



Sesame protein isolate: Fractionation, secondary structure and functional properties

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ABSTRACT

This study investigated the selective fractionation of sesame proteins using sodium chloride and ammonium sulfate. Maximum protein extraction occurred at pH 7–10 and in the presence of 0.6–1 M NaCl. Sesame protein isolate (Ses-PI) yield increased from 12.5% in the absence of NaCl to 54.6% at 1 M NaCl. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) indicated differences in the electrophoretic pattern of water-extracted Ses-PI compared to the NaCl extracts. However, changing the NaCl concentration did not affect the principal components in the profile of the sesame protein extracts. With ammonium sulfate (AS) precipitation, the 11S fraction was preferentially extracted at 30% saturation (AS30%). More 2S and 7S polypeptide bands were found in the AS50% fraction, whereas the AS70% sample had a higher concentration of the 7S fraction. FTIR analysis revealed that heat treatment as well as use of NaCl and AS at lower concentrations induced changes in protein conformation, whereas at higher concentrations the protein conformation was more stable. CD analysis revealed a predominance of α -helices in the water extract which decreased with increasing NaCl content toward more β -strand structures. Changes in secondary structure were also observed as the ammonium sulfate concentration was increased from 30 to 70%. The functional properties of (Ses-PI–1 M NaCl) were compared to that of soy protein isolate (Soy-PI). Ses-PI–1 M NaCl had significantly ($P < 0.05$) lower protein solubility, water holding and fat binding capacities, but much greater emulsifying and foaming properties compared to Soy-PI.

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1. Introduction

Sesame is one of the most important oilseed crops cultivated in Central America, Tropical Africa, and Eastern Asia. Global production in 2008 was 3.6 million tonnes (FAOSTAT, 2005). Sesame seeds and oil are used in the food industry as well as in the pharmaceutical and cosmetic industries. The growing interest in the use of sesame oil is due to its high content of unsaturated fatty acids and antioxidant lignans (sesamin, sesamol and sesaminol) which have been reported to have hypocholesterolemic effects in humans (Hirata et al., 1996). Due to its many other desirable properties, the oil-free meal containing approximately 50% proteins could be a valuable source of proteins, for use as both functional food ingredients and nutritional supplements. Thus, understanding the functional and molecular properties of sesame proteins is of growing interest.

Most of the proteins present in sesame seeds are storage proteins composed of globulins (67.3%), albumins (8.6%), prolamines (1.4%), and glutelins (6.9%) (Nilo Rivas, Dench, & Caygill, 1981). Association–dissociation, aggregation and denaturation of the major protein in sesame, α -globulin, have been extensively investigated (Lakshmi & Nandi, 1977, 1978, 1979; Prakash & Nandi, 1976, 1977a,b,c, 1978). Functional and physicochemical properties of sesame protein

concentrate (Inyang & Iduh, 1996; Zaghloul & Prakash, 2002), isolate (Khalid, Babiker, & El-Tinay, 2003), as well as its use as a nutritional supplement for beverage (Lopez, Flores, Galvez, Quirasco, & Farres, 2003) and bread (EL-Adawy, 1995) applications were also reported. Limited purification and characterization of minor sesame protein fractions, the 2S (Rajendran & Prakash, 1988) and the 7S have been undertaken to improve understanding of the physical, biochemical and immunological properties of these proteins (Orruno & Morgan, 2007).

Several sesame protein subunits (belonging to 2S albumin, 7S and 11S globulins) (Beyer, Bardina, Grishina, & Sampson, 2002; Beyer, Grishina, Bardina, & Sampson, 2007; Navuluri et al., 2006) and different oil body proteins such as Oleosin isoforms (Chen, Lin, Huang, & Tzen, 1997; Leduc et al., 2006) and Sops (Chen, Tai, Peng, & Tzen, 1998; Frandsen, Mundy, & Tzen, 2001; Lin & Tzen, 2004), have been characterized by electrophoresis and described as major allergens. As food allergy is a growing health problem it is also important that the composition and characteristics of these proteins be carefully studied. Variations were observed in the electrophoretic profiles of sesame proteins due to differences in extraction conditions, quality of the extracts and degree of purity (i.e. % purity and presence of other contaminants) as well as solubility in the presence or absence of salts and denaturants. Consequently, much remains unknown about how the composition of sesame proteins is affected by environmental pH, salt concentration and extraction conditions.

The specific objectives of the present study, therefore, were to: (1) investigate the extractability of sesame proteins from defatted meal

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as a function of pH in water and in the presence of various concentrations of NaCl (0.2, 0.6 and 1 M), (2) use selective precipitation at 30%, 50% and 70% ammonium sulfate saturation to separate the different protein fractions (11S, 7S and 2S), (3) study the effect of pH and salt concentration on selected functional properties and assess global changes in the secondary structure of sesame protein isolate (Ses-PI) and (4) compare the functional properties of Ses-PI-1 M NaCl with those of soy protein isolate (Soy-PI), a major commercial plant protein on the market today.

2. Materials and methods

2.1. Materials

White sesame seeds were purchased from the local market (Montreal, Canada). Defatted soybean flakes (Nutrisoy untoasted 7B flakes) were obtained from ADM (Decatur, IL). Spectra/Por® Dialysis membrane (1 kDa molecular cutoff) was from Spectrum Laboratories, Inc. (Rancho-Dominguez, CA, U.S.A.). High and low molecular weight calibration kits were from Amersham Pharmacia Biotech (Uppsala, Sweden). Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All other chemicals used were of analytical grade.

2.2. Preparation of soy protein isolate (Soy-PI)

Soy protein isolate was prepared from commercial defatted soy flakes (Nutrisoy untoasted 7B flakes, ADM) according to the conventional procedure as described by L'Hocine, Boye, and Arcand (2006). Briefly, defatted meal was dispersed in water (ratio 1:15 w/v), and the pH was adjusted and maintained at 9.0 with NaOH, and stirred for 45 min at 55 °C. The slurry was then centrifuged (Beckman J2-21 centrifuge) at 4 °C for 30 min at 14,300×g. The supernatant was collected and the pH was adjusted to 4.5 with HCl and stirred for 45 min at 25 °C. After centrifugation at 2830×g (Beckman CS-6 centrifuge) for 15 min at 4 °C, the precipitate obtained was washed twice, and re-suspended in a minimum amount of water before neutralization to pH 7.0 with NaOH. The sample was then freeze-dried and stored at 4 °C until analyzed.

2.3. Preparation of different sesame protein isolates (Ses-PI)

2.3.1. Defatting of sesame seeds

Sesame seeds were ground in a coffee grinder and defatted with *n*-hexane (1:10, w/v, sesame to solvent ratio) with constant stirring for 2 h at room temperature (RT). The slurry was defatted two more times (using the same sesame to hexane ratio) and then filtered on a glass sinter. After drying, the defatted sesame meal (DSM) was used for protein extraction.

