



Resistance of purified seed storage proteins from sesame (*Sesamum indicum* L.) to proteolytic digestive enzymes

Estibalitz Orruño*, Michael R.A. Morgan

School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, United Kingdom

ARTICLE INFO

Article history:

Received 10 August 2010

Received in revised form 28 January 2011

Accepted 31 March 2011

Available online 5 April 2011

Keywords:

Sesame (*Sesamum indicum* L.)

Seed storage proteins

Protein digestion

Proteolytic stability

2S albumin

7S globulin

11S globulin

Sesame allergens

ABSTRACT

Sesame has been increasingly associated with food allergy. The main seed storage proteins of sesame (the 2S albumin and the 7S and 11S globulins) were purified and subjected to proteolysis with pepsin, trypsin and chymotrypsin. The degree of proteolysis obtained was monitored by SDS–PAGE, followed by densitometry of the main bands. The 2S albumin was found to be stable to proteolysis, being extremely resistant to pepsin, and relatively resistant to trypsin and chymotrypsin. The 7S and 11S proteins were relatively labile to pepsin. Acidic polypeptides from the 11S protein were more susceptible to proteolysis than the basic polypeptides. Both 7S and 11S proteins generated what appeared to be stable polypeptides after proteolysis with trypsin and chymotrypsin. The results are discussed in relation to similar studies on related seed storage proteins, available structural information, and the potential allergenicity of the sesame proteins.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Sesame (*Sesamum indicum* L.) is a valuable seed crop owing to its high content of oil and protein (the latter usually found between 19% and 25%). The majority of the proteins present in sesame seeds are storage proteins classed as albumins (9%), globulins (67%), prolamins (1%) and glutelins (7%) on the basis of their solubility (Rivas, Dench, & Caygill, 1981). The water-insoluble 11S globulin and the soluble 2S albumin are the two major storage proteins of sesame.

The sesame 11S globulin has the typical oligomeric structure of the 11S seed proteins, being composed of six subunits that interact non-covalently (Okubo, Nishimura, & Shibasaki, 1979a). The 11S hexamers contain a random combination of different subunits, with each of these subunits consisting of an acidic polypeptide (30–34 kDa) and a basic polypeptide (20–25 kDa) linked by a single disulphide bond (Okubo, Nishimura, & Shibasaki, 1979b). The sedimentation coefficient of the sesame 11S globulin varies between 11.0 and 13.0, depending on the ionic strength of the buffer in which the measurements are taken. The cDNA sequences encoding four sesame 11S globulin isoforms have been cloned

successfully (Hsiao, Lin, Wang, Liao, & Tzen, 2006; Tai, Lee, Tsai, Yiu, & Tzen, 2001; Tai, Wu, Chen, & Tzen, 1999).

The molecular weight of the sesame 2S albumin has been estimated to be in the range of 13–15 kDa (Rajendran & Prakash, 1988; Tai et al., 1999). Rajendran and Prakash (1988) showed that the 2S albumin was composed of a single polypeptide chain. However, it has been reported that the sesame 2S albumin can be cleaved into a large subunit of around 9 kDa and a smaller subunit of around 4 kDa in the presence of a disulphide reducing agent (Tzen, personal communication), which is in accordance with the typical subunit structure of most 2S albumins. The amino acid sequences of three isoforms of the sesame 2S albumin have been deduced, indicating that not all the 2S albumin isoforms are sulphur-rich (Hsiao et al., 2006; Tai et al., 1999, 2001).

In contrast to the sesame 11S globulin and 2S albumin, information about the 7S globulin, the third major storage protein of sesame, is scarce. The existence of a 7S globulin as a minor constituent of the total storage proteins in sesame was reported briefly by Prakash and Nandi (1978). Tai et al. (2001) obtained a cDNA sequence encoding the sesame 7S globulin and identified its corresponding polypeptide as a minor constituent in sesame seeds via immunodetection. The purification of the 7S globulin from sesame seeds has been previously described showing that the protein is composed of at least eight polypeptide chains ranging from 12.4 to 65.5 kDa judged by SDS–PAGE analysis and does not contain disulphide bonds (Orruño & Morgan, 2007).

* Corresponding author. Present address: Basque Office for Health Technology Assessment, Department of Health and Consumer Affairs, Olaguibel 38, Vitoria-Gasteiz 01004, Spain. Tel.: +34 945 017153; fax: +34 945 017101.

E-mail addresses: e-orruno@ej-gv.es (E. Orruño), m.morgan@food.leeds.ac.uk (M.R.A. Morgan).

Food allergy is of increasing concern to individuals suffering from the syndrome, to food producers and to regulators. Many of the most important allergenic foods (such as peanut, tree nuts, wheat, soybean, mustard, celeriac and sesame) are of plant origin and are consumed as seed or nut products; seed storage proteins have often been associated with specific reactivity towards IgE from patient serum. Understanding more about the properties of the seed storage proteins has been perceived to be important in identifying key molecular characteristics predisposing to allergenicity. During the last few years, the number of sesame allergic individuals seems to have increased significantly in a number of countries (Gangur, Kelly, & Navuluri, 2005; Sicherer & Leung, 2009). Furthermore, the increasing and widespread consumption of sesame-containing foods and the frequent presence of sesame as a hidden allergen are likely to make sesame allergy become even more common in future. Severe reactions to sesame, such as anaphylactic shock, have been reported in the literature (Asero, Mistrello, Roncarolo, Antonotti, & Falagiani, 1999; Kolopp-Sarda et al., 1997).

It is generally believed that a protein, which is resistant to proteolytic digestion in the digestive tract, retains sufficient structural integrity to have an increased probability of stimulating immune reactions. Small amounts of intact or partially digested proteins are absorbed through the intestine and enter the circulatory system under normal circumstances as a physiological process. Hence, the ability of food allergens to reach the intestinal mucosa intact seems to be a likely requirement for allergenicity (Fuchs & Astwood, 1996). Astwood, Leach, and Fuchs (1996) evaluated the digestive stability of a group of known allergens and a group of non-allergenic enzymes in simulated gastric fluid (SGF). The authors concluded that food allergens were more stable than non-allergenic proteins in SGF and that the digestibility parameter can be used to distinguish allergens from non-allergenic proteins. A critical review of assessment of allergenicity has been published (Orruño & Morgan, 2006).

The sesame seed storage proteins have not been investigated previously with regard to their susceptibility to enzymic digestion. In the present study the digestibility of the three main sesame storage proteins, i.e. 2S albumin, 7S globulin and 11S globulin, was investigated and characterised using single digestive enzymes. Protein degradation was monitored by SDS–PAGE and the percentage of undigested protein bands was quantified using densitometry.

