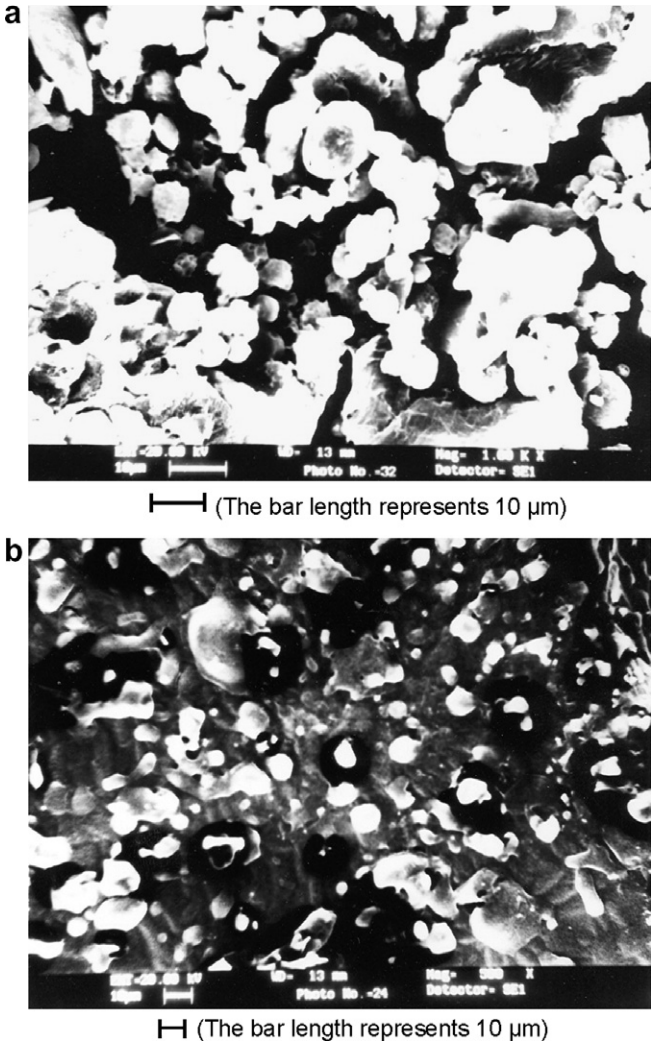

Fig. 3. Scanning electron microscopic picture of (a) untreated flour and (b) mixed flour hydrolysate. LEO 435 VP, Cambridge model Scanning Electron Microscope was used. For untreated mixed flour (a), the scale is (bar length represents) 10 μm; working distance 13 mm; magnification 1 K X; HT 20 K; For mixed flour hydrolysate (b), the scale is (bar length represents)10 μm; working distance 13 mm; magnification 500 X; HT 20 K.

Table 1
Essential Amino acid composition of mixed flour, mixed flour hydrolysate, soy protein hydrolysate and FAO requirement

Amino acid	Mixed flour (g/100 g)	Mixed flour hydrolysate (g/100 g)	Soy protein hydrolysate (g/100 g)	FAO* requirement (g/100 g)
Threonine	3.6 ± 0.16	2.1 ± 0.05	1.9 ± 0.05	3.0
Tyrosine	3.5 ± 0.02	3.0 ± 0.03	1.5 ± 0.03	2.8
Valine	4.6 ± 0.07	3.8 ± 0.06	2.3 ± 0.02	4.2
Methionine	1.7 ± 0.05	1.5 ± 0.02	0.6 ± 0.01	2.2
Isoleucine	3.8 ± 0.12	2.9 ± 0.06	2.1 ± 0.03	4.2
Leucine	7.0 ± 0.23	5.5 ± 0.07	3.8 ± 0.17	4.8
Phenyl alanine	4.0 ± 0.04	3.6 ± 0.04	2.6 ± 0.06	2.8
Lysine	4.3 ± 0.17	3.5 ± 0.08	2.7 ± 0.04	4.2

Values are mean ± SD of three experiments.
* Recommended dietary allowances, 10th edition, Edited by National Research Council, P-57, 1989.

3.7. Nitrogen solubility

Solubility characteristics of protein are among the most important functional properties since many functional performances of proteins depend upon their capacity to go into solution initially. Fig. 4 shows the nitrogen solubility curve of untreated flour, soy protein hydrolysate and mixed flour hydrolysate over a pH range of 2–10. As shown in the figure, the solubility of the hydrolysates is enhanced

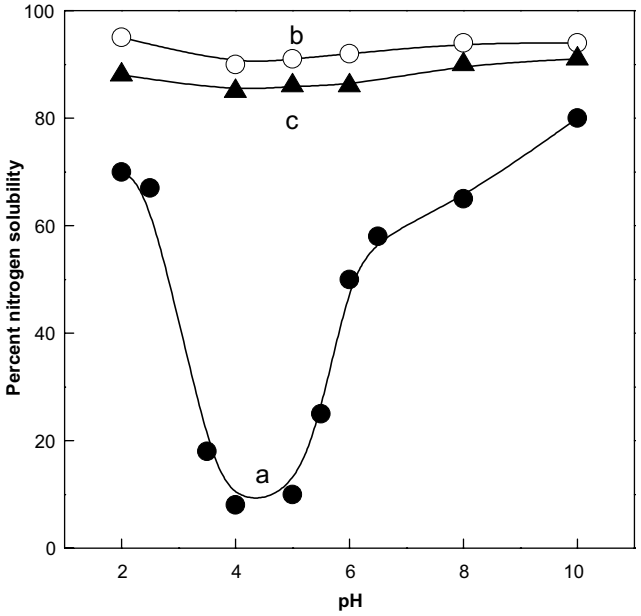

Fig. 4. Nitrogen solubility profile of the flour in water as a function of pH (ranging from 2.0 to 10.0) before and after hydrolysis. The sample concentration was 1% (w/v). (a) untreated mixed flour, (b) mixed flour hydrolysate, and (c) soy protein hydrolysate.

