



Thermal processing, salt and high pressure treatment effects on molecular structure and antigenicity of sesame protein isolate

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ABSTRACT

Sesame protein is a recognized food allergen in many countries. Conditions used during processing can affect its detection in foods. In this study, the effect of different extraction conditions, pH, high pressure (HP) (100–500 MPa) and thermal processing (boiling, microwave heating and dry roasting) on the antigenic properties of sesame protein isolates were studied. Isolates prepared using water, sodium chloride at different concentrations, and ammonium sulfate at different percent saturation showed different immunoreactivity responses. Higher immunoreactivity was observed with the water, low salt (0.2 M NaCl), and ammonium sulfate isolated extracts compared to those extracted with higher salt (0.6–1 M NaCl) likely due to differences in the types of proteins extracted. High pressure treatment markedly decreased antigenicity at all pH values. Boiling and dry roasting increased the ELISA response, whereas microwaving decreased it. FTIR results showed significant changes in sesame protein conformation due to unfolding and/or denaturation which could have modified the antigenic properties of the sesame proteins.

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1. Introduction

The eight foods traditionally considered to be responsible for more than 90% of food allergic reaction are dairy, eggs, fish, shellfish, peanuts, tree nuts, wheat and soy (Hefle, Nordlee, & Taylor, 1996). Sesame has recently been added to the list of priority allergens both in Canada and in the European Union. As a result, concerns about exposure to sesame proteins by sensitized individuals has increased especially due to its frequent use in a variety of food products (e.g., oils, breads, processed meats and even prescription drugs). Although the global prevalence of sesame allergy is unknown, there is a growing number of publications and reports of sesame allergy in Europe, Israel, Japan (Goto et al., 2008) and the USA (Alexis, Safier, Rosenthal, & Cavuoto, 2009). Currently, Canada and the European Union require mandatory labelling when sesame is used as an ingredient in foods (Chassaing, Brohée, Norgaard, & van Hengel, 2007; Gangur, Kelly, & Navuluri, 2005).

Studies describing the IgE-binding properties of sesame revealed that allergic patients recognize a wide range of polypeptides with different molecular weight (MW), indicating the existence of multiple allergens in sesame seeds (Permaul et al., 2009; Zavalkoff, Kagan, Joseph, St-Pierre, & Clarke, 2008). At least 7 potential sesame allergens have been identified, including Ses i 1 (Pastorello et al., 2001; Wolff et al., 2003, 2004), Ses i 2 and Ses i 3 (Beyer, Bardina, Grishina, & Sampson, 2002) with MWs of 9–14 kDa, 7 kDa and 45 kDa, respectively. Two oil body-associated proteins (oleosins) were found to bind IgE from

sesame allergic individuals. These oleosins were sequenced and named Ses i 4 (17 kDa) and Ses i 5 (15 kDa), in accordance with the International Union of Immunological Societies (IUIS) Nomenclature (Leduc et al., 2006). More recently, 2 additional sesame seed allergens, Ses i 6 (52.2 kDa) and Ses i 7 (56.6 kDa) have been identified by Beyer, Grishina, Bardina, and Sampson (2007a,b) confirming the allergenic potential of the 11S globulin. Numerous other sesame allergens with MWs of 20–25 kDa and 35 kDa have been observed by two-dimensional electrophoresis and immunoblotting. However, further studies are needed to confirm the biological activity of these newly identified 11S allergenic subunits (Beyer et al., 2002; Fremont et al., 2002; Kolopp-Sarda et al., 1997; Navuluri et al., 2006). Furthermore, purification, biochemical and immunological properties of the sesame 7S globulin were also investigated (Orriño & Morgan, 2007), and cross-reactivity between sesame and other plant seeds, such as soybean and peanut (Stutius et al., 2009), tree-nuts (Lencer et al., 2006), and more recently with walnut (Wallowitz, Chen, Tzen, & Teuber, 2006, 2007) have been reported.

Several reports have shown that the ability of food proteins to elicit an allergic reaction can be modified by food processing. Food processing can alter the structure of food proteins either through unfolding and aggregation, or through covalent interactions with other food components (Besler, Steinhart, & Paschke, 2001; Mills, Sancho, Rigby, Jenkins, & Mackie, 2006; Mondoulet et al., 2005). Processing conditions could either increase, stabilize or decrease the allergenicity of a protein depending on both the structure and chemical properties of the allergen, the thermal processing used, e.g., dry vs wet, the temperature and duration of treatment, as well as the composition and interactions

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of the proteins with other constituents of the food matrix (Mondoulet et al., 2005; Sanchez & Frémont, 2003). Moreno, Maldonado, Wellner, and Mills (2005) using a physiological relevant *in vitro* gastrointestinal model showed that the major 2S albumin allergen, Ses i 1 had very high thermostability at 100 °C (at both acid or neutral pH) and was very resistant to digestion. This observation is, however, not universal for all allergenic proteins.

Although several allergens have been identified in sesame seeds, discrepancies persist in the literature about the protein fractions involved due to a number of factors such as the extraction procedure, quality of extracts, variety and analytical techniques. To our knowledge, there is also no published work presenting the effect of food processing treatments on the allergenicity of sesame proteins. In addition, the relationship between structural properties and antigenicity of sesame proteins has not been investigated yet. Infrared spectroscopy (FTIR) is a very powerful technique that can be used in such studies as it can provide information on the effects of food processing treatment on the global secondary structure of proteins which can be correlated to changes in immunoreactivity of treated sesame proteins.

The aim of this study, therefore, was to (1) assess the immunogenicity of different protein extracts of sesame prepared using various extraction conditions, (2) investigate the effects of high pressure (HP) and thermal processing on the immunoreactivity of the sesame protein isolate and sesame seeds, and (3) evaluate the use of FTIR spectroscopy to investigate the conformational changes induced as a results of these treatments and their correlation with the observed changes in immunoreactivity.

2. Materials and methods

2.1. Materials

White sesame seeds (*Sesamum indicum*) imported from Africa and distributed by “La Meunerie Milanaise Inc.” to local supermarkets in Montreal, Canada. 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 defatted sesame meal

Sesame seeds (500 g) 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 the mixture was allowed to decant, after which the solvent layer was siphoned. The mixture (meal plus residual solvent) was filtered on a glass sinter and then left overnight to dry at room temperature under a fume hood. After drying, the defatted sesame meal (DSM) was re-ground with a coffee grinder and used to prepare the sesame protein isolate (Ses-PI).

2.3. Fractionation of sesame proteins

Selective extraction of the different sesame protein fractions (2S, 7S and 11S) was investigated using two different protocols as described by Achouri, Nail, and Boye (2012). Protocol # 1: sesame protein isolates were prepared by dispersing 100 g of DSM in water or in 0.2 M, 0.6 M and 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 at 14,300 ×g for 30 min at 5 °C. The precipitates obtained were washed twice (with the same buffer used for extraction), 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:

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

Protocol # 2: consisted of a sequential fractionation of the different sesame protein fractions (11S, 7S and 2S globulins) using ammonium sulfate at 30%, 50% and 70% saturation. Briefly, 100 g of defatted meal was dispersed in phosphate buffer (0.02 M, pH 7.5) containing 1 M NaCl (1:10 w/v, meal to buffer ratio), 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 the extraction buffer, dialyzed against de-ionized water for 48 h at 4 °C and freeze-dried (Fraction 1-AS30%). The supernatant obtained after centrifugation was then adjusted to 50% ammonium sulfate saturation and to a final concentration of 70% ammonium sulfate saturation at 0 °C in an ice bath by the addition of solid ammonium sulfate in order to prepare two other fractions, namely, Fraction 2-AS50% and Fraction 3-AS70%, respectively. The obtained pellets were re-dissolved in the extraction buffer, dialyzed against distilled water and freeze-dried. Total protein content of the different isolates (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.