2. Materials and methods

2.1. Chemicals

Acrylogel-3-solution, ethanol and hydrochloric acid were purchased from BDH Ltd., Poole, UK. Glacial acetic acid, glycerol, *n*-hexane and sodium dodecyl sulphate (SDS) were purchased from Fisons/Fisher, Loughborough, UK. Dithiothreitol (DTT) was purchased from Lancaster, Morecambe, UK. Ammonium persulphate, ammonium sulphate, bromophenol blue, *n*-butanol, calcium chloride, disodium hydrogen orthophosphate, electrophoresis molecular weight markers (wide-range and low-range), glycine, sodium carbonate, sodium chloride, sodium dihydrogen orthophosphate, *N,N,N,N'*-tetramethyl-ethylene diamine (TEMED), and Trizma™ base were purchased from Sigma–Aldrich Chemicals Co., Poole, UK.

2.2. Purification of sesame proteins

The seed storage proteins from sesame were purified following the procedures described by Orruño and Morgan (2007). White sesame seeds (purchased from local retailers) were ground and

defatted with hexane for 16 h using a Soxhlet apparatus. Sesame proteins were extracted from the defatted flour with phosphate buffer (20 mM, pH 7.5) containing 1 M sodium chloride (1:10, w:v, sesame flour to solvent ratio) under constant stirring for 2 h at room temperature. The slurry was centrifuged at 19,800g for 1 h at 4 °C. The supernatant obtained after centrifugation was filtered through Watman No. 1 filter paper. Following a number of ammonium sulphate fractionation steps, the sesame 2S albumin was purified by gel filtration chromatography on Sephadex G-100 followed by re-chromatography on Sephadex G-50, and the 11S globulin by using Sephacryl S-200 HR followed by re-chromatography on Sephacryl S-300 HR. The sesame 7S globulin was purified by anion exchange chromatography on Q Sepharose Fast Flow. Chromatographic materials were purchased from Sigma–Aldrich Chemical Co., Poole, UK. Each of the three protein preparations gave single symmetrical peaks on column chromatography, the expected bands and subunit composition by SDS–PAGE and single bands by native PAGE.

2.3. Protein determination

Estimation of protein concentration was performed with Coomassie Plus Protein Assay reagent (Perbio Science UK Ltd., Cramlington, UK) according to the method of Bradford (1976).

2.4. Enzymic hydrolysis

The enzyme digestions of sesame 11S and 7S globulins and 2S albumin were performed for 30 min at 37 °C with pepsin, chymotrypsin and trypsin (TLCK-treated α -chymotrypsin type VII from bovine pancreas, pepsin A from porcine stomach mucosa, and TPCK-treated trypsin from bovine pancreas, all obtained from Sigma–Aldrich Chemical Co., Poole, UK). The pH, temperature and reaction time were kept constant and a range of enzyme to sesame protein ratios (1:5, 1:10, 1:50 and 1:100 (w:w)) were tested. Pepsin digestions were carried out in saline (0.03 M) adjusted with hydrochloric acid to pH 1.2; trypsin digestions in Tris–HCl buffer (0.1 M, pH 8.0) containing 20 mM calcium chloride; and chymotrypsin digestions in Tris–HCl buffer (0.1 M, pH 8.0). Each of the sesame protein solutions (70 μ l) containing 100 μ g of protein were added to eppendorf tubes (Camlab, Cambridge, UK) and pre-incubated for 10 min in a water bath set at 37 °C. The appropriate enzyme solutions (30 μ l) were added at 1 min intervals to each of the tubes, the reaction mixture was vortex mixed and placed into a water bath for 30 min at 37 °C. Enzymatic digestions were stopped by the addition of 100 μ l of electrophoresis sample buffer containing DTT and boiling of the samples for 5 min. Sodium carbonate solution (1 M, 10 μ l) was added to the pepsin containing samples prior to the addition of sample buffer. Sesame 11S, 7S and 2S protein blanks were prepared by replacing the enzyme solutions with an equal volume of appropriate buffer. Each enzyme stock solution (1 mg/ml; 100 μ l) was used as the enzyme blank. All blanks were incubated in the same conditions as the samples.

2.5. SDS–PAGE analysis

The digested samples were analysed by SDS–PAGE on a Mini-Protean® II electrophoresis cell (Bio-Rad, Hemel Hempstead, UK) according to a modification of the method of Laemmli (1970). The samples and blanks containing 15 μ g of protein (30 μ l) were loaded in the gel wells and electrophoresis was carried out at constant current of 75 mA on 15% T acrylamide mini gels. The protein bands were stained with Coomassie Brilliant Blue R-250 (Sigma–Aldrich Chemical Co., Poole, UK). Gels were photographed digitally (University of Leeds Media Services).

2.6. Densitometric analysis of gels

The densitometric analysis of protein bands on SDS–PAGE gels was carried out using the QuantiScan version 2.1 software (Bio-software®, Cambridge, UK) in order to quantify the extent of proteolysis observed in the gels. The relationship between the protein concentration and the areas of the peaks obtained by QuantiScan was determined by running a range of different concentrations (2.5–25 µg of protein per well) of sesame 11S globulin on SDS–PAGE and subjecting the bands to analysis. The peak areas showed a linear relationship with the concentration of protein across the whole range tested. For the bands correlating to the acidic subunits, the graph for area against protein concentration was represented by $y = 34.5x$ with $R^2 = 0.977$, for the basic subunits $y = 31.5x$ with $R^2 = 0.982$, and for both acidic and basic subunits combined $y = 66.0x$ with $R^2 = 0.980$. Digestibility results for particular proteins for each enzyme used were expressed as the ratio (as a percentage) of the area given by the digested band to that given by the same protein as a control (obtained with no enzyme present).

One important limitation of the densitometric methodology applied was that in some cases certain proteins and enzymes had a very similar molecular weight as observed on SDS–PAGE and, therefore, the distinction between enzyme and subject proteins in specific molecular weight regions was not always possible. Instances of possible difficulties of identification are noted where relevant. In addition, only the main protein bands were quantified by QuantiScan. Thus, stable peptide fragments of low molecular weight that may have been created as a result of proteolytic degradation were not considered for the QuantiScan analysis. It should be noted that in several *in vitro* digestibility studies of allergenic proteins, the presence of small IgE-reactive proteolytic fragments below 10 kDa, have been observed (Vieths, Reindl, Müller, Hoffmann, & Hausteiner, 1999).