2.3.2. Optimal pH for protein extractability

To optimize the protein extraction (recovery), extractability of sesame total proteins from defatted meal as a function of pH in various concentrations of sodium chloride (0–1 M) was investigated. Four SDM suspensions (10% w/v) were prepared in water. The solutions were allowed for stirring for 2 h. Different amounts of NaCl were added to prepare suspensions at various NaCl concentrations of 0.2 M, 0.6 M and 1 M. One suspension was retained as control (no salt added). The control and NaCl suspensions (0.2, 0.6 and 1 M NaCl) were aliquoted into test tubes and their pH was adjusted to cover the range from 1 to 10 (in 1-unit increment) using HCl or NaOH. The dispersions were stirred continuously and maintained at the desired pH level for 2 h at RT. The samples were sonicated (VWR-Aquasonic, Model 750D) for 30 min, and centrifuged (Beckman CS-6 centrifuge) for 30 min at 2060×g. The protein content of supernatants was measured using the method of Bradford (1976)

and total protein by Leco using the Dumas method (AOAC, 1995). Protein extractability (solubility) was calculated as follows: Extractability (%) = (soluble protein in supernatant/total protein of DSM) × 100.

2.3.3. Preparation of sesame protein isolates at various NaCl concentrations (Protocol # 1)

The determined optimum pH of extraction (pH 7.5) was then used to prepare different Ses-PIs in order to investigate any changes in the composition of proteins as a result of various NaCl concentrations, as well as on the yield. DSM was suspended in 0.0 to 1 M NaCl at a ratio of 1:6 (w/v) at pH 7.5 for 2 h at 35 °C. The supernatants obtained after centrifugation (14,000×g for 30 min at RT) were adjusted to pH 4.5 with HCl, left to stand overnight at 4 °C for 18 h, and then centrifuged (Beckman J2-21 centrifuge) at 14,300×g for 30 min at 5 °C. The precipitates obtained were washed twice, neutralized to pH 7.0 with 2 N NaOH and dialyzed extensively against distilled water for 48 h. The protein solutions were then frozen with liquid nitrogen and freeze-dried. The protein yield was calculated as follows;

$$\text{Protein yield (\%)} = \frac{\text{weight (g) of Ses-PI} \times \text{protein content (\%)} \text{ of Ses-PI}}{\text{weight (g) of DSM} \times \text{protein content (\%)} \text{ of DSM}} \times 100.$$

2.3.4. Extraction of sesame proteins using ammonium sulfate precipitation (Protocol # 2)

Selective fractionation of the different sesame protein fractions (11S, 7S globulins and 2S albumins) using ammonium sulfate at 30%, 50% and 70% saturation was carried out. DSM (100 g) was dispersed in water (1:10 w/v) containing 1 M NaCl (pH 7.5) and with constant stirring for 2 h at 35 °C. The slurry was centrifuged at 14,000×g for 30 min at RT. The supernatant was collected and ammonium sulfate was added to 30% saturation in an ice bath. The solution was left to stand for 1 h and subsequently centrifuged at 14,000×g for 30 min at 4 °C. The pellet obtained was re-dissolved in water, dialyzed for 48 h at 4 °C and freeze-dried (Fraction AS30%). The supernatant obtained after centrifugation was then adjusted to 50% ammonium sulfate saturation and then to a final concentration of 70% saturation at 0 °C in an ice bath by the addition of ammonium sulfate in order to prepare two other fractions, namely, Fraction AS50% and Fraction AS70%, respectively. The obtained pellets were re-dissolved in water, dialyzed and freeze-dried.

2.4. Proximate analysis

Total protein content of the different extracts (freeze-dried powders) was determined by Leco (Leco FP-428, Leco Corp., St. Joseph, Mich., U.S.A.) using the Dumas method (AOAC, 1995) and a nitrogen conversion factor of 6.25. Fat content was determined using a Soxtec apparatus (Foss Tecator Soxtec System HT-6, 1043 extraction unit, Brampton, Ont., Canada) according to AACC method 30-25 (AACC, 2003a). Ash content was determined according to AACC official methods 08-03 (AACC, 2003b), and moisture was determined by drying 2 g sample in a Fisher Isotemp Vacuum Oven (Fisher Scientific, Montreal, Qc, Canada) for 5 h at 100 °C (AACC, 1983a). All determinations were done at least in triplicate and average values were computed.

2.5. Protein characterization

2.5.1. Analytical 1D gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and native polyacrylamide gel electrophoresis (Native-PAGE) were carried out by the method of Laemmli (1970) on Bio-Rad (St. Louis, Mo., U.S.A.) pre-cast 10–20% and 4–15% gradient

polyacrylamide Tris–HCl gel, respectively, using the Bio-Rad Criterion cell. Protein samples (1 mg) were dispersed in 1 ml of either Laemmli native or SDS sample buffers (Bio-Rad) and vortexed for 15 min. For SDS-PAGE, 5% β -mercaptoethanol was added to the dispersions prior to boiling for 5 min. After centrifugation for 5 min at $1000\times g$, 15 μ l of sample solution was loaded in each well and gels run at constant voltage (200 V). The gels were stained with Coomassie brilliant blue R-250. Bio-Rad Precision Plus Protein Standards (10 to 250 kDa) and Amersham Pharmacia Biotech (Uppsala, Sweden) High Molecular Weight Calibration Kit (66 to 669 kDa) were used as molecular markers.

2.5.2. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was carried out on the sesame protein extracts prepared in water and under various NaCl and ammonium sulfate concentrations. Protein solutions (100 mg/ml) were prepared in D₂O-phosphate buffer at pD 7.4 (pD = pH + 0.4). D₂O was used instead of water, since D₂O has greater transparency in the infrared amide I' region (Susi & Byler, 1983). To ensure complete hydrogen–deuterium exchange, sample dispersions were incubated at 4 °C for 1 day before analysis and equilibrated to RT prior to measurements. Aliquots (10 μ l) of each sample were held in an IR cell between two CaF₂ windows, with a 25 μ m path length, and infrared spectra were recorded at 25 °C with an Excalibur FTIR spectrometer (system FTS 3000) equipped with a deuterated triglycine sulfate (DTGS) detector (Bio-Rad Laboratories, Cambridge, MA). A total of 256 scans were averaged at 2 cm^{−1} resolution. The signal-to-noise ratio was >20,000:1. Deconvolution was performed using the Digilab Merlin FTIR software (version 3.4) with a half-bandwidth of 13 cm^{−1} and an enhancement factor of 2.4 (Kauppinen, Moffatt, Mantsch, & Cameron, 1981). Band assignment in the amide I' region (1600–1700 cm^{−1}) was according to Byler and Susi (1986) and Choi and Ma (2005). For heating experiments, an Omega temperature controller (Omega Engineering, Stamford, CA) was used. The samples were heated from 25 °C to 95 °C at 5 °C intervals and allowed to equilibrate for 7 min at each temperature prior to data acquisition. Spectra were also recorded when the sample was cooled back to the initial temperature (25 °C).

2.5.3. Circular dichroism (CD)

Far UV CD spectra of sesame extracts were recorded with a JASCO J-815 spectropolarimeter (Jasco Corporation, Japan) at 25 °C with thermostatically controlled cell holders. Protein solutions were prepared at concentration of 1 mg/ml in 200 mM phosphate buffer (pH 7.5), and poured into a quartz cuvette (Hellma, Mullheim, Baden, Germany) of 0.05 cm path length. Absorption between 178 and 250 nm was recorded using the following instrumental parameters: bandwidth = 1.0 nm, sensitivity = standard, time constant = 1 s and scanning speed = 20 nm/min. The obtained spectra represent an average of five consecutive scans. The compositions (%) of α -helix, β -sheet, turn and unordered structures in the protein mixtures were calculated with the CDPro analysis software and the SELCON3 program which are part of the Jasco software.