2.4. High pressure and thermal processing treatments

For the high pressure treatment, Ses-PI-1 M NaCl (1%, w/v) was first dispersed in water for 2 h at 35 °C. Aliquots of the sesame protein dispersion were then prepared at various pHs (2, 5, 7, and 10) and subjected to a range of high pressures (100, 200, 300, 400, 500 MPa) for 10 min at RT in a hydrostatic pressure unit (GEC Alstom ACB 900 HP, Nantes, France). High pressure treatment was conducted directly on “protein dispersions” rather than on whole sesame seeds, as this facilitated subsequent observation of changes induced in protein structure (i.e., unfolding, aggregation) as measured by FTIR. Furthermore, pressurization of whole seeds can result in loss of water soluble proteins such as 2S albumin which has been identified as the most potent sesame allergen. Various workers have, indeed, reported notable losses in soluble proteins in pressurized rice and soybean seeds (Kato, Katayama, Matsubara, Omi, & Matsuda, 2000; Omi, Kato, Ishida, Kato, & Matsuda, 1996).

For the other investigated treatments (roasting, boiling, microwave heating), however, whole sesame seeds were used as sesame seeds are mostly ingested in processed form (example in confectionary, bakery products, Asian soups and paste (tahini)). For thermal processing treatments therefore, whole sesame seeds were subjected to the followings: (a) boiling (100 °C) for 5 and 10 min. The samples were patted dry on paper towels and further air-dried at RT in a fume hood; (b) microwave heating in a Panasonic microwave oven (Panasonic Company, Secaucus, NJ, USA) at 1000 W for 1 and 3 min; (c) dry roasting at 150 °C for 7.5 and 15 min in a rotational oven roaster (Model DISCAF TN 1 Roaster (Sabadell, Barcelona, Spain)) previously set at the desired temperature. Unprocessed sesame seed was used as control. All the thermally treated samples were ground in a coffee grinder and defatted with *n*-hexane at a ratio of 1 to 10 (3 washes). Residual solvent was removed under vacuum, and the defatted sesame meals (DSM) were reground and stored at 4 °C until analyzed.

It is important to mention that although roasting, boiling and microwaving are all thermal treatments, their impacts can vary due to the treatment environment (wet versus dry heat) and the time

and extent of heating. As these treatments are regularly used in food processing and preparation it was therefore of importance to assess how each treatment affected the stability of the sesame allergens.

2.5. Protein solubility of treated sesame samples

The protein extractability (%) of the sesame meals prepared from control and thermally processed samples was determined by dispersing the samples in water (10% w/v) and pH adjusted to the studied values. The dispersions were stirred with a magnetic stirrer at 25 °C for 2 h and then centrifuged at 3000 × g for 30 min. The protein content of the supernatants was determined by the Bradford (1976) method using bovine serum albumin (BSA) as a protein standard. Protein extractability was calculated as: $PE (\%) = 100 \times PS / PT$, where PS is the protein content in the supernatant after centrifugation, and PT is the total protein content present in the DSM samples previously determined by Leco (AOAC, 1995). For the high pressure treatment, aliquots of protein dispersions (100 µl) prepared with Ses-PI-1 M NaCl (1%, w/v) at different pHs (2, 5, 7 and 10) were subjected to HP (100 to 500 MPa). After centrifugation, the protein content of the supernatants was determined by the Bradford method and divided by the total protein (PT) content of Ses-PI-1 M NaCl determined by Leco to obtain the % PE.

2.6. Enzyme-linked immunosorbent assay (ELISA) assessment of sesame antigenicity

The immunoreactivity of the sesame protein isolates, thermally processed and pressurized samples was determined by non-competitive, sandwich-type ELISA using a commercial sesame protein test kit (Tepnel BioSystems Ltd., Deeside, Flintshire, United Kingdom). Thermally processed defatted meal samples (10 g) were extracted using a salt-Tris/HCl extraction buffer (0.05 M Tris–0.2 M NaCl–1 M HCl, pH 8.25) at a sample to buffer ratio of 1:5. The mixture was blended for 2 min at RT, and the slurry was centrifuged to collect the supernatant. Prior to immunoassay, the concentration of the soluble protein in the supernatants was first determined by the method of Bradford (1976) using BSA as the standard protein. Protein aliquots were then normalized to 25 ppm prior to the immunoassay. The ELISA Tepnel sesame protein kit used contained test strips (96 microwells coated with purified anti-sesame antibodies), a negative control (a buffered base) and 2 kit-provided standards of 10 and 100 ppm sesame. Briefly, 100 µl of serially diluted sesame standards (100, 50, 25, 12.5, 6.25 ppm) and sample protein extract were pipetted into the anti-sesame sensitized microwell. After incubation at RT for 1 h, the wells were washed five times with the wash solution. Anti-sesame biotin (50 µl) was added into each well and incubated for another hour. After washing, sesame protein bounded to the antibody coated-well was detected by reaction with avidin-peroxidase conjugate (for 15 min at 25 °C). The excess reagent was removed by washing as previously indicated, and bound peroxidase enzyme activity was determined by the addition of a 3,3',5,5'-tetramethylbenzidine (TMB) substrate, which developed a blue coloration during the 45 min incubation period. The reaction was stopped with the stop solution which changes the color from blue to yellow. The absorbance was determined at 450 nm using a Bench Mark Plus Microplate Reader (Bio-Rad) controlled by the Microplate Manager Software (Bio-Rad). Triplicate analyses were performed for each sample. Quantitative estimates of sesame content (ppm) were calculated from the sesame calibration curve within the quantifiable range (6.25–100 ppm) using the sesame standards provided with the kit. A conversion factor of 5.5 was used to convert the sesame material (ppm) to sesame protein (ppm) as indicated in the sesame assay kit instructions.

2.7. Polyacrylamide gel electrophoresis (native and SDS-PAGE).

Native and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were performed according to the method of Laemmli (1970) using a Protean II electrophoresis apparatus (Bio-Rad Laboratories, Hercules, CA, USA), and Ready Gel precast gels of 4–15% and 10–20% polyacrylamide Tris–HCl, respectively. For the pressurized protein solutions, aliquots were directly diluted with the Laemmli native buffer (62.5 mM Tris–HCl buffer, pH 6.8 from Bio-Rad) to the desired protein concentration prior to loading into the gel. However, for the thermally processed defatted samples, 1 mg of each meal was dispersed in 1 ml of Laemmli native buffer for native-PAGE, whereas for SDS-PAGE the same buffer was used with the addition of 2% SDS and 2% β-mercaptoethanol. After centrifugation for 5 min at 1000 × g, 15 µl of sample solution was loaded in each well and gels run at constant voltage (200 V) in a Tris–glycine buffer for native-PAGE and in Tris–glycine buffer containing 0.1% SDS for the SDS-PAGE. Gels were stained with Coomassie Brilliant Blue R-250. Low (14 to 97 kDa) and high (10 to 250 kDa) molecular weight calibration kits from Amersham Pharmacia Biotech (Uppsala, Sweden) were used as molecular markers.

2.8. Fourier transform infrared spectroscopy (FTIR)

For FTIR analysis, sesame protein dispersions were prepared by dissolving Ses-PI-1 M NaCl (10% w/v) in D₂O-phosphate buffer prepared at various pH ranges of 2, 5, 7 and pH 10. D₂O was used instead of H₂O because of its greater transparency in the infrared amide I' region (Susi & Byler, 1983). Another set of samples were then subjected to high pressure treatment of 100, 200, 300, 400 and 500 MPa for 10 min at RT in a hydrostatic pressure unit (GEC Alsthom ACB 900 HP, Nantes, France) prior to FTIR analysis. The thermally processed samples (boiled, microwaved and roasted defatted meals) were dissolved (10% w/v) in D₂O-phosphate buffer (pH 7) and analyzed. After centrifugation for removal of any insoluble residues, aliquots (10 µl) of each sample were held in an IR cell with a 25 µm pathlength, between two CaF₂ windows 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). The spectrometer was purged with dry air for 10 min before recording the spectrum. A total of 256 scans were averaged at 4 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). The spectra were baseline-corrected between 1720 and 1525 cm^{−1} and normalized by dividing the absorbance value at each wavenumber in this range by the integrated area over this range, with the use of OMNIC software (Nicolet, Thermo Electron Cooperation).