3. Results and discussion

3.1. General

The relative resistance of food proteins to enzymic hydrolysis has been observed to be a characteristic of many food allergens. In the present study it is characterisation of the relative resistance to hydrolysis by individual digestive enzymes that was of interest rather than digestibility *per se*. Indeed, the latter is likely to be a much more complicated issue *in vivo* than it might appear *in vitro*, particularly since intact sesame seeds, being so small, are quite likely to undergo minimal damage through chewing which may well result in the seed contents remaining inaccessible to proteolytic enzymes. Consequently it should be noted that proteins that may appear unstable when isolated from the food matrix and assayed by SGF or individual enzymes could, in fact, possess enhanced stability *in vivo* due to their location within the seed protein body or within a tough seed coat. In the present study, resistance to proteolysis of the sesame 2S, 7S and 11S proteins by the main gastric enzyme, pepsin, and two pancreatic enzymes, trypsin and chymotrypsin was investigated. The degree of proteolysis obtained at different enzyme to protein ratios was monitored by SDS–PAGE.

3.2. Stability of the sesame 2S albumin to proteolytic enzymes

The sesame 2S albumin showed a remarkable stability to proteolytic digestion with each of the three enzymes tested. The 2S albumin was extremely resistant to pepsin digestion (Fig. 1), which clearly contrasted with the susceptibility to pepsin proteolysis exhibited by the two other sesame proteins assessed in the present

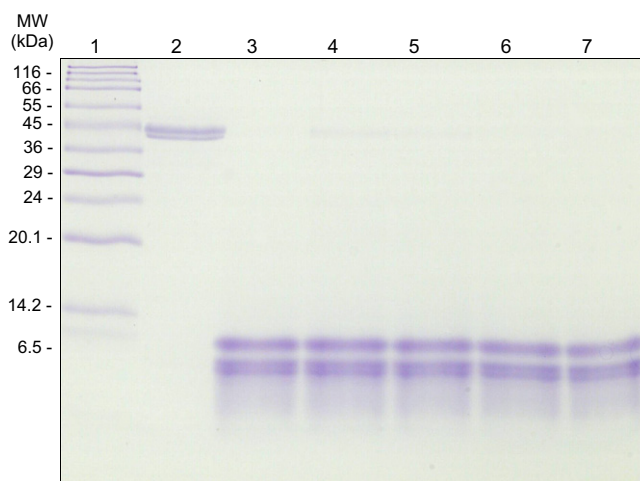


Fig. 1. SDS–PAGE of the digestions of sesame 2S albumin with pepsin. Samples were run on a 15% acrylamide gel under reducing conditions. Lane 1: wide-range molecular weight markers; Lane 2: pepsin blank; Lane 3: non-digested 2S albumin; Lane 4: digestion with 1:5 (w:w) pepsin to 2S albumin ratio; Lane 5: digestion with 1:10 (w:w) pepsin to 2S albumin ratio; Lane 6: digestion with 1:50 (w:w) pepsin to 2S albumin ratio; Lane 7: digestion with 1:100 (w:w) pepsin to 2S albumin ratio.

study. Similarly, the 2S albumin was also more stable than the 7S and 11S proteins to digestion with trypsin and chymotrypsin. Indeed, even at the highest enzyme to protein ratios, i.e. 1:5 (w:w), a high percentage (over 58% of the small polypeptide) of the 2S albumin remained undigested (Fig. 2). The behaviour of the large and small polypeptides derived from the 2S protein was fairly similar; both subunits were resistant to proteolytic digestion. Nevertheless, the small subunit was found to be more susceptible to trypsin hydrolysis than the large subunit at 1:10 (w:w) and 1:5 (w:w) enzyme to protein ratios. These findings coincide with the high resistance to digestion reported for *Ses i 1*, the major sesame 2S albumin allergen, with an *in vitro* gastrointestinal model system (Moreno, Maldonado, Wellner, & Mills, 2005). The authors reported that after 2 h of gastric digestion, the 2S albumin allergen remained completely intact and only the small subunit was cleaved during 2 h of subsequent duodenal digestion.

Several 2S albumins from other plant species have been reported to be stable to proteolysis by digestive enzymes. Lehmann et al. (2006) demonstrated the high stability of *Ara h 2* and *Ara h 6*, the two 2S albumin allergens from peanut, to proteolytic duodenal enzymes. In a previous investigation, *Ara h 2* was found to be very resistant to pepsin, trypsin and chymotrypsin digestion (Sen et al., 2002). The high stability of *Ara h 2* against pepsin hydrolysis for over 60 min was also reported by Astwood and co-workers (1996). The 2S albumin from yellow mustard, *Sin a 1*, was highly resistant to extensive treatments over 24 h with trypsin, chymotrypsin or pepsin (Oñaderra et al., 1994). The extraordinary stability to digestion of the 2S albumins appears to be due to the high cysteine content of the proteins and, consequently, to the presence of inter- and intra-molecular disulphide bridges that hold the protein together forming a compact structure which limits the accessibility of the proteolytic enzymes to susceptible peptide bonds (Sen et al., 2002). However, protease inhibitory activity has been identified in some of the 2S albumins of different plant species (Maleki et al., 2003; Vioque et al., 1999) and, therefore, it is possible that such inhibitory activity can also contribute to the high stability of the 2S albumins to proteolytic digestion. The 2S albumin storage protein family appears to be an important class of common allergenic proteins in many plant species including several oilseeds, legumes and nuts (Menéndez-Arias, Moneo, Domínguez, & Rodríguez, 1988; Teuber, Dankedar, Peterson, & Sellers, 1998). In

