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Food Chemistry 100 (2007) 926–934
Food Chemistry
www.elsevier.com/locate/foodchem

Purification and characterisation of the 7S globulin storage protein from sesame (*Sesamum indicum* L.)

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Received 18 July 2005; received in revised form 28 October 2005; accepted 28 October 2005

Abstract

The 7S globulin from sesame seeds was purified by means of selective precipitation and anion-exchange chromatography on Q Sepharose Fast Flow. The 7S globulin migrated as a single band on native PAGE, which suggested homogeneity of the sample. The isolated protein was composed of at least eight polypeptide chains, ranging from 12.4 to 65.5 kDa, judged by SDS–PAGE analysis, and did not contain disulphide bonds. Furthermore, comparison of the polypeptide bands of the 7S and 11S globulins by SDS–PAGE indicated that the purified 7S globulin was free of legumin-like contaminant polypeptides and of 2S albumin. The identity of the purified polypeptides was verified by comparing the N-terminal amino acid sequences of the main polypeptide bands with the amino acid sequence deduced from a cDNA clone, which encoded the sesame 7S globulin precursor. Purification of the 7S globulin from sesame has not previously been reported.

Keywords: Sesame (*Sesamum indicum* L.); Seed storage proteins; Protein purification; 7S globulin; 11S globulin; 2S albumin; Food allergens

1. Introduction

Sesame (*Sesamum indicum* L.) is probably the most ancient oilseed cultivated by mankind. The crop has been grown for centuries owing to its high content of excellent quality oil (42–54%) and protein (22–25%). The amino acid composition of the sesame seeds is unique and unusual among the oilseed proteins, due to its high content of sulphur-containing amino acids (methionine and cysteine) and low content of lysine (Johnson, Suleiman, & Lusas, 1979). Because of its amino acid profile, sesame has been recommended as a protein supplement for legumes (Bolo-orforooshan & Markakis, 1979; Brito & Nuñez, 1982). In addition, sesame is also a rich source of niacin, folic acid, vitamin E, calcium and phosphorus.

Most of the proteins present in sesame seeds are storage proteins found as albumins (8.9%), globulins (67.3%), prolamins (1.3%) and glutelins (6.9%) on the basis of their solubility (Rivas, Dench, & Caygill, 1981). The water-insoluble 11S globulin and the soluble 2S albumin, conventionally termed α-globulin and β-globulin, respectively, are the two major storage proteins and constitute 80–90% of the total seed proteins in sesame.

Although the sesame 11S globulin, and to a lesser extent its water-soluble 2S albumin counterpart, have been purified and well investigated, very little is known about the third major sesame storage protein, i.e., 7S globulin. The existence of a 7S globulin as a minor constituent of the total storage proteins in sesame has briefly been reported in several investigations (Beyer, Bardina, Grishina, & Sampson, 2002; Prakash, 1986; Prakash & Nandi, 1978; Tai, Lee, Tsai, Yiu, & Tzen, 2001). The 7S globulin constitutes approximately 5% of the total sesame protein (Prakash & Nandi, 1978). More recently, Tai et al. (2001) obtained a cDNA sequence encoding the sesame 7S

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doi:10.1016/j.foodchem.2005.10.051

globulin and identified its corresponding polypeptide as a minor constituent in sesame seeds via immunodetection. Nevertheless, the purification of sesame 7S globulin has not been previously reported.

Sesame has been increasingly associated with food allergy. According to recent investigations, the storage proteins appear to be the major allergens of sesame (Beyer et al., 2002; Pastorello et al., 2001; Wolff et al., 2003). Furthermore, despite the 7S globulin only accounting for 5% of the total sesame proteins, a 45 kDa polypeptide corresponding to the 7S globulin has recently been shown to be a major allergen for a group of twenty individuals with sesame allergy (Beyer et al., 2002). In this respect, the purification of the sesame 7S globulin could constitute an important first step for the investigation of the inherent physical, biochemical and immunological properties of the storage protein.

In the present investigation, we report the purification of the 7S globulin from sesame, its characterisation and a comparison of the 7S protein with the 2S albumin and the 11S globulin, the other sesame storage proteins.