Throughout this study, FTIR spectrum of Ses-PI-1 M NaCl in D₂O (as control, non-pressurized) was recorded and used for comparison against spectra of pressurized samples at various pH conditions. For thermally processed samples, the FTIR spectrum of a second control prepared from unprocessed sesame seed (Ses-PI-control) was recorded for comparison with processed sesame seeds.

2.9. 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. Protein content and yields of different sesame samples

Table 1 shows the protein content of whole sesame seed, defatted meal, and the sesame protein isolates (Ses-PI) extracted in water and under various sodium chloride concentrations, as well as their protein yields. The detailed proximate analyses of the sesame seeds and defatted sesame meal were previously reported in our earlier work (Achouri et al., 2012). The results indicated that the prepared sesame isolates had high protein content (>93%), and 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. Isolates prepared with saturated ammonium sulfate had protein contents of 100%, 100% and 97.5% for AS30%, AS50% and AS70%, respectively. Several studies have reported that during extraction, the extent of solubilization of the different protein components is greatly affected by their physicochemical properties, pH and ionic strength of the dispersing medium at a given temperature (Kinsella, 1979).

3.2. Effect of salt concentration on the immunoreactivity of prepared sesame isolates

ELISA results of the immunoreactivity of the different sesame protein isolates prepared using sodium chloride and ammonium sulfate are presented in Fig. 1. The assay revealed that the relationship between absorbance (OD) and analyte concentration was nonlinear and fitted the Logistic-Log mathematical model. The model function was calculated by the Bio-Rad Microplate Manager Data analysis software as follows:

$$\text{Abs} = (0.227 - 3.49) / (1 + (\text{Conc}/31.1)^{1.84}) + 3.49.$$

The precision of the ELISA kit was assessed by determining the intra-assay and inter-assay coefficients of variation (% CV), which measure the variability of the result for the same sample evaluated repeatedly in the same assay (run) and in separate assays (runs), respectively. It is generally agreed that intra- and inter-assay variability of less than 10% to 15% is acceptable (FDA, 2001). In the present study, the coefficient of variation (% CV) obtained with the standards and samples ranged from 1.8% to 3.5% and 1.4% to 9.0%, respectively, demonstrating a good precision for the test kit. The accuracy of the

Table 1

Protein content^a and yield of whole sesame seed, defatted sesame meal, Ses-PI and the different protein extracts (% wet basis).

Sample	Protein content (%)	Protein yield (%)
Whole sesame seed	24.8	–
Defatted sesame meal (DSM)	59.0	47.0
Ses-PI-H ₂ O (0 M NaCl)	100	12.5
^b 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
^c Ammonium sulfate saturation		
Fraction AS30%	100	22.7
Fraction AS50%	100	5.80
Fraction AS70%	97.5	1.17

^a The protein content values were determined using the Dumas method (AOAC, 1995) and a nitrogen conversion factor of 6.25 (ash content not determined).

^b 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.

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

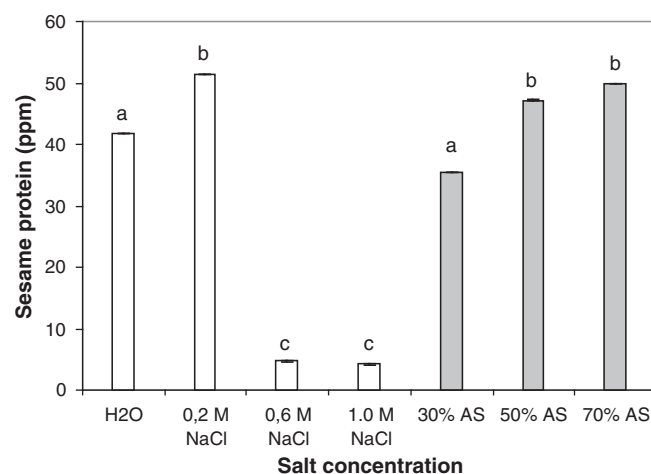


Fig. 1. ELISA response of sesame protein extracts prepared using different extraction protocols (expressed as ppm of sesame protein). H₂O (aqueous extract); 0.2, 0.6 and 1 M NaCl refers to sesame protein isolates (Ses-PI) extracted at different sodium chloride concentrations. 30% AS, 50% AS and 70% AS refers to Ses-PI prepared at different percent ammonium saturation. Different letters indicate statistically significant differences among samples ($P < 0.05$).

test kit was also performed by determining the recovery of samples spiked with known concentrations, and the results revealed acceptable recovery rates with values superiors to 98%.

Isolates prepared by using water and different concentrations of sodium chloride showed different immunoreactivity responses. Higher immunoreactivity was observed with isolates prepared using water and 0.2 M NaCl compared to those prepared with 0.6 M and 1 M NaCl. This is likely due to differences in the types of proteins extracted

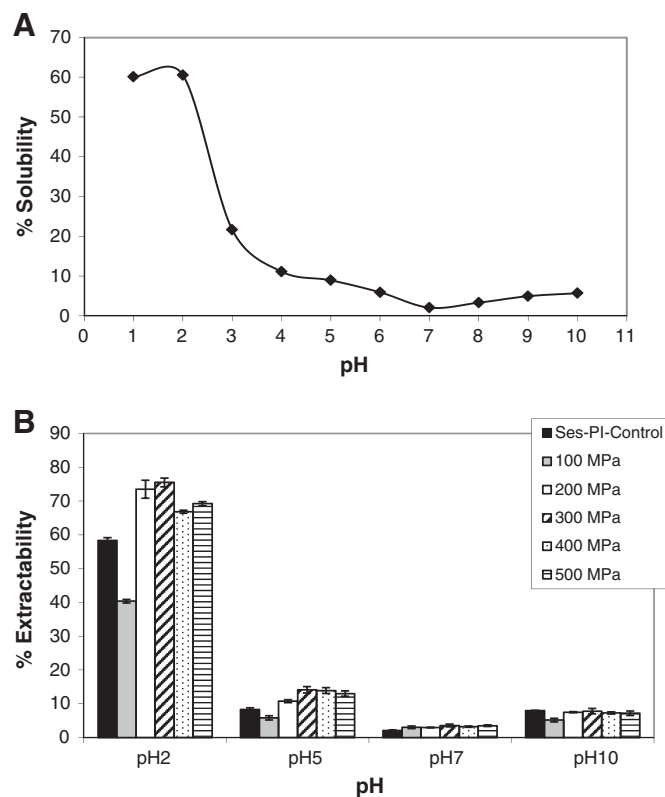


Fig. 2. Solubility-profile of sesame protein isolate (Ses-PI-1 M NaCl) at various pHs (1–10) (A), and (B) percent protein extractability as a function of pH and high pressure treatment.