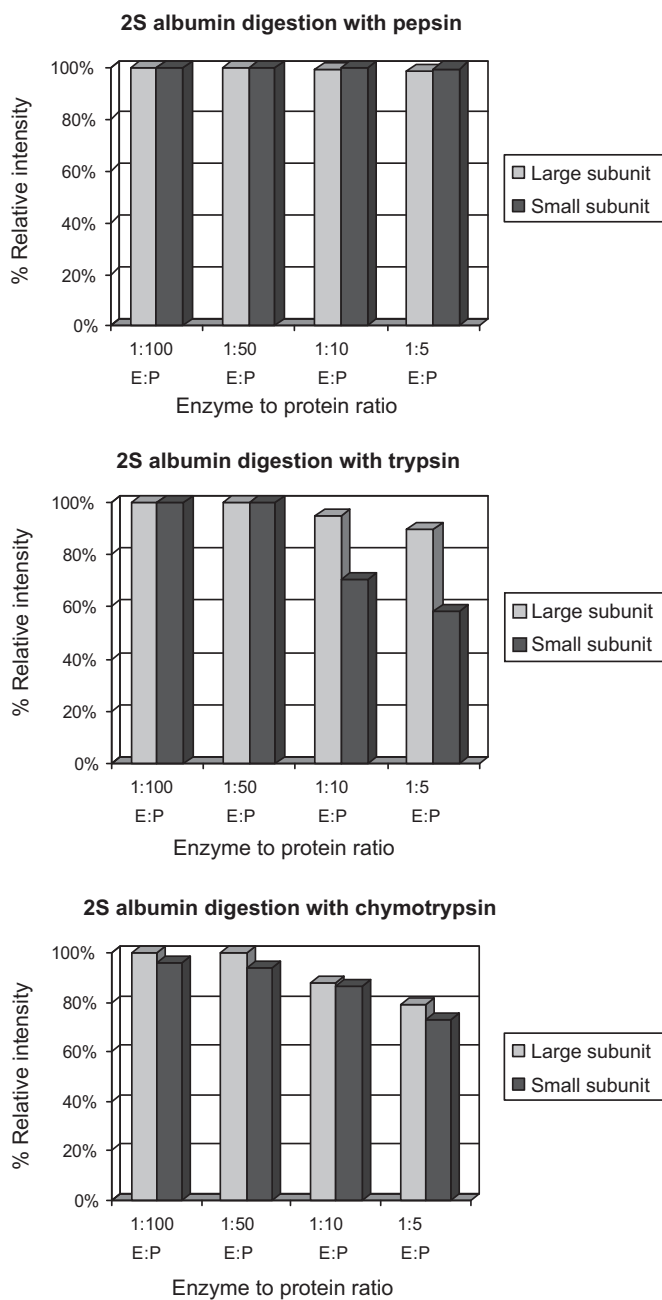


Fig. 2. Digestibility of sesame 2S albumin with pepsin, trypsin and chymotrypsin. Peak area values obtained with the QuantiScan were used for the calculation of the relative protein band intensity percentages. Non-digested 2S albumin was used as reference (100% intensity) to calculate the decrease of intensity of the large and small polypeptides after enzymic digestion (E:P; Enzyme to protein ratio).

particular, two 2S albumins, *Ses i 1* (Pastorello et al., 2001) and *Ses i 2* (Beyer, Bardina, Grishina, & Sampson, 2002), have been reported to be the major allergens of sesame based on patient IgE recognition.

3.3. Stability of the sesame 11S globulin to proteolytic enzymes

With regard to the digestion stability of sesame globulins, both 7S and 11S proteins were considerably more resistant to digestion by trypsin and chymotrypsin compared with pepsin. Where

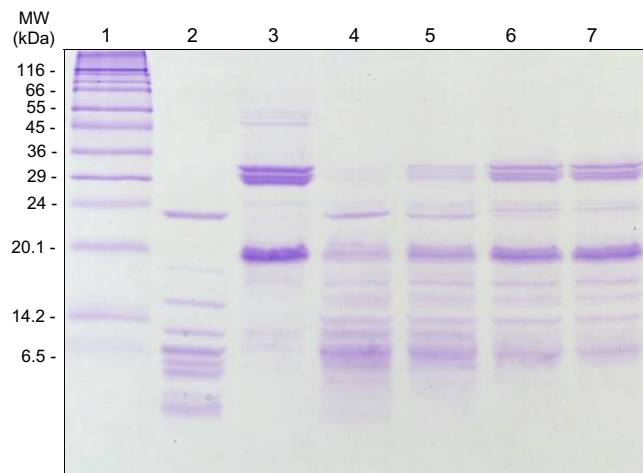


Fig. 3. SDS-PAGE of the digestions of sesame 11S globulin with trypsin. Samples were run on a 15% acrylamide gel under reducing conditions. Lane 1: wide-range molecular weight markers; Lane 2: trypsin blank; Lane 3: non-digested 11S globulin; Lane 4: digestion with 1:5 (w:w) trypsin to 11S globulin ratio; Lane 5: digestion with 1:10 (w:w) trypsin to 11S globulin ratio; Lane 6: digestion with 1:50 (w:w) trypsin to 11S globulin ratio; Lane 7: digestion with 1:100 (w:w) trypsin to 11S globulin ratio.

proteolysis was occurring in the 11S and 7S globulins, a significant amount of low molecular weight peptides as digestion products could be observed. Such peptides were apparently relatively stable to further hydrolysis and could, therefore be of further interest in this context.

The 11S globulin showed relative resistance to digestion with duodenal enzymes. In this respect, a higher amount of undigested acidic and basic polypeptides of the 11S globulin could be observed by SDS-PAGE analysis after trypsin digestion (Fig. 3) as compared to chymotrypsin digestion (data not shown). The 11S globulin was, however, extremely labile to pepsin attack, yet low molecular weight peptides of <12 kDa and small amounts of undigested acidic and basic polypeptides could be visualised on the gels (data not shown). The digestibility of the acidic polypeptides of the 11S globulin differed considerably from that of the basic polypeptides. For all the enzymes tested, the acidic polypeptides were more susceptible to proteolysis than the basic polypeptides (Fig. 4). Such differences may be related to the surface location of the acidic polypeptides within the 11S globulin molecule (Plietz, Drescher, & Damaschun, 1987) and, thus, an increased exposure to enzymes. In addition, the higher resistance of the basic polypeptides to enzyme hydrolysis could be explained by the hydrophobic character of the basic polypeptides (Okubo et al., 1979b). The higher susceptibility of the acidic polypeptides to enzymatic hydrolysis as compared to the basic polypeptides has also been observed *in vitro* in soybean glycinin (Lynch, Rha, & Catsimopoulos, 1977) and pea legumin (Plumb, Carr, Newby, & Lambert, 1989).

The almost complete degradation of the sesame 11S globulin by pepsin could be attributed to the very low pH (1.2) at which the enzymatic incubation was carried out as well as to the broad specificity of the enzyme. The 11S protein has been reported to undergo dissociation and denaturation at acidic pH and to re-associate as the pH decreases to 1.5 (Prakash & Nandi, 1977). Such conformational changes, providing increased access of enzymes, could enhance the proteolytic susceptibility of the protein. Glycinin, the 11S globulin of soybean, and the 11S globulin of cashew nut have also been reported to be extensively degraded by pepsin (Nielsen, Deshpande, Hermodson, & Scout, 1988; Sathe, Sze-Tao, Wolf, & Hamaker, 1997). In contrast, the higher stability of the sesame 11S