2.6. Functional properties

The pH-solubility profile of sesame extracts over a pH range from 1 to 10 was determined as described by Betschart (1974). Briefly protein solutions were prepared in water (1%, w/v) and their pH adjusted from 1 to 10. The protein solutions were stirred with a magnetic stirrer at 25 °C for 2 h, centrifuged at $3000\times g$ for 30 min. The protein content of the supernatants was determined by Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Protein solubility was calculated as: PS (%) = $100 \times \text{PS} / \text{PT}$, where PS is the protein content in the supernatant after centrifugation, and PT is the total protein content present in the protein sample previously determined by Leco (Dumas method). Fat absorption capacity (FAC) was

assessed using the procedure of Lin and Humbert (1974). Water holding capacity (WHC) was assessed by the AACC procedure, method 88-04 (AACC, 1983b) and reported as the maximum amount of water retained by 1 g of sample under low-speed centrifugation ($2060\times g$ for 10 min, Beckman CS-6 centrifuge).

The procedure proposed by Waniska and Kinsella (1979) with some modifications was followed for measuring foaming properties. The protein was dispersed in 0.01 M phosphate buffer (pH 7) at concentration of 0.5% (w/v) with stirring for 10 min. The experiment was conducted at room temperature (25 °C). The protein solution (15 ml) was then injected into the sparging chamber of a water-jacketed glass condenser via the septum-stoppered inlet. Nitrogen gas was sparged into the protein solution until the foam chamber (55 ml) was filled with foam, while simultaneously maintaining the volume of liquid in the sparging chamber by addition of protein solution. The required time to form 55 ml of foam, and the volume of protein solution added were recorded. After 5 min the volume of liquid drained from the foam was also noted. Several parameters were used to predict foaming properties as follows: Gi (%), which is the percent of gas entrapped in the foam; FE (%), which is the percent foam expansion and R5 (%), which represents the percent of liquid retained in the foam after 5 min (Achouri, Boye, Yaylayan, & Yeboah, 2005). Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by the turbidimetric method of Pearce and Kinsella (1978). Briefly, 1.5 ml of corn oil was added to 4.5 ml of 0.5% (w/v) protein solution prepared in 0.01 M phosphate buffer (pH 7), after which the mixture was homogenized at 20,000 rpm at room temperature for 1 min with a PT 2100 Polytron homogenizer (Kinematica AG, Littau-luzern, Switzerland). 250 μ l of the emulsion was taken out from the bottom at different times and diluted with 50 ml of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was then determined at 500 nm with a Cary 300 Bio, UV-Visible Spectrophotometer (Varian Canada, Inc., St-Laurent, Quebec city, Canada). All measurements were assessed in triplicate.

2.7. Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) using the PRISM software, version 3.02 (Graph Pad Software, Inc. San Diego, CA, USA). Significant differences between means were determined by Tukey's Multiple Comparison Test procedure at the 5% significance level.

3. Results and discussion

3.1. Composition analysis

Table 1 shows the proximate composition of whole sesame seed, DSM, and the sesame protein isolates (Ses-PI) prepared under various sodium chloride concentrations, as well as their protein yields. As expected, extraction of oil from sesame flour led to increases in other constituents in the defatted meal, particularly in protein content, which increased from 24.8% in the sesame seed to 59% in the DSM. Processing of the DSM to protein isolate, resulted in a further increase in protein content from 59.0% to 94.6%.

3.2. Effects of pH and sodium chloride on the extractability of sesame proteins

The major storage globulins, namely the 7S and 11S globulins, are oligomeric proteins and exhibit dissociation/association behavior, depending on the pH and ionic strength of the medium, which affects the solubilization of these proteins and their ratios in the protein isolate. Fig. 1 shows the % protein extractability as a function of pH in water and at various concentrations of NaCl. The results indicated that increasing the NaCl concentration from 0 to 1 M enhanced

Table 1

Proximate analysis of whole sesame seed, defatted sesame meal, Ses-PI and protein yield of different protein extracts.

Sample	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	^c Protein yield (%)
Whole sesame seed	24.8	52.0	2.89	2.70	–
Defatted sesame meal (DSM)	59.0	1.44	7.17	8.8	47.0
Ses-PI-H ₂ O (0 M NaCl)	100	–	–	–	12.5
^a NaCl concentration					
Ses-PI-0.2 M NaCl	100	–	–	–	22.8
Ses-PI-0.6 M NaCl	93.2	–	–	–	53.0
Ses-PI-1 M NaCl	94.6	–	–	–	54.6
^b Ammonium sulfate saturation					
Fraction AS30%	100	–	–	–	22.7
Fraction AS50%	100	–	–	–	5.80
Fraction AS70%	97.5	–	–	–	1.17

^a Sesame protein isolates (Ses-PI) were prepared by extraction in water (H₂O) (pH 7.5 at 35 °C) and in the presence of various concentrations of NaCl (0.2, 0.6 and 1 M), followed by acid precipitation to pH 4.5 (25 °C), neutralization to pH 7.0, dialysis and freeze-drying.

^b Sesame protein fractions prepared using ammonium sulfate at 30%, 50% and 70% saturation.

^c Protein yield was calculated based on the protein mass obtained divided by the protein mass of the starting material.

protein extractability over the pH range of 4–10. The pH range for maximum extractability was around 7–10 and in the presence of 0.6–1 M NaCl. pH had little effect on the extractability of Ses-proteins in the absence of added salt (i.e., water extract), with an observed maximum recovery of ~14% within the acidic pH range of 1–4. Similar variations in sesame protein extractability with pH and salt concentration were also reported by Nilo Rivas et al. (1981). Prakash (1986) reported that the pH of minimum extractability drifted toward more acidic pH (from pH 4 to pH 2) as sodium chloride concentration was increased from 0.05 to 2.0 M. Our results did not confirm this finding. In fact, our study showed that for all NaCl concentrations, a slight increase in extractability occurred as the pH was decreased to 1 but this increase was only marginal and appeared to be independent of NaCl concentration.

The results for protein yield are presented in Table 1. At pH 7.5 (the optimal pH for protein extraction), the obtained data clearly showed that increasing the NaCl concentration from 0 to 1 M progressively increased the yield of the protein isolate from 12.5% at 0 M concentration to 54.6% at 1 M NaCl concentration.

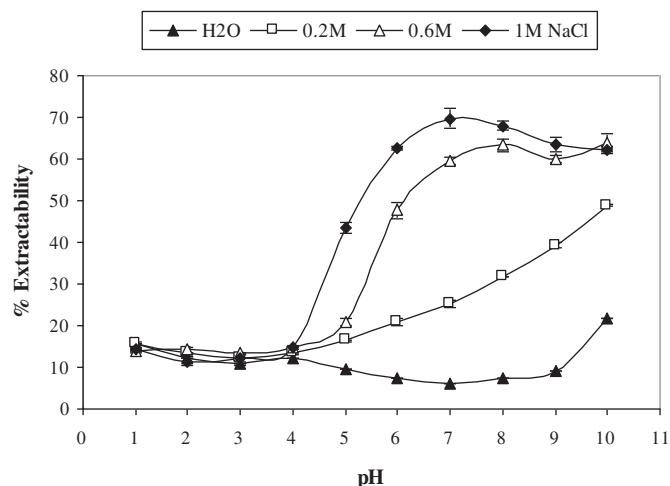


Fig. 1. Extractability (%) of total sesame proteins as a function of pH and in the presence of sodium chloride at different concentrations.