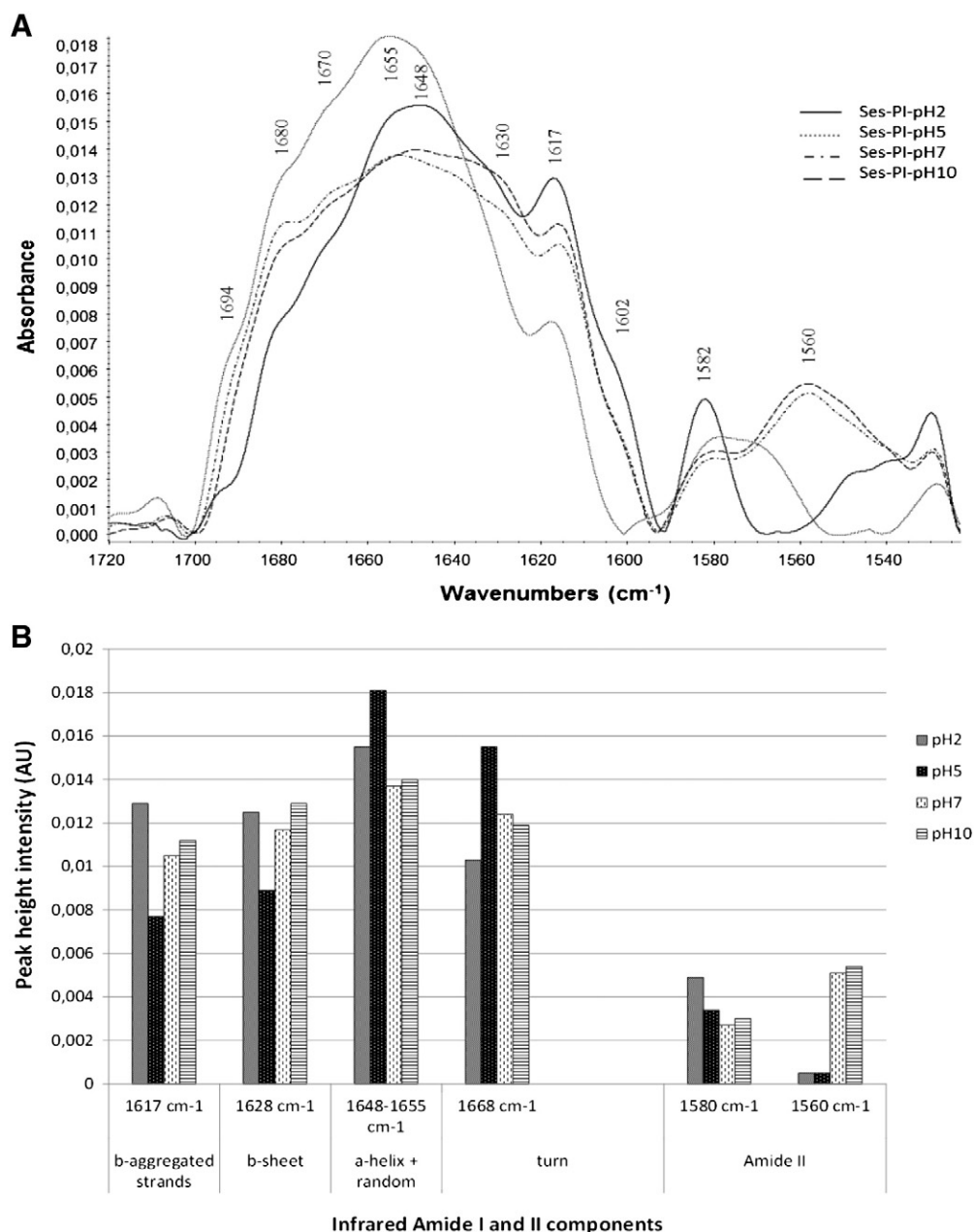


Fig. 3. FTIR-spectra (at 25 °C) of Ses-PI-1 M NaCl (10% w/v, in deuterated phosphate buffer): (A) at different pH values, and (B) the effect on the peak height intensities of different Amide I and II components.

under the different conditions studied as well as differences in the solubility of the proteins extracted (Achouri et al., 2012). Earlier studies (Prakash, 1986; Prakash & Nandi, 1978) showed, for example, that in water and at low NaCl concentrations (0–0.2 M), the 2S protein fraction is preferentially extracted, while at higher NaCl concentrations, there is greater solubilization of the 11S globulin and some 7S proteins as well as higher 16S protein aggregates. Salts can perturb protein conformational structure by influencing the physical state of water, interrupting the hydrogen-bonded structure of water, and by affecting electrostatic and hydrophobic interactions between proteins (Damodaran & Kinsella, 1982; Von-Hippel & Scheich, 1969). Our results also showed that when the concentration of NaCl was increased to 0.6 M and 1 M NaCl the immunoreactivity decreased significantly ($P < 0.001$) by nearly 90%. Although, the solubility of higher molecular weight components increased with increasing salt concentrations (0.6–1 M), conformational changes occurring due to salt introduction could have potentially prevented epitopes from reacting with the IgG antibodies. Indeed, our

previously reported SDS-PAGE, FTIR, and circular dichroism (CD) results on salt extracted sesame isolates confirmed the conformational changes in the protein and the formation of aggregates with increasing salt concentration (Achouri et al., 2012).

In contrast to the observations for sodium chloride at high concentrations, the 30%, 50% and 70%AS protein isolates all showed high immunoreactivity. Under ammonium sulfate precipitation, Scopes (1987) earlier reported that the material precipitating before 25% saturation is generally, pre-aggregates or very high molecular weight protein (11S and 16S), whereas higher percent saturation, results in the precipitation of lower molecular weight proteins (2S and 7S). This may explain why AS50% and AS70% showed higher immunoreactivity than the AS30% isolate. Indeed, the immunoreactivity of proteins is reportedly influenced by a multiplicity of factors particularly, the polypeptide profile, changes in secondary structure, protein flexibility, the extraction medium and epitope accessibility for antibody binding.