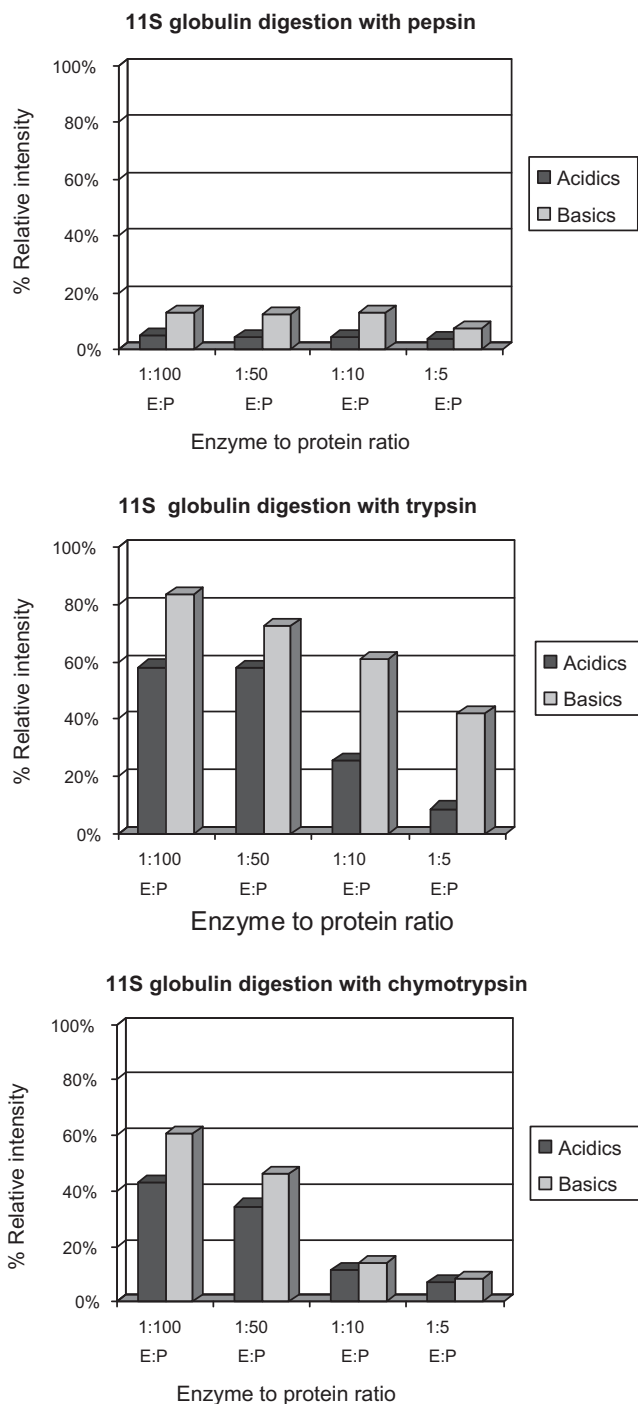


Fig. 4. Digestibility of sesame 11S globulin with pepsin, trypsin and chymotrypsin. Peak area values obtained with the QuantiScan were used for the calculation of the relative protein band intensity percentages. Non-digested 11S globulin was used as reference (100% intensity) to calculate the decrease of intensity of the acidic and basic polypeptides after enzymic digestion (E:P; Enzyme to protein ratio).

globulin against trypsin and chymotrypsin hydrolysis could be due to the globulin being conformationally stable at the pH at which the digestions were carried out, i.e. pH 8.0 (Prakash & Nandi, 1977). Therefore, as a consequence, a number of susceptible peptide bonds would be located in the interior of the protein protected by the hexameric conformation, thus, restricting the availability of cleavage sites for the enzymes.

A number of legumin-like proteins have been identified as allergens. Examples include, *Ara h 3*, the 11S globulin of peanut

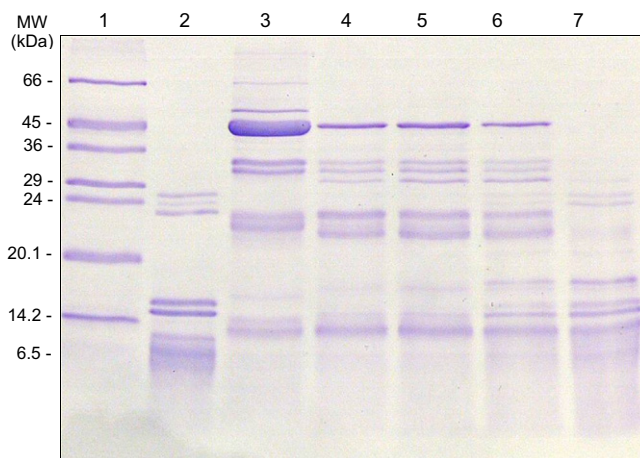


Fig. 5. SDS-PAGE of the digestions of sesame 7S globulin with chymotrypsin. Samples were run on a 15% acrylamide gel under reducing conditions. Lane 1: low-range molecular weight markers; Lane 2: chymotrypsin blank; Lane 3: non-digested 7S globulin; Lane 4: digestion with 1:100 (w:w) chymotrypsin to 7S globulin ratio; Lane 5: digestion with 1:50 (w:w) chymotrypsin to 7S globulin ratio; Lane 6: digestion with 1:10 (w:w) chymotrypsin to 7S globulin ratio; Lane 7: digestion with 1:5 (w:w) chymotrypsin to 7S globulin ratio.

(Rabjohn et al., 1999) and the acidic and basic polypeptides of the 11S globulin of cashew nut (Teuber, Sathe, Peterson, & Roux, 2002). Regarding the allergenicity of the sesame 11S globulin, legumin-like proteins (*Ses i 6* and *Ses i 7*) have been identified as allergens on the basis of IgE recognition from the sera of allergic individuals (Beyer, Grishina, Bardina, & Sampson, 2007; Navuluri et al., 2006; Wallowitz, Chen, Tzen, & Teuber, 2007).