considerably at pH 4–5, as compared to that of the intact proteins. The solubility pattern of mixed flour hydrolysate is in agreement with that of soy protein hydrolysate. However, the nitrogen solubility of mixed flour hydrolysate at all pH values was higher than that of the untreated flour. The nitrogen solubilities of mixed flour at pH 2.0, 4.0, 6.0, 8.0 and 10.0 were 70%, 8%, 50%, 75% and 80%, respectively, while those of mixed flour hydrolysate were 94%, 90%, 92%, 94% and 94%, respectively.

It has been suggested that an increase in the solubility of protein hydrolysate over that of the original protein 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; Phillips & Beuchat, 1981; Turgeon, Gauthier, & Paquin, 1992). Both the hydrolysed sample showed a similar pattern of nitrogen solubility values, which could be due to the fact that the specificity of the enzyme used was the same in both the cases. Nitrogen solubilities of these hydrolysates were pH independent 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 the isoelectric pH (Cheftel, Cuq, & Lorient, 1985). This is an important feature, which could increase the use of the hydrolysates in many food and nonfood applications.

Oilseed proteins do not exhibit similar amino acid patterns. For example, soybeans are deficient in methionine but are a good source of lysine, sesame is deficient in lysine but a good source of sulfur-containing amino acids, peanut is deficient in both these amino acids (Amaya, Young, Mixon, & Norden, 1977). Eventhough peanut is deficient in above two amino acids, defatted peanut flour contains 47–55% high quality protein with other high essential amino acid content (Basha & Pancholy, 1982; USDA-NAL, 2005) and lends itself being used in many food applications (Prinyawiwatkul et al., 1993). The development of a peanut protein concentrate (PPC) from defatted peanut flour would also provide the food industry with a new high protein food ingredient for product formulation requiring high emulsifying capacity and this could be a good source of protein fortification for a variety of food products for protein deficient consumers in developing countries. (Yu, Ahmedna, & Goktepe, 2007). The mixed flour compensates for the limiting amino acids of these oil seeds (Table 1). The main advantage of enzyme hydrolysis over acid and alkali hydrolysis is that the nutritive quality of the protein remains practically the same as that of the starting protein without altering the amino acid profile.

3.8. Foam capacity (FC) and foam stability (FS)

Foam capacities and foam stabilities of the mixed flour hydrolysate, mixed flour, soybean flour, sesame flour and peanut flour are shown in Table 2. The foam capacity and foam stability of mixed flour hydrolysate were $122 \pm 5\%$ and 90 ± 3 mL, respectively. The results shows a significant increase in the foaming capacity of the mixed flour hydrolysate, as compared to the respective flour and individual flours. The foam capacity and foam stability values of the individual flours (soy, peanut and sesame)were lesser than that of the mixed flour hydrolysate. Peanut flour shows lesser foam capacity and foam stability ($40 \pm 2\%$ and 10 ± 1 mL) compared to foam capacities and foam stabilities of soy flour and sesame flour. Proteins in dispersions cause a lowering of the surface tension at the water air interface thus creating foaming capacity (Surowke & Fik, 1994). Foaming capacity is also determined by molecular flexibility and physico-chemical properties (hydrophobicity, net charge and charge distribution, hydrodynamic properties) of proteins and to form efficiently (i.e. to possess high foamability), a protein needs to adsorb rapidly during the transient stage of foam formation (Gonzalez-Perez, Vereijken, Van Koningsveld, Gruppen, & Voragen, 2005; Graham & Philips, 1976; Martin, Grolle, Bos, Cohen Stuart, & van Vilet, 2002). These results suggest an increase in surface activity, probably due to the initially greater

Table 2
Foam capacities and foam stabilities of the mixed flour protein hydrolysate, mixed flour and individual oilseed flours

Protein	Foam Capacity (FC) (%)	Foam Stability (FS) (mL)
Mixed flour protein hydrolysate	122 ± 5	90 ± 3
Mixed flour	42 ± 2	34 ± 2
Soy flour	56 ± 3	42 ± 2
Peanut flour	40 ± 2	10 ± 1
Sesame flour	52 ± 3	16 ± 2

Values are mean ± SD of three experiments.

number of polypeptide chains (Kuehlor & Stine, 1974), which are formed from partial proteolysis, allowing more air to be incorporated as shown in Fig. 3 of scanning electron microscopy results.

4. Conclusion

This paper describes a novel methodology of mixing oilseed flours based on its amino acid composition and then hydrolyzing it to get a composite hydrolysate which is rich in essential amino acids on the one side and quality functional hydrolysate on the other side. The hydrolysates that are obtained also has an effect on improving the functionality such as solubility, foaming properties and other important properties of proteins. Not only these hydrolysates can be used as food additives to improve the functionality but also improving the nutritional profile and better protein quality of foods by incorporating them in selected foods. It has a wide range of applications depending upon the end food product.

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