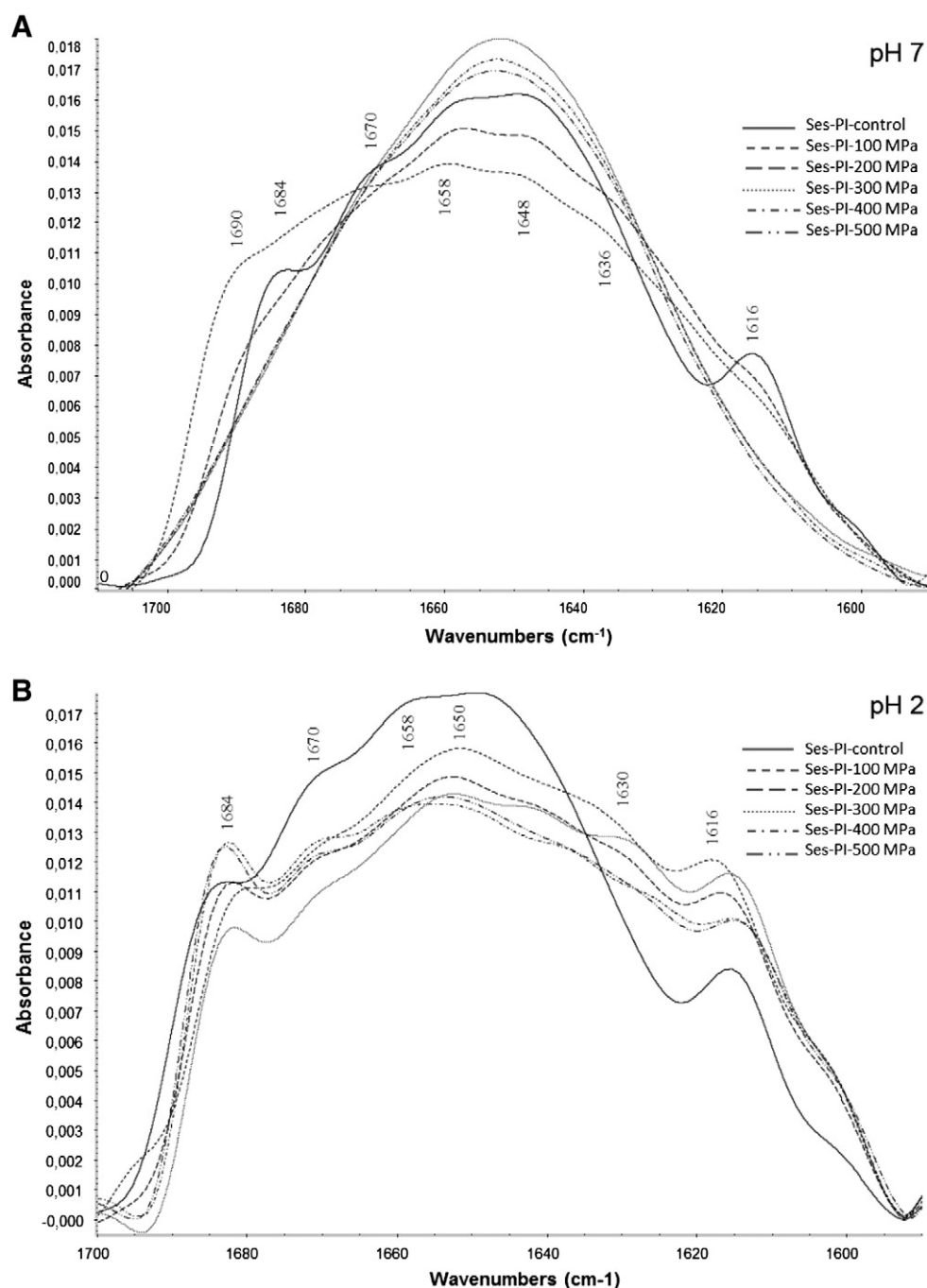


Fig. 4. FTIR-spectra (at 25°C) of Ses-PI-1 M NaCl (10% w/v, in deuterated phosphate buffer) subjected to high pressure treatment (100–500 MPa): (A) at pH 7.0, and (B) at pH 2.0.

3.3. Effect of high pressure on the immunoreactivity of sesame protein solutions

High pressure processing is a relatively novel process which is increasingly becoming a technology of interest in food application and development (Rastogi, Raghavarao, Balasubramaniam, Niranjana, & Knorr, 2007). In the area of allergy research, some preliminary research has suggested that the use of high pressure may modify the allergenicity of some foods (Kato et al., 2000; Penas, Préstamo, Baeza, Martínez-Molero, & Gomez, 2006; Penas, Snel, Floris, Préstamo, & Gomez, 2006). In this regard, protein solutions made with sesame protein isolate (Ses-PI-1 M NaCl) at different pHs were subjected to high pressure treatment to assess the influence on physico-chemical and antigenic properties.

3.3.1. Protein solubility

The pH-solubility profile of Ses-PI-1 M NaCl (Fig. 2A), showed a decrease in solubility with increasing pH values, particularly above the pI. The decrease in Ses-PI solubility at high NaCl concentration (1 M) could be explained by the fact that, the addition of sodium chloride compresses the solvation layer formed by the water around the hydrophilic residues on the protein's surface and increases protein-protein interactions. As the salt concentration increases, the charges on the surface of the protein interact with each other, not the water, and the protein falls out of solution (precipitates). In other words, addition of salt during aqueous extraction results in less water partaking in the solvation layer around the protein and exposure of hydrophobic patches on the protein surface. Proteins may then exhibit hydrophobic interactions, aggregate and precipitate out of solution. These interactions form

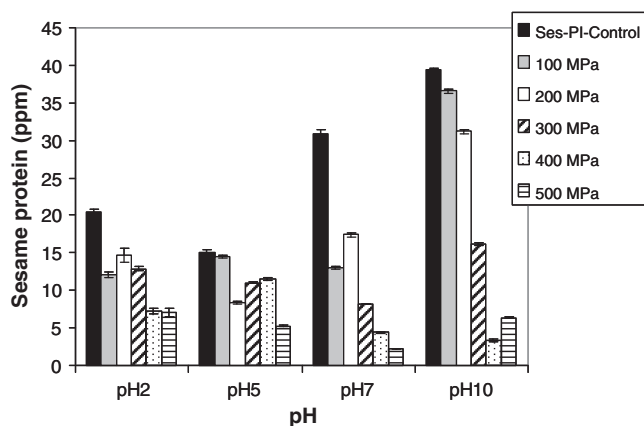


Fig. 5. ELISA assay of sesame protein solutions as a function of pH and high pressure treatment (100–500 MPa).

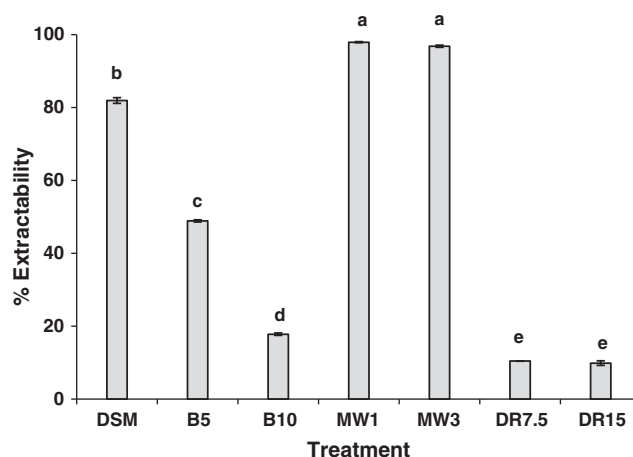


Fig. 7. Percent protein extractability of sesame seeds subjected to different thermal treatments. DSM: defatted sesame meal; B5 and B10: boiling for 5 and 10 min; MW1 and MW3: microwaving for 1 and 3 min; DR7.5 and DR15: dry roasting for 7.5 and 15 min.

the basis of “salting-out” interactions (Arakawa & Timasheff, 1984). To circumvent this low solubility at pH 7, we decided to conduct the high pressure treatment on sesame protein solutions prepared at various pH values of 2, 5, 7 and 10, at pressures of up to 500 MPa.

The results (Fig. 2B), indicated that the solubility of Ses-PI increased particularly at pH 2 and pH 5 with increasing pressure treatment above 100 MPa. This increase in solubility induced by high pressure may result from the unfolding of the sesame proteins, resulting in higher protein–solvent interactions. In addition, disassociation of large aggregates or precipitates into small molecular weight soluble aggregates could have contributed to the increase in solubility. The solubility increase at pH 7 was marginal. Although at pH 10 the protein solubility was originally low (~10%), more turbidity (milky appearance) was visually observed in the protein solutions treated at higher pressures suggestive of further aggregation, which may explain the slight decrease in solubility. A similar increase in turbidity resulting from the formation of aggregates has been reported for soy proteins (Molina, Papadopoulou, & Ledward, 2001) and lupin proteins (Chapleau & De Lamballerie-Anton, 2003).

3.3.2. FTIR spectroscopy

Fig. 3A and B shows the normalized FTIR spectra of the Ses-PI-1 M NaCl (control, non-pressurized) at various pH values and the peak height intensities of the major amide I' and II bands. The infrared absorption was normalized to allow for comparison. Overall, similarities were observed in the FTIR spectrums between pH 2 and pH 5, and between pH 7 and pH 10. At neutral pH of 7 (Fig. 3A), the Ses-PI protein structure consisted of several bands, predominantly α -helix (1648 – 1650 cm^{-1}), random coil (1642 cm^{-1}), anti-parallel β -aggregates (1617 and 1680 cm^{-1}), and turns (1668 cm^{-1}). Increasing the pH to 10 resulted in a shift to lower wavenumbers and an increase in intramolecular β -sheet band intensity (1630 cm^{-1}), suggesting changes in secondary structure.