3.4. Stability of the sesame 7S globulin to proteolytic enzymes

The five major polypeptides of the sesame 7S globulin showed a very different susceptibility to the digestive enzymes tested. The 7S polypeptides with molecular weights of 45, 35, 33 and 23 kDa showed moderate stability to chymotrypsin with 30–50% of the polypeptides remaining un-degraded after proteolytic attack (Figs. 5 and 6). In contrast, the 13 kDa polypeptide was extremely resistant to chymotrypsin degradation. Moreover, several new polypeptides were generated after proteolysis of the 7S globulin with chymotrypsin. One newly generated peptide appeared as a 22 kDa band, which could also be observed in the SDS-PAGE gels of the trypsin digests of the 7S globulin (data not shown). A 32 kDa polypeptide, probably generated from the proteolytic degradation of one of the higher molecular weight polypeptides, could be visualised at enzyme to protein ratios ranging from 1:100 to 1:10 (w:w). Finally, a new polypeptide of around 16 kDa could be observed on SDS-PAGE when the 7S globulin was subjected to extensive chymotrypsin degradation. With regard to trypsin digestion, the 23 and 35 kDa polypeptides showed moderate stability to proteolysis, whereas both the 33 and 45 kDa bands were completely degraded even at the lowest enzyme concentration used for the assay (Fig. 6). As observed for the chymotrypsin digestions, the proteolytic breakdown of the 7S globulin polypeptides was accompanied by the generation of new polypeptides with molecular weights around 24, 22, 14 and <10 kDa. The 13 kDa band showed significant stability to tryptic digestion. In fact, the intensity of the 13–14 kDa polypeptide increased after trypsin digestion, presumably as a result of the proteolytic degradation of the larger polypeptides of the sesame 7S globulin. The 24 kDa band generated after digestion appeared to correspond to the main trypsin polypeptide rather than to a degradation product of the 7S globulin. The identity of such a polypeptide, however, could not be confirmed by gel electrophoresis alone. As with the 11S globulin, the

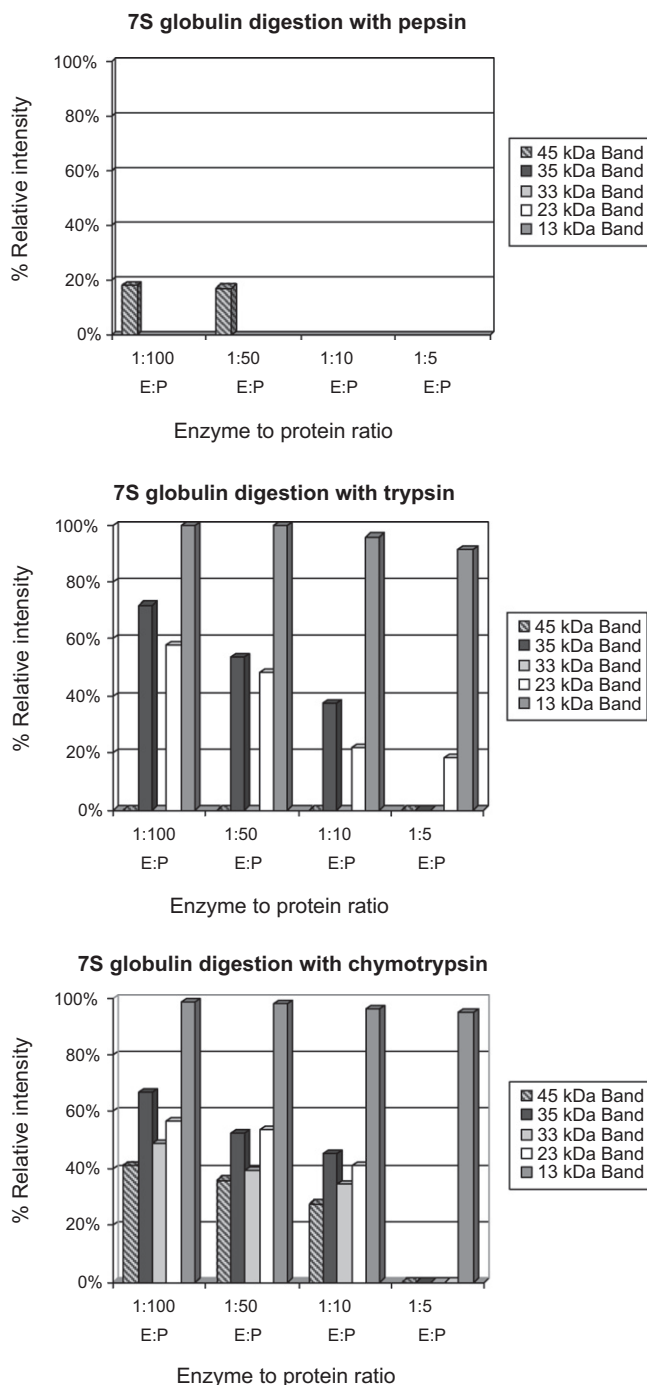


Fig. 6. Digestibility of sesame 7S globulin with pepsin, trypsin and chymotrypsin. Peak area values obtained with the QuantiScan were used for the calculation of the relative protein band intensity percentages. Non-digested 7S globulin was used as reference (100% intensity) to calculate the decrease of intensity of the five main 7S globulin polypeptides after enzymic digestion (E:P; Enzyme to protein ratio).

sesame 7S globulin was extremely susceptible to pepsin digestion. Indeed, barely 18% of the 45 kDa polypeptide remained un-degraded after pepsin digestion at low concentrations of enzyme (Fig. 6). As the enzyme concentration was increased, extensive degradation of all the 7S polypeptides was observed together with the generation of very low molecular weight peptides.

To the best of our knowledge, only the 7S globulins of peanut (*Ara h 1*) and soybean (β -conglycinin) have been investigated for reactivity towards individual digestive enzymes or under SGF conditions. Astwood et al. (1996) reported that the β -subunit of