3.3. Fractionation of sesame proteins using ammonium sulfate

Ammonium sulfate (AS) is the most common salt used to fractionate complex protein samples by step-wise precipitation (England & Seifter, 1990). Results presented in Table 1 show that the prepared protein fractions (AS30%, AS50% and AS70%) had high protein contents ranging from 97.5 to 100%. Fraction AS30% gave the highest yield (22.7%), whereas lower yields of 5.8% and 1.17% were obtained for Fraction AS50% and Fraction AS70%, respectively.

3.4. Native and SDS-PAGE profiles of the protein isolates

Fig. 2 shows the native-PAGE of the sesame protein isolates (Ses-PIs) which had been prepared in water and using different NaCl and ammonium sulfate concentrations. The Ses-PI-H₂O extract revealed protein bands mainly comprising of 7S and 11S proteins. Samples prepared in the presence of NaCl (0.2–1 M) contained higher amount of aggregated proteins (~669 kDa) and faint bands of 7S globulins (140 kDa) and 2S albumins. A similar pattern was observed for the Fraction AS30% and the Fraction AS50% which were composed mainly of high molecular weight bands of 669 kDa (possibly 11S aggregates) and 135 kDa (7S globulins). Larger quantities of 7S globulins, lower polypeptides and 2S albumins bands were observed in the Fraction AS70%. Scopes (1987) earlier reported that with ammonium sulfate fractionation, the material precipitating before 25% saturation is generally, pre-aggregates or very high molecular weight protein, while higher percent saturation, results in the precipitation of lower molecular weight proteins with a lower yield.

SDS-PAGE under reducing conditions indicated differences in the electrophoretic pattern between the protein extracts prepared in water and those extracted in the presence of NaCl (Fig. 3A). The water extract showed different components with molecular weights ranging from ~9 to 100 kDa. The 2S and 7S globulins, with respective molecular weight of 13 and 45–50 kDa, were the two major components. The sesame 2S albumin, conventionally termed β -globulin, is composed of two subunits of 9 and 4 kDa linked by disulfide bonds, which co-fractionate under reducing condition (Orruno & Morgan, 2007; Tai, Wu, Chen, & Tzen, 1999). The 7S globulin, present in sesame seed as a minor constituent (1–2% of the total proteins), was recently characterized as being composed of two isoforms (7S-I and 7S-II) encoded by a gene of the 7S vicilin-like globulin family (Tai, Lee, Tsai, Yiu, & Tzen, 2001). Several other bands were also observed exclusively in the aqueous extract. Based on literature, these polypeptide bands correspond to Oleosin isoforms (Chen et al., 1997; Frandsen et al., 2001; Tai, Chen, Peng, & Tzen, 2002) and to three minor proteins, Sop 1, 2 and 3 which have been identified in the oil bodies of sesame seeds (Chen et al., 1998; Lin & Tzen, 2004). Some unknown bands were also present in the water extract. The extracts prepared with 0.2 M, 0.6 M and 1 M NaCl showed similar elution profiles. The extracts were composed of similar polypeptide bands belonging mainly to the acidic subunits (30–35 kDa) and the basic subunit (20–25 kDa) of 11S globulin of sesame seeds as reported previously (Tai et al., 1999) and in other known 11S globulins of diverse species (Jung, Nam, Saalbach, Muntz, & Nielsen, 1997). Ses-PI-0.2 M NaCl showed higher ratios of 2S and 7S components (intense bands) than the Ses-PI-0.6 M and 1 M NaCl extracts which had more 11S subunits. Thus, low salt concentration (0.2 M NaCl) appeared to affect the composition of proteins extracted compared to the water extract, but no significant changes in the composition were observed with higher levels of NaCl.

Under ammonium sulfate precipitation, the protein fractions showed very different SDS-PAGE patterns (Fig. 3B). The Fraction AS30% was mainly composed of 11S globulin subunits and some 2S albumin and 7S globulin, somewhat similar to the profile of the extracts prepared with NaCl at various concentrations. However, as the ammonium sulfate increased to 50% and 70% saturation, the extracts were

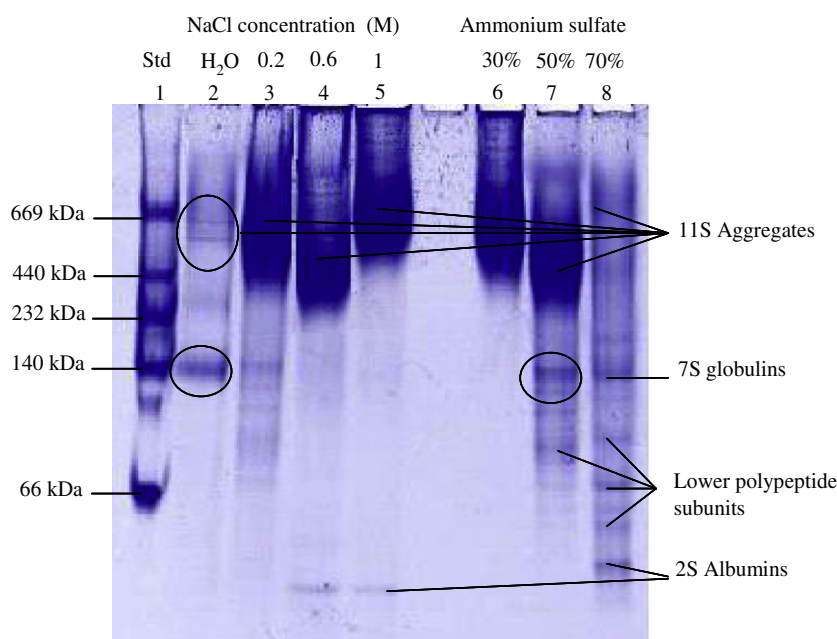


Fig. 2. Native-PAGE of sesame protein extracts prepared in water, at different NaCl concentrations and under ammonium sulfate precipitation. Samples were run on 4–15% gradient acrylamide gel. Lane 1-Std: molecular weight markers (66–669 kDa); Line 2: H₂O extract; Lines 3, 4 and 5: protein extracts prepared in the presence of 0.2 M, 0.6 M and 1 M NaCl, respectively. Lines 6, 7 and 8: protein fractions prepared under ammonium sulfate precipitation.

composed mainly of 2S and 7S polypeptide bands. The Fraction AS70% had a greater amount of the 7S globulin fraction with some contamination of 2S albumin. Orruno and Morgan (2007) recently reported that SDS-PAGE of purified 7S globulin (initially extraction at 80% ammonium sulfate saturation) was composed of at least eight polypeptides with molecular weights ranging from 12.4 to 65.5 kDa. 7S globulins from several species such as pea (Gatehouse, Croy, Morton, Tyler, & Boulter, 1981), broad bean (Scholz, Manteuffel, Muntz, & Rudolph, 1983) and amaranth seeds (Marcone, 1999) have also been found to possess a complex polypeptide composition over a wide range of molecular weights. This selective precipitation of sesame proteins using ammonium sulfate seemed to be an interesting approach for the separation of sesame protein fractions, which could serve as starting material for further purification using chromatographic techniques.