The FTIR spectra recorded at pH 5 and pH 2 (Fig. 3A) showed a shift toward higher wavenumbers with the formation of a more defined peak with band range intensity of 1640 – 1655 cm^{-1} which represents the overlap of absorptions of α -helical and random coil structures. The data clearly demonstrated an effect of pH particularly

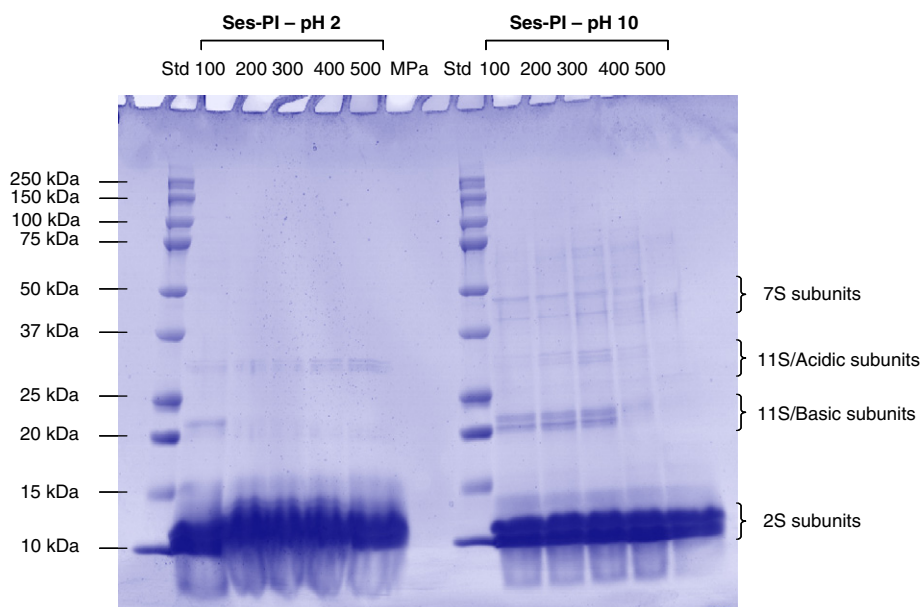


Fig. 6. Native-PAGE pattern of sesame protein solutions subjected to different high pressure treatment. Samples were run on 10–20% gradient acrylamide gel. Std: molecular weight markers (10–250 kDa); Ses-PI-1 M NaCl (10% w/v) at pH 2 and pH 10 subjected to high pressure treatment (100–500 MPa).

at pH 5 and pH 2 on the conformational structure of Ses-PI-1 M NaCl with a transition toward more α -helical (1652 cm^{-1}) and random coil (1648 cm^{-1}) structures, as shown by the increase in their respective peak height intensities (Fig. 3B). At extreme pH conditions (pH 2), the intensity of the aggregated β -strands (1617 cm^{-1}) increased, whereas for other isolates the band was not affected by the pH change. This 1617 cm^{-1} band as well as the one observed at 1692 cm^{-1} in the FTIR spectrum have been assigned to amide groups of antiparallel β -sheet(s) that are hidden in the interior of the protein and undergo very slow H-D exchange at ambient temperatures (Boye, Ismail, & Alli, 1996), and changes in the intensity of this band serve as a probe of changes in the tertiary structure that allow D_2O to enter the interior of the protein.

There was also a loss in the intensity of the band at 1560 cm^{-1} at highly acidic conditions (pH 2 and pH 5) and further increase in the intensity of the band at 1580 cm^{-1} in the amide II region, indicating a higher H-D exchange of buried $-\text{NH}$ groups (L'Hocine, Boye, & Jouve, 2007). The amide II region ($1500\text{--}1600\text{ cm}^{-1}$), corresponds to N-H bending vibration coupled to C-N stretching, and can be used to assess protein conformation. However, as it rises from complex vibrations involving multiple functional groups they are less useful for protein prediction than bands in the amide I region (Jackson & Mantch, 2000).

3.3.3. High pressure and pH effects on sesame secondary structure

The combined effect of high pressure treatment and pH on the FTIR spectral characteristics of Ses-PI-1 M NaCl (10% w/v, in deuterated phosphate buffer) is presented in Fig. 4A and B. Under neutral condition (pH 7.0), HP treatment induced changes in the secondary structure of Ses-PI (Fig. 4A) as indicated by the decrease in band intensities coupled with the transition toward more defined and ordered structures (β -sheet, α -helix, random coil and β -strands), specially at lower pressures (100 and 200 MPa). At higher pressures (300, 400 and 500 MPa), we observed further changes in the secondary structure with a more pronounced shift to a higher proportion of random coil (1645 cm^{-1}) structures. Zhang, Li, Tatsumi, and Kotwal (2003) found similar observations using circular dichroism (CD) in regard to the conversion of ordered structures (α -helix and β -structure) to random coil at higher pressure level (500 MPa) of soybean glycinin. In addition, a remarkable decrease in the intensities of the anti-parallel β -sheets bands at 1616 and 1684 cm^{-1} was observed which is indicative of further protein dissociation/disaggregation. Previous studies have indicated that HP treatment has a disruptive effect on the tertiary and quaternary structure of most globular proteins. At high pressures (above 300 MPa), many proteins tend to unfold, and dissociation can occur (Puppo et al., 2004; Silva & Weber, 1993).

At extreme acidic condition (pH 2.0) (Fig. 4B), HP treatment (100, 200 and 300 MPa) also altered the secondary structure and resulted in a pronounced transition toward a more balanced proportion of the principal bands (β -sheet, α -helix, random coil, turn and β -strands). At higher HP levels (400–500 MPa) the sesame protein structure showed a more stable conformation. These results are in accordance with those of Puppo et al. (2004) for soy protein isolate, where the changes provoked by high-pressure treatments were more important at pH 8 than at pH 3.

3.3.4. High pressure and pH effects on sesame immunoreactivity

The ELISA response of the sesame treated samples (which were all normalized to 25 ppm) showed that high pressure treatment decreased the immunoreactivity of sesame protein isolate (at all the pH values studied) compared to the untreated Ses-PI-control. Overall the results indicated that the higher the pressure, the higher the decrease in immunoreactivity, particularly at pH 7.0 and 10.0 (Fig. 5). However, careful observation of the results reveals some differences in immunoreactivity response for the same pH and specific pressure

treatment (e.g. lower immunoreactivity at 100 MPa than that at 200 MPa at pH 2 or pH 7). This fluctuation in immunoreactivity could be related to the observed structural changes at these pHs as previously reported in Fig. 4A and B. Low to medium pressure treatments are well-known to promote protein denaturation leading to unfolding and conformational changes (Iametti et al., 1997). At higher HP treatments further conformational changes in sesame proteins toward a more ordered or disordered structures (depending on the environmental pH) may have forced the protein into a more tightly packed structure obscuring the allergen epitopes, thus decreasing their immunoreactivity. This hypothesis agrees with the FTIR results which showed greater structural changes at high pressure treatments at pH 2 and 7. The role of HP and its effects on allergenicity and nutritional quality is currently a topic of much interest and investigation. To date promising results in the reduction of allergenicity were only observed upon combined high pressure and proteolytic treatments (synergistic effects) on bovine β -lactoglobulin (Bonomi et al., 2003), whey protein (Chicon, Belloque, Alonso, & Lopez-Fandino, 2009; Penas, Préstamo, et al., 2006; Penas, Snel, et al., 2006), as well as upon combined high pressure and heat treatments on rice (Kato et al., 2000) and egg in processed meat (Hildebrandt et al., 2010) rather than the effect of the single treatments conducted separately.