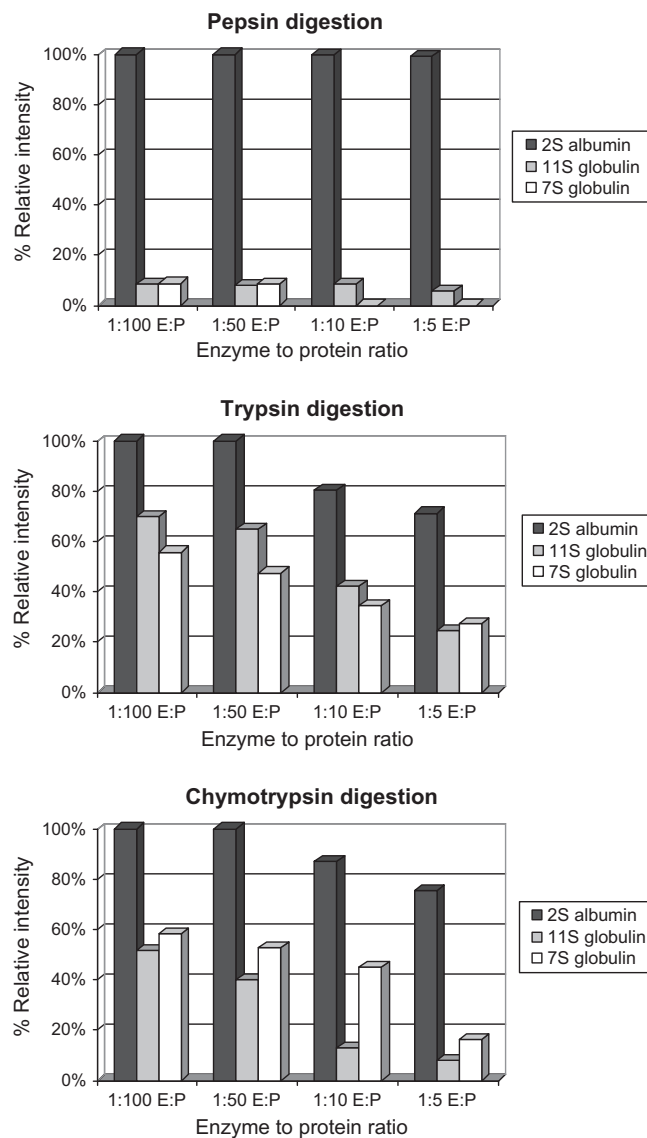


Fig. 7. Comparative digestibility of sesame storage proteins with gastric and pancreatic enzymes expressed as relative protein band intensity. The plotted intensity values represent the overall digestive stability of all the subunits of each storage protein (E:P; Enzyme to protein ratio).

β -conglycinin was stable for 60 min in SGF, whereas the α -subunit was digested within 2 min. As for the peanut *Ara h 1*, several protein fragments were stable to SGF for 60 min (Astwood et al., 1996). Maleki et al. (2000) identified various protease-resistant polypeptide fragments containing IgE-binding sites after incubation of *Ara h 1* with pepsin, trypsin and chymotrypsin. The authors suggested that the trimeric quaternary structure of the *Ara h 1* may confer some protection against the attack from proteolytic enzymes. 7S Seed storage proteins have been reported to be the major allergens found in, amongst others, peanuts, *Ara h 1* (Burks et al., 1991). In addition, *Ses i 3*, a 45 kDa polypeptide which was recognised by 75% of the IgE of a sesame-allergic population, was found to be a 7S vicilin-type protein (Beyer et al., 2002).

3.5. Concluding remarks

The results of this investigation shed some light on the stability of the three main sesame seed storage proteins to digestive enzymes. A comparison of the digestibility results obtained for the

three sesame proteins is shown in Fig. 7. The results seem to indicate that the pattern of stability by different enzymes varies widely and in a manner correlating with reported IgE-binding data. From all the proteins studied, the sesame 2S albumin was the only one highly stable to digestion by all the enzymes tested. The sesame globulins were more stable to degradation by the pancreatic enzymes than by pepsin. Thus, not all the sesame allergenic proteins analysed showed high stability to pepsin digestion. In this sense, most of the polypeptides of the 7S globulin and the acidic and basic polypeptides of the 11S globulin were extensively degraded by the gastric enzyme. Therefore, despite the data acquired appearing to indicate that the sesame 2S albumin is more likely than the globulin proteins to be allergenic due to its high stability to digestive enzymes, the IgE-binding capacity and *in vivo* allergenicity of the sesame digests remain to be tested.

Acknowledgements

We are grateful to the Education, University and Research Department of the Basque Government for a research Grant (BF198.106/AK) to Dr. E. Orruño.