3.5. FTIR spectroscopic analysis

Fourier transform infrared (FTIR) spectroscopy provides a valuable method for studying conformational changes of proteins in solid, crystal and solution states. However, a limitation for this method is that the protein materials studied are often protein extracts (isolates, concentrates and meals) composed of different individual proteins (2S albumins, 7S and 11S globulins) rather than purified fractions, and unfortunately, many plant proteins and other molecules important to food industry cannot be purified since it is costly and time-consuming. In this study we used the technique on the protein extracts in order to glean information about global changes occurring in the secondary structure of the different proteins present in the mixture.

3.5.1. Effect of NaCl and ammonium sulfate

FTIR spectra of Ses-PI prepared in water, at different NaCl concentrations and under ammonium sulfate precipitation are presented in Fig. 4. The deconvoluted sesame protein IR spectrum showed typical protein amide I (1600–1700 cm⁻¹) and amide II (1600–1560 cm⁻¹) bands, which arise from specific stretching and bending vibrations of the protein backbone (Byler & Susi, 1986). Table 2 presents the assignments of the deconvoluted amide I' bands of Ses-PI-H₂O extract. From the

relative intensities of the infrared bands, the secondary structure of Ses-PI-H₂O at 25 °C consisted of several bands, predominantly α -helix (1648–1650 cm⁻¹), random coil (1642 cm⁻¹), antiparallel β -sheet aggregates (1618 and 1685 cm⁻¹), turns (1660 and 1670 cm⁻¹) and side chain (1604 cm⁻¹) structures. Two other bands of the amide II region were also observed at 1584 and 1568 cm⁻¹ (predominantly resulting from an N–H bending vibration coupled to C–N stretching). However as these arise from complex vibrations involving multiple functional groups they are less useful for protein structure prediction than the amide I bands (Jackson & Mantsch, 1995).

Addition of sodium chloride caused conformational changes in sesame protein structure, particularly at lower concentration (Fig. 4A). Proteins extracted using 0.2 M NaCl showed a shift to lower wavenumbers, an increase in random coil (1642 cm⁻¹) band intensity and a prominent reduction in antiparallel β -sheet aggregates (1618 and 1685 cm⁻¹), suggesting protein unfolding, dissociation and rearrangement of protein molecules. It has been demonstrated that salts perturb protein conformational structures by influencing the physical state of water, interrupting the hydrogen-bonded structure of water, and by affecting the electrostatic and hydrophobic interactions of proteins (Damodaran & Kinsella, 1982; Von-Hippel & Scheich, 1969). As the salt concentration increased from 0.2 M to 0.6 and 1 M NaCl, proteins showed the formation of aggregates characterized by the presence of increased anti-parallel β -sheet structures (increase in the intensity of the 1618 cm⁻¹ band). Choi and Ma (2005) reported that addition of sodium chloride can promote salting-out and aggregation due to higher intramolecular interactions, which may stabilize protein conformation. This may explain why Ses-PI-0.6 M NaCl and Ses-PI-1 M NaCl samples showed similar IR spectra with a more stabilized structure than at low salt concentration (0.2 M NaCl).

The effect of ammonium sulfate fractionation on sesame protein structure is shown in Fig. 4B. Prominent peak shifts were observed for the proteins extracted in the presence of 30%AS and 50AS% (Fraction AS30% and Fraction AS50%) compared to Ses-PI-H₂O extract, suggesting changes in protein conformation. Specifically, the bands at 1652 and 1648 cm⁻¹ shifted to lower wavenumbers (1650 and 1640 cm⁻¹). In general the IR spectra for Fractions AS30% and AS50% revealed several bands representing random coils (bands at 1640–1648 cm⁻¹), α -helices (1651–1655 cm⁻¹), turns (1671–1673 cm⁻¹) and aggregated

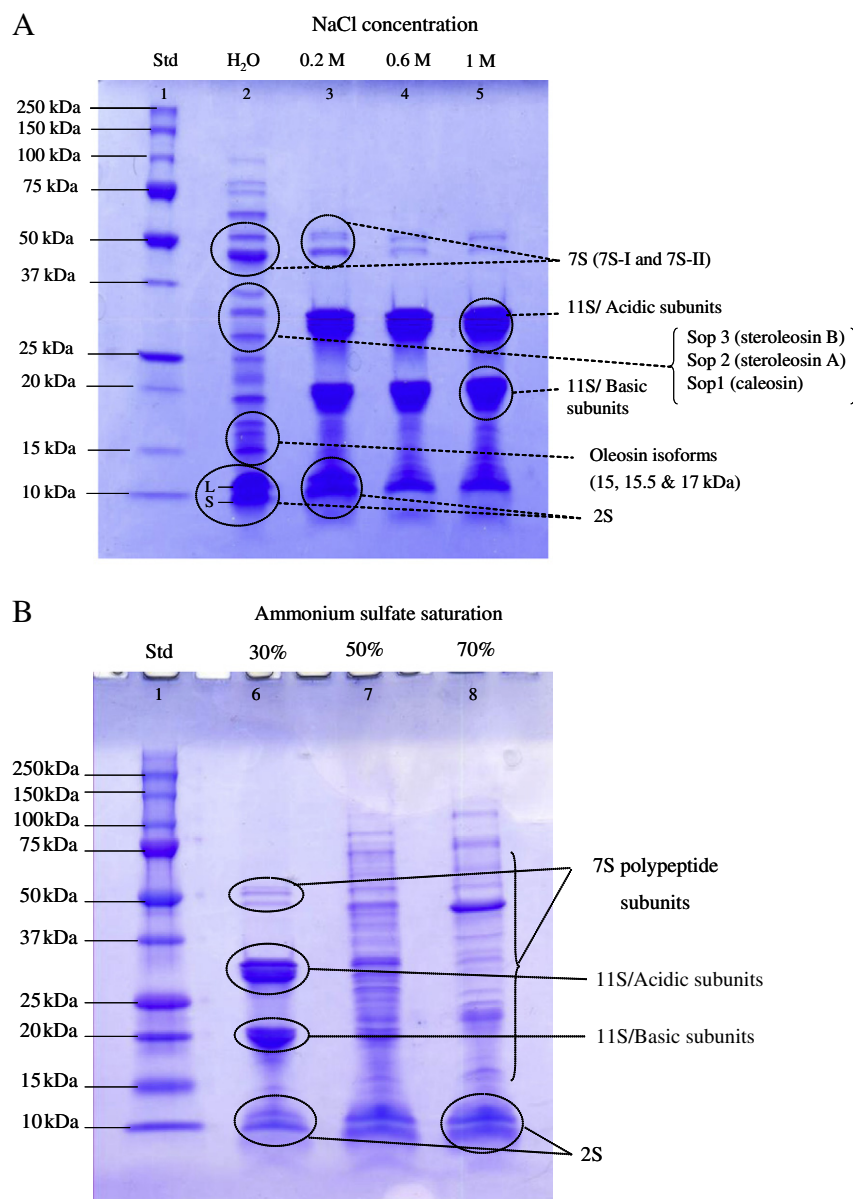


Fig. 3. SDS-PAGE of sesame protein fractions: (A) prepared in water and at different NaCl concentrations; (B) under ammonium sulfate precipitation. Samples were run on 10–20% gradient acrylamide gel. Lane 1-Std: molecular weight markers (10–250 kDa); Lines 2: H₂O extract; Lines 3, 4 and 5: protein extracts prepared in the presence of 0.2 M, 0.6 M and 1 M NaCl, respectively; Lines 6, 7 and 8: protein fractions prepared under ammonium sulfate precipitation. L, large subunit; S, small subunit of 2S albumin.