Furthermore, the results also revealed that the antigenic properties of the sesame proteins were pH-dependent (Fig. 5). Greater IgG binding were observed at pH 7.0 and pH 10.0 than at pH 5.0 and pH 2.0. This enhanced immunoreactivity could be related to a higher binding of IgG antibodies with multiple polypeptide subunits belonging to the different sesame protein fractions (2S, 7S and 11S). In fact, native-PAGE pattern of Ses-PI at pH 10, as shown in Fig. 6, showed more polypeptide subunits of 7S and 11S compared to the pattern of Ses-PI at pH 2. In addition, the re-organization in the sesame protein structure (conformational changes) with the increase in HP treatment, which is in good agreement with the previously reported FTIR results (Fig. 4A), may have allowed the antibodies greater access to hidden epitopes, and may thus have contributed to the observed increase in immunoreactivity. Previously, L'Hocine et al. (2007) also reported that the immunoreactivity response of soy 11S glycinin is strongly modulated by its conformational structure which in turn is greatly affected by pH and ionic strength of the dispersing medium.

3.4. Effect of thermal processing on the immunogenicity of sesame proteins

In general, thermal processes used in food production can either lead to an increase or loss of protein solubility as a result of denaturation and subsequent association/aggregation reactions. The extent is,

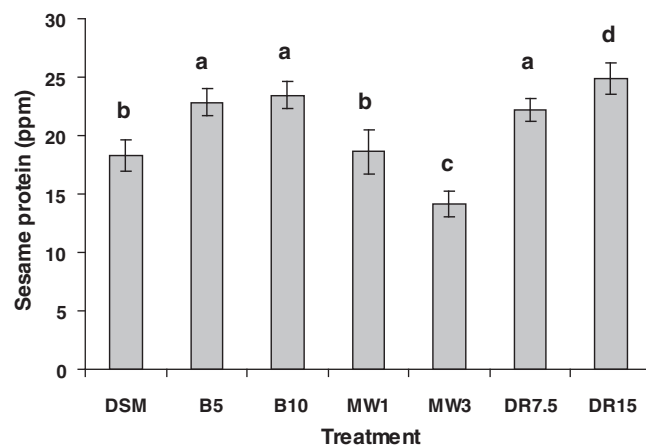


Fig. 8. ELISA assay of sesame proteins extracted from control and thermally processed sesame seeds. DSM: defatted sesame meal (control, no treatment); B5 and B10: boiling for 5 and 10 min; MW1 and MW3: microwaving for 1 and 3 min; DR7.5 and DR15: dry roasting for 7.5 and 15 min.

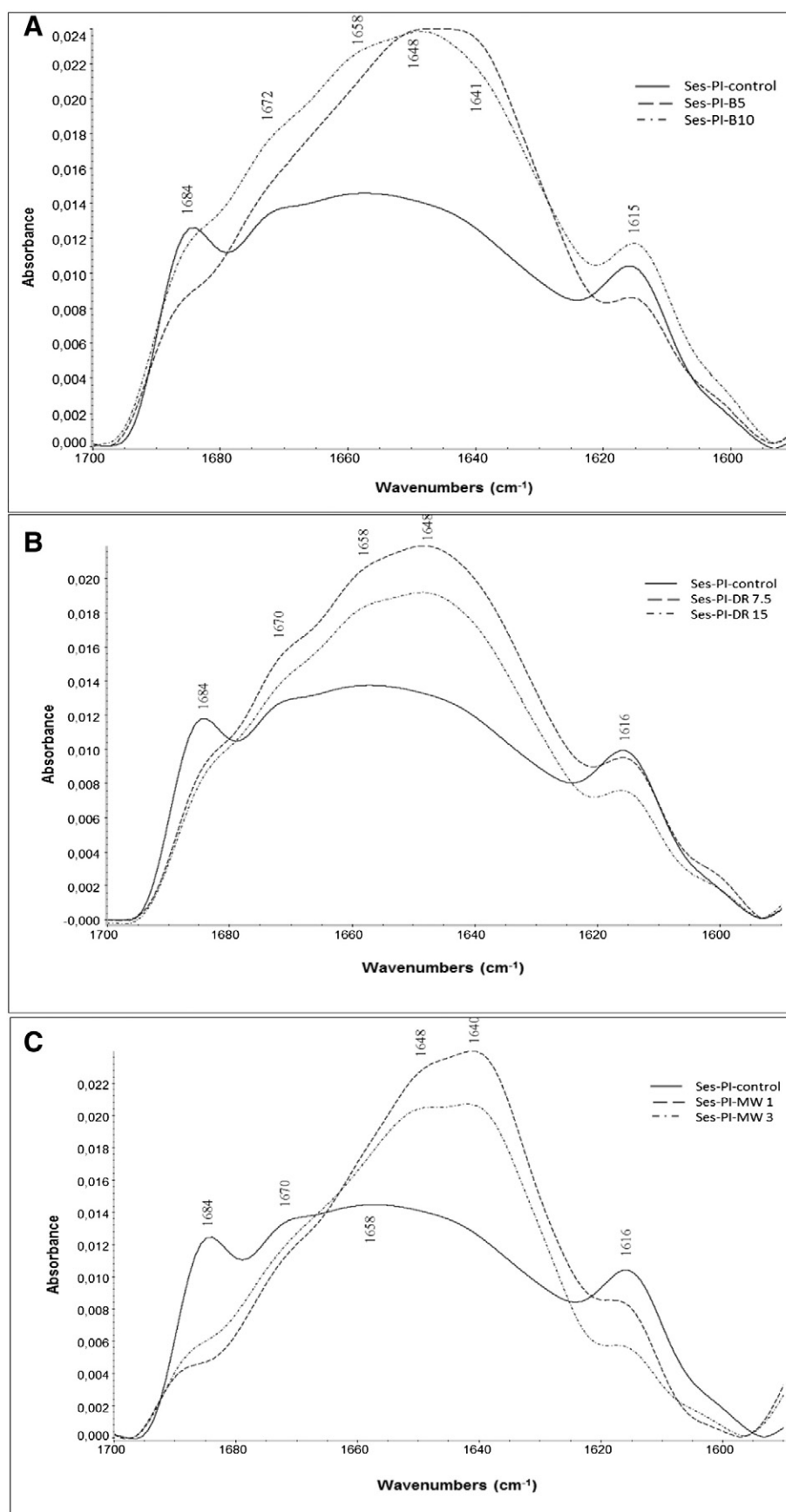


Fig. 9. Stacked plot of deconvoluted infrared spectra of sesame proteins extracted from thermally processed sesame seeds. (A) Control: proteins extracted from defatted sesame meal without treatment; B5 and B10: boiling for 5 and 10 min; (B) DR7.5 and DR15: dry roasting for 7.5 and 15 min; (C) MW1 and MW3: microwaving for 1 and 3 min. (10% w/v, in deuterated phosphate buffer, pH 7.0).

however, dependent on the severity and duration of processing (Maleki & Sathe, 2006). Different types of thermal processing (blanching, boiling, roasting or frying) will alter protein structure in different ways, potentially changing immunoreactivity responses. The extent of the change observed will be influenced by a multiplicity of factors including solubility, pH and the ionic strength of the dispersing medium.

3.4.1. Solubility

Our study results, as represented in Fig. 7, showed a remarkable decrease ($P < 0.05$) in sesame protein solubility by 40–78% and ~88% following boiling and dry roasting treatments, respectively. However, an increase (20%) in solubility was observed with microwave treatment. Heating can cause either reversible or irreversible protein denaturation. Reversible changes of protein structure mostly occur in the temperature range up to 60 °C and may affect the association or dissociation behavior of some protein polypeptides. These changes often are ascribed to pre-denaturation transitions caused by a partial loss of the three-dimensional protein structure and changes of protein hydration. Irreversible changes of protein structure may occur above the denaturation temperature of a protein and are influenced by the type and time of heating and further by environmental conditions such as pH, ionic strength, and protein concentration. Irreversible denaturation effects mostly result in reduced protein solubility leading to association and precipitation of the polypeptide chains and the formation of higher molecular weight compounds (De Wit & Klarenbeek, 1984). These changes in solubility could affect the antigenic response due to either exposure or masking of allergenic epitopes. These findings are in agreement with those of Schmitt and Maleki (2004) and Kopper et al. (2005), who reported that frying, boiling and roasting peanuts over increasing time periods decreases the solubility, and IgE binding of most peanut proteins including Ara h 1 and Ara h 2.).