References

- Asero, R., Mistrello, G., Roncarolo, D., Antonotti, P. L., & Falagiani, P. (1999). A case of sesame seed-induced anaphylaxis. *Allergy*, 54, 526–527.
- Astwood, J. D., Leach, J. N., & Fuchs, R. L. (1996). Stability of food allergens to digestion *in vitro*. *Nature Biotechnology*, 14, 1269–1273.
- Beyer, K., Bardina, L., Grishina, G., & Sampson, H. A. (2002). Identification of sesame seed allergens by 2-dimensional proteomics and Edman sequencing: Seed storage proteins as common food allergens. *Journal of Allergy and Clinical Immunology*, 110, 154–159.
- Beyer, K., Grishina, G., Bardina, L., & Sampson, H. A. (2007). Identification of 2 new sesame seed allergens: *Ses i 6* and *Ses i 7*. *Journal of Allergy and Clinical Immunology*, 119(6), 1554–1556.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye binding. *Analytical Biochemistry*, 72, 248–254.
- Burks, A. W., Williams, L. W., Helm, R. M., Connaughton, C., Cockrell, G., & O'Brien, T. (1991). Identification of a major peanut allergen, *Ara h 1*, in patients with atopic dermatitis and positive peanut challenges. *Journal of Allergy and Clinical Immunology*, 88, 172–179.
- Fuchs, R. L., & Astwood, J. D. (1996). Allergenicity assessment of foods derived from genetically modified plants. *Food Technology*, 50, 83–88.
- Gangur, V., Kelly, C., & Navuluri, L. (2005). Sesame allergy: A growing food allergy of global proportions? *Annals of Allergy Asthma and Immunology*, 95(1), 4–11.
- Hsiao, E. S., Lin, L. J., Wang, M. M., Liao, M. Y., & Tzen, J. T. (2006). Gene families encoding isoforms of two major sesame seed storage proteins, 11S globulin and 2S albumin. *Journal of Agricultural and Food Chemistry*, 54(25), 9544–9550.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Lehmann, K., Schweimer, K., Reese, G., Randow, S., Suhr, M., Becker, W. M., et al. (2006). Structure and stability of 2S albumin-type peanut allergens: Implications for the severity of peanut allergic reactions. *Biochemical Journal*, 395, 463–472.
- Kolopp-Sarda, M. N., Moneret-Vautrin, D. A., Gobert, B., Kanny, G., Brodschii, M., Bene, M. C., et al. (1997). Specific humoral immune responses in 12 cases of food sensitisation to sesame seed. *Clinical and Experimental Allergy*, 27, 1285–1291.
- Lynch, C. L., Rha, C. K., & Catsimopoulos, N. (1977). Note on the rapid proteolysis of glycinin by pepsin and trypsin. *Cereal Chemistry*, 54, 1282–1285.
- Maleki, S. J., Kopper, R. A., Shin, D. S., Park, C.-W., Compadre, C. M., Sampson, H., et al. (2000). Structure of the major peanut allergen *Ara h 1* may protect IgE-binding epitopes from degradation. *Journal of Immunology*, 164, 5844–5849.
- Maleki, S. J., Viquez, O., Jacks, T., Dodo, H., Champagne, E. T., Chung, S.-Y., et al. (2003). The major peanut allergen, *Ara h 2*, functions as a trypsin inhibitor, and roasting enhances this function. *Journal of Allergy and Clinical Immunology*, 112, 190–195.
- Menéndez-Arias, L., Moneo, I., Domínguez, J., & Rodríguez, R. (1988). Primary structure of the major allergen of yellow mustard (*Sinapis alba* L.) seed, *Sin a 1*. *European Journal of Biochemistry*, 177, 159–166.
- Moreno, F. J., Maldonado, B. M., Wellner, N., & Mills, E. N. (2005). Thermostability and *in vitro* digestibility of a purified major allergen 2S albumin (*Ses i 1*) from white sesame seeds (*Sesamum indicum* L.). *Biochimica et Biophysica Acta*, 1752(2), 142–153.
- Navuluri, L., Parvataneni, S., Hassan, H., Birmingham, N. P., Kelly, C., & Gangur, V. (2006). Allergic and anaphylactic response to sesame seeds in mice. Identification of *Ses i 3* and basis subunit of 11S globulins as allergens. *International Archives of Allergy and Immunology*, 140(3), 270–276.
- Nielsen, S. S., Deshpande, S. S., Hermodson, M. A., & Scout, M. P. (1988). Comparative digestibility of legume storage proteins. *Journal of Agricultural and Food Chemistry*, 36, 896–902.
- Okubo, K., Nishimura, N., & Shibasaki, K. (1979a). Composition of sesame seed protein components and purification of the main globulin. *Cereal Chemistry*, 56, 100–104.
- Okubo, K., Nishimura, N., & Shibasaki, K. (1979b). Separation of the 13S globulin in sesame seeds into two groups of acidic and basic subunits, and their physicochemical properties. *Cereal Chemistry*, 56, 317–320.
- Oñaderra, M., Monsalve, R. I., Mancheño, J. M., Villalba, M., Martínez del Pozo, A., Gavilanes, J. G., et al. (1994). Food mustard allergen interaction with phospholipid vesicles. *European Journal of Biochemistry*, 225, 609–615.
- Orruño, E., & Morgan, M. R. A. (2006). IgE binding to proteins from sesame and assessment of allergenicity: Implications for biotechnology? *Biotechnology Letters*, 28(23), 1877–1888.
- Orruño, E., & Morgan, M. R. A. (2007). Purification and characterisation of the 7S globulin storage protein from sesame (*Sesamum indicum* L.). *Food Chemistry*, 100, 926–934.
- Pastorello, E. A., Varin, E., Farioli, L., Pravettoni, V., Ortolani, C., Trambaioli, C., et al. (2001). The major allergen of sesame seeds (*Sesamum indicum*) is a 2S albumin. *Journal of Chromatography B*, 756, 85–93.
- Plietz, P., Drescher, B., & Damaschun, G. (1987). Relationship between the amino acid sequence and the domain structure of the subunits of the 11S seed globulins. *International Journal of Biological Macromolecules*, 9, 161–165.
- Plumb, G. W., Carr, H. J., Newby, V. K., & Lambert, N. A. (1989). A study of the trypsinolysis of pea 11S globulin. *Biochimica et Biophysica Acta*, 999, 281–288.
- Prakash, V., & Nandi, P. K. (1977). Association-dissociation and denaturation behaviour of an oligomeric seed protein α -globulin of *Sesamum indicum* L. In acid and alkaline solutions. *International Journal of Peptide and Protein Research*, 9, 319–328.
- Prakash, V., & Nandi, P. K. (1978). Isolation and characterization of α -globulin of sesame seed (*Sesamum indicum* L.). *Journal of Agricultural and Food Chemistry*, 26, 320–322.
- Rabjohn, P., Helm, E. M., Stanley, J. S., Michael, W. C., Sampson, H. A., Burks, A. W., et al. (1999). Molecular cloning and epitope analysis of the peanut allergens *Ara h 3*. *Journal of Clinical Investigation*, 103, 535–542.
- Rajendran, S., & Prakash, V. (1988). Isolation and characterization of β -globulin low molecular weight protein fraction from sesame seed (*Sesamum indicum* L.). *Journal of Agricultural and Food Chemistry*, 36, 269–275.
- Rivas, N. R., Dench, J. E., & Caygill, J. C. (1981). Nitrogen extractability of sesame (*Sesamum indicum* L.) seed and the preparation of two protein isolates. *Journal of the Science of Food and Agriculture*, 32, 565–571.
- Sathe, S. K., Sze-Tao, K. W. C., Wolf, W. J., & Hamaker, B. R. (1997). Biochemical characterization and *in vitro* digestibility of the major globulin in cashew nut (*Anacardium occidentale*). *Journal of Agricultural and Food Chemistry*, 45, 2854–2860.
- Sen, M., Kopper, R., Pons, L., Abraham, E. C., Burks, A. W., & Bannon, G. A. (2002). Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. *Journal of Immunology*, 169, 882–887.
- Sicherer, S. H., & Leung, D. Y. M. (2009). Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2008. *Journal of Allergy and Clinical Immunology*, 123(2), 319–327.
- Tai, S. S. K., Lee, T. T. T., Tsai, C. C. Y., Yiu, T. J., & Tzen, J. T. C. (2001). Expression pattern and deposition of three storage proteins, 11S globulin, 2S albumin, and 7S globulin in maturing sesame seeds. *Plant Physiology and Biochemistry*, 39, 981–992.
- Tai, S. S. K., Wu, L. S. H., Chen, E. C. F., & Tzen, J. T. C. (1999). Molecular cloning of 11S globulin and 2S albumin, the two major seed storage proteins in sesame. *Journal of Agricultural and Food Chemistry*, 47, 4932–4938.
- Teuber, S. S., Dankedar, A. M., Peterson, W. R., & Sellers, C. L. (1998). Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut (*Juglans regia*), a major food allergen. *Journal of Allergy and Clinical Immunology*, 101, 807–814.
- Teuber, S. S., Sathe, S. K., Peterson, W. R., & Roux, K. H. (2002). Characterization of the soluble allergenic proteins of cashew nut (*Anacardium occidentale* L.). *Journal of Agricultural and Food Chemistry*, 50, 6543–6549.
- Vieths, S., Reindl, J., Müller, U., Hoffmann, A., & Hausteiner, D. (1999). Digestibility of peanut and hazelnut allergens investigated by a simple *in vitro* procedure. *European Food Research and Technology*, 209, 379–388.
- Vioque, J., Sánchez-Vioque, R., Clemente, A., Pedroche, J., Bautista, J., & Millán, F. (1999). Purification and partial characterization of chickpea 2S albumin. *Journal of Agricultural and Food Chemistry*, 47, 1405–1409.
- Wallowitz, M. L., Chen, R. J. Y., Tzen, J. T. C., & Teuber, S. S. (2007). *Ses i 6*, the sesame 11S globulin, can activate basophils and shows cross-reactivity with walnut *in vitro*. *Clinical and Experimental Allergy*, 37, 929–938.