strands (shoulders at 1688 cm^{-1} and 1615 cm^{-1}). Compared to Fraction AS30% and Fraction AS50%, Fraction AS70% showed an increase in the intensity of the random coil band ($1640\text{--}1650\text{ cm}^{-1}$), with a concomitant increase of the α -helical band (1653 cm^{-1}), suggesting a transition from random coil to more ordered structure. The spectra also indicated the presence of antiparallel β -sheet aggregates at 1618 cm^{-1} . Two other bands of the amide II region were also observed at 1584 and 1568 cm^{-1} , similar to that observed for the aqueous and sodium chloride extracted samples.

3.5.2. Effect of heating temperature

To study the effect of thermal treatment on the secondary structure of Ses-PI, the FTIR spectra of Ses-PI-1 M NaCl heated at temperatures ranging from $25\text{ }^{\circ}\text{C}$ to $95\text{ }^{\circ}\text{C}$ were collected (Fig. 5A). The data showed marked changes in the conformation of sesame when the protein was heated above $50\text{ }^{\circ}\text{C}$. The major changes observed during heating were a pronounced transition to more β -sheet structures and a shift toward lower wavenumbers, suggesting protein structure reorganization (Choi & Ma,

2005). The intensity of the bands associated with the formation of intramolecular β -sheet structures ($1622\text{--}1636\text{ cm}^{-1}$) increased upon heating. On the other hand, the intensities of the aggregated strands (1616 and 1685 cm^{-1}) progressively increased and then decreased above 50 to $95\text{ }^{\circ}\text{C}$, indicating association/aggregation (Boye, Ma, & Harwalker, 1997) and subsequent dissociation. In addition, the FTIR results also showed that on cooling the Ses-PI-1 M NaCl sample back to $25\text{ }^{\circ}\text{C}$ (Fig. 5B), it assumed a secondary structure that was very different from the ones observed during heating, with a transition toward more random coil (1648 cm^{-1}), α -helix (1661 cm^{-1}) and turn (1672 cm^{-1}) structures. The pronounced decrease in band intensities and slight shift toward lower wavenumbers suggest extensive unfolding and thermal denaturation of the sesame proteins which was not reversed on cooling. Additionally, the spectra showed further development of aggregate structures on cooling (i.e., bands at 1614 and 1684 cm^{-1} representing antiparallel β -sheet structure).

The presence of amide II absorption indicates that not all of the protein's peptide hydrogen atoms have been exchanged for

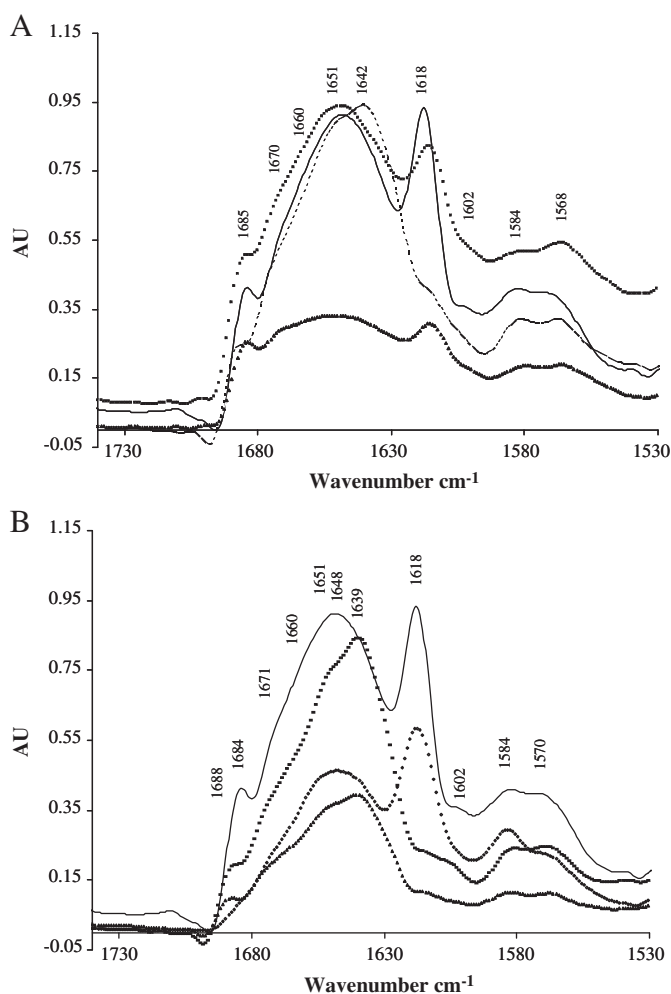


Fig. 4. FTIR spectra of sesame protein isolates (Ses-PI): (A) prepared in water and at different NaCl concentrations; (B) under ammonium sulfate precipitation. (—) Ses-PI-H₂O; (---) Ses-PI-0.2 M NaCl; (□) Ses-PI-0.6 M NaCl; (△) Ses-PI-1 M NaCl; (▲) Fraction AS30%; (■) Fraction AS50%; (○) Fraction AS70%.

deuterium, likely due to the compact inaccessible form of some native, folded protein molecules (Clark, Saunderson, & Suggett, 1981; Kavanagh, Clark, & Ross-Murphy, 2000). The amide II bands (centered around 1550 cm⁻¹) have been shown by some authors to decrease on heating due to H-D exchange, thereby indicating progressive unfolding of the protein (Boye, Ismail, & Alli, 1996; L'Hocine, Boye, & Jouve, 2007). Interestingly, our results showed an increase of the amide II bands on heating. One of the possible reasons for this increase could be exposure of more sites that had not undergone H-D exchange as a result of the heat treatment due to a more compact protein structure. Some authors attribute this to a concentration effect induced by heating (Kavanagh et al., 2000).

Table 2
Assignment of amide I' bands in the FTIR spectrum of Ses-PI.^a

Wavenumber (cm ⁻¹)	Assignment
1600–1604	Side-chain vibrations
1616–1618	Anti-parallel β-sheet, aggregated strands
1632–1639	Intramolecular β-sheet
1640–1644	Random coil
1648–1652	α-Helix
1660	β-Turns
1670–1673	β-Turns
1680–1988	Anti-parallel β-sheet, aggregated strands

^a Ten percent in deuterated phosphate buffer, pD 7.0.

3.5.3. Circular dichroism measurements

Secondary structure elements such as α-helix and β-sheet have dichroic activity in the wavelength range from 190 to 260 nm (Kelly & Price, 1997). Fig. 6 shows far-UV CD spectra of DSM, water (Ses-PI-H₂O), salt (Ses-PI-0.2 M NaCl, Ses-PI-0.6 M NaCl, Ses-PI-1 M NaCl) and ammonium sulfate extracts (Fraction AS30%, AS50% and AS70%), in the far-UV region, 178–250 nm. The secondary structure compositions (α-helix, β-sheet, turns and random coil) of these samples were calculated from the far-UV CD spectra using the SELCON3 program in the CDPro software, and are displayed in Table 3.