3.4.2. Effect of thermal processing on immunoreactivity, secondary structure and SDS-PAGE

The antigenic response of the sesame proteins after subjecting them to thermal treatments was therefore assessed by ELISA and the results are shown in Fig. 8. Boiling of sesame seeds resulted in a significant increase in the ELISA response. However, the immunoreactivity was not time-dependent (i.e., no further increase in ELISA response was observed when boiling time was increased from 5 to 10 min). Microwave treatment for 1 min had no significant effect on the immunoreactivity of the sesame proteins compared to the untreated sample, whereas a significant decrease in immunoreactivity was observed after 3 min holding time. In contrast to microwaving, roasting for both 7.5 and 15 min induced a significant increase in immunoreactivity. In general, thermal processes by using moist heat or dry heat have been found to significantly either decrease or increase the IgG reactivity of well known allergens, most likely as the result of unfolding and aggregation mechanisms (Besler et al., 2001; Kleber, Krause, Illgner, & Hinrichs, 2004; Sathe, Teuber, & Roux, 2005).

To understand the relationship between the effect of thermal processing and IgG binding, changes in protein conformation were monitored by FTIR. The FTIR spectra of thermally processed samples indeed showed significant changes in protein conformation based on the type of treatment applied (Fig. 9). Boiling and roasting treatments showed similar patterns which included a shift toward lower wavenumbers of the major bands, coupled with the transition of predominant α -helical structures toward more unordered structures (random coil at 1647 cm^{-1}) and a progressive decrease in the intensity of the bands associated with the anti-parallel β -sheet structures and aggregated strands (1616 and 1684 cm^{-1}) (Fig. 9A and B). Microwave heating also induced major changes in the sesame protein structure compared to the untreated sample (Ses-PI-control). In this case, a transition from α -helical structure toward more ordered β -sheet structures and a decrease in intensities of the antiparallel

β -sheet (1618 cm^{-1}) and aggregated strands (1684 cm^{-1}) were observed, which are all indicative of protein unfolding and denaturation (Fig. 9 C). Thus, depending on the location of the allergenic epitopes, thermal processing (boiling, microwaving and dry roasting) of sesame proteins could result in changes in the antigenic response of treated samples, due to unfolding, denaturation or aggregation depending upon whether the conformational epitopes are exposed or hidden within the structure of the molecule.

Proteins extracted from the defatted sesame meal prepared from the boiled, microwave-heated and roasted sesame seeds were compared with the untreated control sample by native and SDS-PAGE analysis (Fig. 10). The protein polypeptide profiles remained essentially unchanged. Similar results were previously reported by

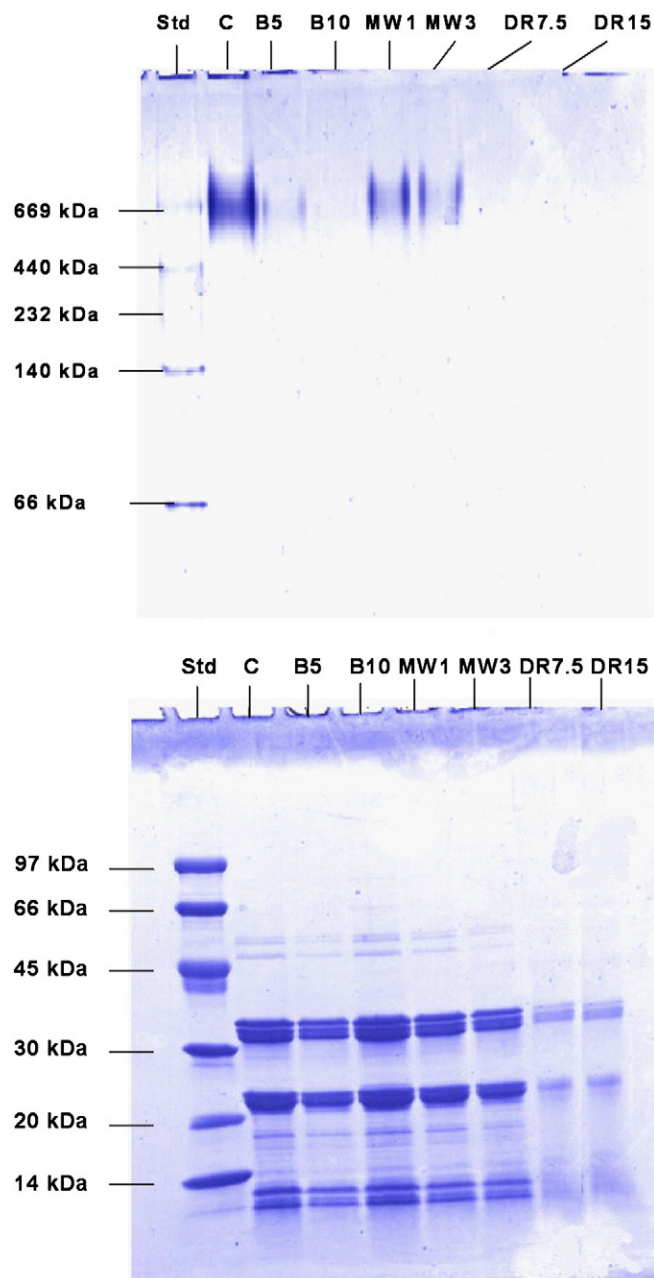


Fig. 10. Native (A) and SDS-PAGE (B) of sesame proteins extracted from thermally processed sesame seeds. Samples were run on 4–15% and 10–20% gradient acrylamide gel, respectively. Lane 1-Std: molecular weight markers (66–669 kDa for Native and 14–97 kDa for SDS-PAGE); Line 2: control (untreated); Lines 3 and 4: boiling for 5 and 10 min; Lines 5 and 6: microwaving for 1 and 3 min; Line 7: dry roasting for 15 min.

Alvarez-Alvarez et al. (2005) whose study demonstrated that processing procedures, such as microwave heating, boiling, and extrusion cooking, produce minimum changes on the SDS-PAGE protein profile of lupine protein extracts. However, some protein bands were absent in roasted sesame extracts, particularly the 7S bands (above 45 kDa) and 2S albumins (bands below 14 kDa).

4. Conclusions

Sesame protein isolates prepared with water and at different NaCl concentrations showed different immunoreactivity responses. Higher immunoreactivity was observed with isolates prepared using water and lower salt concentration (0.2 M NaCl) compared to samples prepared with 0.6 M and 1 M NaCl likely due to differences in the types of proteins extracted. Furthermore, use of higher salt concentrations promote salting-out and aggregation which could potentially prevent epitopes from reacting with the IgG antibodies. The ELISA results revealed major differences in the immunoreactivity of sesame protein as a function of both pH and HP, with HP having a more dominant effect. High pressure treatment ranging from 100 up to 500 MPa markedly decreased the antigenicity of sesame proteins at all pH values. Greater IgG binding was observed at pH 7.0 and pH 10.0 than at pH 5.0 and pH 2.0. On the other hand thermal processing methods such as boiling and dry roasting increased the ELISA response, while microwaving decreased it. Alteration in protein structure as revealed by FTIR results may have led to epitope destruction, masking, or unmasking thereby decreasing or increasing the immunogenic response. As sesame products used by the food industry are processed with a variety of thermal treatments, it is important to know the effect of these treatments on the immunogenicity of sesame proteins and their impact on allergen detection. Future work on differences in the ability of the processed seeds to provoke allergic responses *in vivo* and *ex vivo* will be useful.

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