The CD spectrum of the sesame extracts in the far-UV region showed two negative bands at 209 and 221 nm, as well as a predominant positive band at 191 nm (Fig. 6). These features of the spectrum are sufficient indicators of a highly ordered structure, including the α-helices and β-types (Yang, Wu, & Martinez, 1986; Zirwer, Gast, Welfle, Schlesier, & Schwenke, 1985). In general, the CD spectrum of α-helix has an intensive positive band at 191–193 nm and a typical double minimum (negative) at 208–210 and 222 nm, whilst that of β-sheet has fairly intense positive band at 195–200 nm and a negative band about 216–218 nm (Yang et al., 1986).

The secondary structure composition of DSM was 24.4% α-helix, 20.3% β-sheet, 20.6% turns and 29.6% random coil (Table 3). In contrast, the water extract (Ses-PI-H₂O) showed a prominent α-helix content, which is in good agreement with the FTIR results. Extraction with salt (0–1 M NaCl) resulted in an extract with decreased α-helical content and increased β-strands, turn and random coil contents. In the case of ammonium sulfate precipitation, the protein fraction

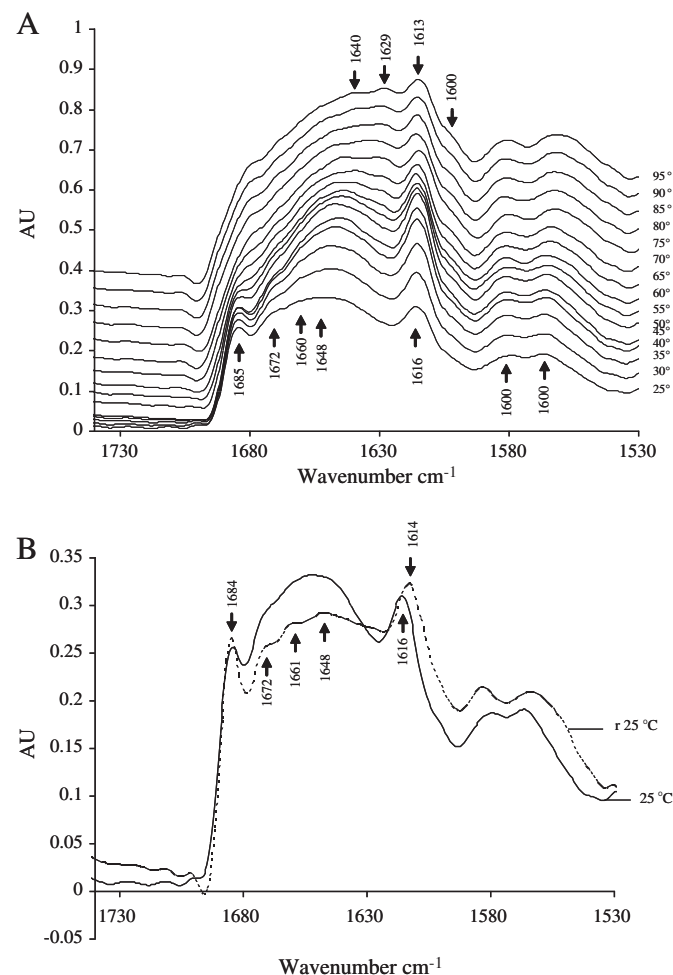


Fig. 5. Stacked plot of deconvoluted infrared spectra (1740–1530 cm⁻¹) of (A) 10% Ses-PI-1 M NaCl heated from 25 °C to 95 °C at 5 °C intervals, and (B) cooled back to 25 °C (r 25 °C).

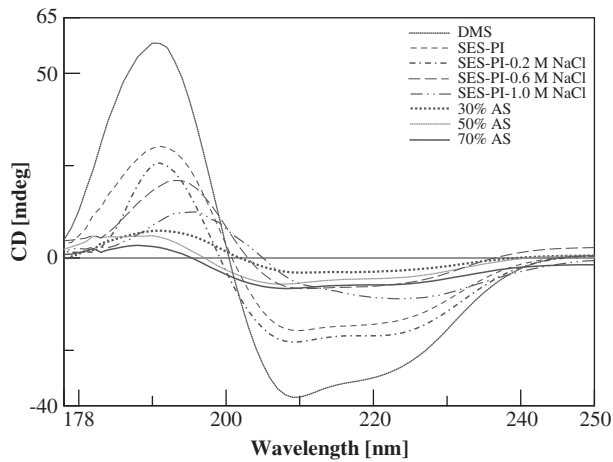


Fig. 6. CD spectra (Far-UV) of SDM and sesame protein extracts prepared using water (Ses-PI-H₂O), different salt concentrations (Ses-PI-0.2 M NaCl, Ses-PI-0.6 M NaCl, Ses-PI-1 M NaCl) and ammonium sulfate precipitation (Fraction AS30%, Fraction AS50%, Fraction AS70%).

(AS30%) had more α -helix. The helical content increased slightly at 50% saturation and then decreased at 70% saturation. These differences in the secondary structure compositions suggest conformational changes as a result of difference in the protein composition of the extracts.

3.6. Functional properties of sesame protein isolate

3.6.1. Solubility

Fig. 7 shows the pH-solubility curves of the sesame protein isolates (Ses-PI) compared to soy protein isolate (Soy-PI). A comparison with soy was considered relevant due to its extensive utilization by the industry. Both Ses-PI-H₂O and Soy-PI showed similar solubility trends with a minimum solubility in the region of the isoelectric point (pH 4–5), however, Soy-PI had higher solubility below and above the pI regions than the Ses-PI. At pH values between 1 and 3, the solubility for Ses-PI was 20–43% compared to an average of 53% for Soy-PI. Above the pI region, the solubility was 5–45% for Ses-PI compared to 46–59% for Soy-PI.

Sesame protein isolates prepared in the presence of sodium chloride showed similar solubility profiles independent of NaCl concentration. Compared to Ses-PI-H₂O and Soy-PI, the maximum solubility ($\approx$ 58–71%) occurred at very acidic pH (pH 1 to 2). At pH above 2, solubility markedly decreased to around 6 to 13% at pH 4.0 and continued to decrease up to pH 10.0 with a maximum solubility below 9% for the three sesame protein extracts.

Table 3

Secondary structure composition of sesame protein extracts as calculated from far-UV CD spectra using the SELCON3 program in CDPro software.

Sample	α -Helix (%)	β -Sheet (%)	Turns (%)	Random coil (%)
Defatted sesame meal (DSM)	24.4	20.3	20.6	29.6
Ses-PI-H ₂ O (0 M NaCl)	80.0	0.0	5.5	14.7
Ses-PI-0.2 M NaCl	54.9	6.1	26.1	28.0
Ses-PI-0.6 M NaCl	35.9	7.5	21.7	36.3
Ses-PI-1 M NaCl	36.0	7.9	22.4	34.2
^b Ammonium sulfate saturation				
Fraction AS30%	48.5	10.5	18.8	24.4
Fraction AS50%	50.3	6.2	13.8	20.0
Fraction AS70%	41.3	13.6	19.3	26.8

^a Combined regular and distorted α -helices.

^b Combined regular and distorted β -strands.

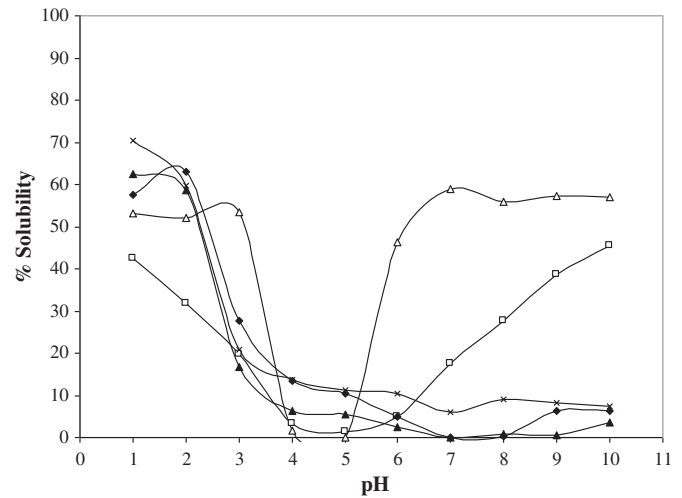


Fig. 7. pH-solubility profiles of sesame protein isolate (Ses-PI) in water ($\square$), 0.2 M NaCl ($\bullet$), 0.6 M NaCl ($\blacktriangle$) and 1 M NaCl ($\times$) compared to soy protein isolate ($\triangle$).

In general, for most proteins at pH values above and below the isoelectric pH, the proteins net charge, electrostatic repulsion and ionic hydration promote their solubilization. This is in good correlation with our observed solubilities of Ses-PI-H₂O and Soy-PI samples. However, for samples prepared in the presence of 0.2, 0.6 and 1 M NaCl, the salting-out effect of NaCl was significant above pH 4 (isoelectric point) and in the alkaline region resulting in a drastic decrease in solubility. Thus, high adsorption of chloride ions by the proteins may have lowered repulsive interactions between protein molecules, contributing to aggregation. In fact, Damodaran and Kinsella (1982) reported that the decrease in the electrostatic charge repulsion between proteins may enhance hydrophobic interaction between their non-polar surfaces and lead to formation of insoluble aggregates.

3.6.2. Functionality

A brief comparative study of other functional properties between Ses-PI-1 M NaCl and Soy-PI samples was also performed. This study focused only on the Ses-PI-1 M NaCl sample due to the lower protein yield obtained for the other Ses-PI samples (0.2 and 0.6 M NaCl). Functional properties were studied at pH 2.0, 5.0 and 7.0 in order to evaluate the effect of pH. The results (Table 4) showed Ses-PI had lower water holding and fat binding capacities compared to Soy-PI. Both fat and water absorption have been described as a physical entrapment of oil and water, respectively (Kinsella, 1979), and several

Table 4

Functional properties of sesame and soy protein isolates.

Sample	WHC (%)	FBC (%)	EAI (m ² /g)	ESI (min)	FE (%)	FS (%)
Ses-PI-1 M NaCl						
pH 2	–	–	13.0 $\pm$ 0.2c	20.8 $\pm$ 0.5a	431 $\pm$ 0.0d	48.5 $\pm$ 0.2c
pH 5	–	–	20.7 $\pm$ 0.8a	20.7 $\pm$ 0.9a	465 $\pm$ 2.1b	86.3 $\pm$ 0.0b
pH 7	327 $\pm$ 1.9b	323 $\pm$ 3.4b	16.8 $\pm$ 0.2b	17.4 $\pm$ 0.3b	537 $\pm$ 0.0a	87.8 $\pm$ 0.0a
Soy-PI						
pH 7	440 $\pm$ 1.4a	998 $\pm$ 7.0a	12.2 $\pm$ 0.1c	18.6 $\pm$ 0.1b	449 $\pm$ 0.9c	47.7 $\pm$ 0.6c

Different letters within the same column indicate statistically significant differences among samples ($P < 0.05$). Data are means $\pm$ standard deviation of triplicates.

WHC – water hydration capacity; FBC – fat binding capacity; EAI – emulsifying activity index; ESI – emulsifying stability index; FE – foam expansion; FS – foam stability (% liquid retained in foam after 5 min); Ses-PI-1 M NaCl – sesame protein isolate; Soy-PI – soy protein isolate.

authors have related these properties to the polarity of the protein side chains as well as to hydrophobicity, degree of denaturation, size and flexibility of the protein (Lin & Zayas, 1987; Nakai, 1983). At pH 7.0, the emulsifying activity and stability indices, as well as foam expansion and foam stability, of sesame protein isolate were significantly higher than that of Soy-PI ($P < 0.05$). Results also revealed that the emulsifying and foaming properties of Ses-PI were pH-dependent. The emulsifying activity, stability, foam expansion and foam stability were significantly ($P < 0.05$) higher at pH 5.0 compared to at pH 2.0. Foaming properties were, however, significantly higher at neutral pH than at both pH 5.0 and pH 2.0, whereas the emulsifying properties were reduced at pH 7.0 compared to pH 5.0. These results are in good agreement with those reported for sesame protein concentrate by Inyang and Iduh (1996) who showed an increase in these functional properties with pH. The higher % solubility (61%) observed particularly at pH 2.0, does not fully explain the improved surface properties of the sesame proteins; thus, other factors such as the hydrophilic-lipophilic balance, protein conformation and the composition of protein fraction, which could vary depending on the pH of protein resolubilization, may be key factors affecting the functional properties of the sesame proteins.

4. Conclusions

In summary, the results from this study have shown that extractability of sesame proteins is pH sensitive specifically above pH 4, and increases with increasing sodium chloride concentration. The type of proteins extracted was also affected by the addition of either NaCl or ammonium sulfate in the dispersing medium. FTIR showed marked changes in the conformation of the different Ses-PI extracts and heating above 50 °C caused pronounced unfolding and denaturation which was irreversible upon cooling and was associated with subsequent dissociation of protein aggregates. CD analysis revealed a predominance of helical structure, while addition of salt (NaCl) and ammonium sulfate decreased α -helix content and increased β -strands, turns and random coil structures. The present FTIR and CD studies provide valuable information on the global secondary structure of sesame proteins which could be a significant support for a further understanding of relationship between structural and functional properties and improving the utilization of sesame proteins as functional ingredient. Additionally, Ses-PI-1 M NaCl extracts had lower solubility, water holding and fat binding capacities, but superior emulsifying and foaming properties compared to Soy-PI. Emulsifying and foaming properties were affected by pH, while solubility was greatly affected by salt concentration and pH values above the isoelectric point.

As protein type and structure can affect functionality as well as properties such as immunogenicity and allergenicity, it is important that the effect of these conditions on the extraction and function of sesame proteins be carefully studied. In this paper the functionality of sesame has also been compared to soy as they are both plant proteins and both are considered as priority allergens requiring labeling when used in foods. Whereas soy has been extensively studied as an allergen, only few studies have looked at the structure–function–allergen interrelationship of sesame proteins. An understanding of the way these proteins behave in foods and how they compare to each other could help manufacturers and regulators to develop appropriate tools for the detection of sesame proteins when used in foods.

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