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, methanol 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, disodium hydrogen orthophosphate, glycine, sodium chloride, sodium dihydrogen orthophosphate, *N*,*N*,*N*′,*N*′-Tetramethylethylene diamine (TEMED), Trizma™ base, Q Sepharose Fast Flow, Sephadex G-50 and G-100, Sephacryl S-200 HR and S-300 HR were purchased from Sigma–Aldrich Chemical Co., Poole, UK.

2.2. Purification of sesame proteins

2.2.1. Extraction and fractionation of sesame proteins

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), with constant stirring for 2 h at room temperature. The slurry was centrifuged at 19,800*g* for 1 h at 4 °C. The supernatant obtained after centrifugation was filtered through Whatman no. 1 filter paper and used for the isolation of sesame proteins. The proteins were then fractionated by a modification of the procedures described by Rajendran and Prakash (1988) and Prakash and Nandi (1978). Briefly, the supernatant was diluted with distilled water (1:5.5, v:v), allowed to stand for 1 h and centrifuged at 19,800*g* for 1 h at 4 °C. The water-insoluble pellet was re-dissolved in the extraction buffer and ammonium sulphate was added to 35% saturation. The solution was left to stand for 2 h and subsequently centrifuged at 11,600*g* for 30 min at 4 °C. The pellet obtained was re-dissolved in the extraction buffer, dialyzed extensively against distilled water in the cold and freeze-dried. This fraction was the starting material for the purification of the sesame 11S globulin by column chromatography. The water-soluble supernatant obtained after dilution was used for the purification of the 7S globulin and the 2S albumin. The 11S globulin was removed from the water-soluble fraction by precipitation with 40% ammonium sulphate and both the 7S globulin and 2S albumin were recovered by increasing the ammonium sulphate concentration to 80% saturation. The pellet obtained from the 80% ammonium sulphate fraction was re-dissolved in the extraction buffer, dialysed against distilled water and freeze-dried.

2.2.2. Purification of sesame proteins by column chromatography

The sesame 7S globulin was purified by anion-exchange chromatography on Q Sepharose Fast Flow. The column (18 × 1.6 cm) was equilibrated with phosphate buffer (0.1 M, pH 8.0). Freeze-dried water-soluble 80% ammonium sulphate fraction was dissolved in phosphate buffer (0.1 M, pH 8.0) at a concentration of 1 mg/ml. The solution was centrifuged at 11,600*g* and the clarified sample (2 ml) was loaded onto the Q Sepharose Fast Flow column at a flow rate of 0.5 ml min^{-1}. The column was then flushed with 70 ml phosphate buffer (0.1 M, pH 8.0) to wash away unbound materials. Elution was carried out using a linear gradient of 0–0.5 M sodium chloride in phosphate buffer (0.1 M, pH 8.0) in a total volume of 300 ml and fractions of 5 ml were collected. The protein content of the eluate was monitored at 280 nm.

The sesame 2S albumin and 11S globulin were purified by gel filtration chromatography. Briefly, the 2S albumin was purified on a Sephadex G-100 column (94 × 1.6 cm) equilibrated with phosphate buffer (60 mM, pH 7.5) containing 0.15 M sodium chloride. The freeze-dried, water-soluble, 80% ammonium sulphate fraction (10 mg) was dissolved in 2 ml of the same buffer, loaded onto the gel filtration column at 0.2 ml min^{-1} and eluted with 300 ml of phosphate buffer. Fractions of 6 ml were collected. Samples containing 2S albumin were pooled, concentrated by ultrafiltration, applied to a Sephadex G-50 column (88 × 1.6 cm) and eluted with 300 ml of phosphate buffer (60 mM, pH 7.5) containing 0.15 M sodium chloride. The 11S globulin was purified on a Sephacryl S-200 HR column (98 × 1.6 cm), followed by re-chromatography on Sephacryl S-300 HR (96 × 1.6 cm) equilibrated with Tris–HCl buffer (0.1 M, pH 7.5) containing 0.3 M sodium chloride. The 11S globulin was purified from the freeze-dried, water-insoluble, 35% ammonium sulphate fraction using the chromatographic conditions described previously.

2.3. Protein determination

Estimation of protein concentration was performed with Coomassie Plus Protein Assay reagent (Pierce and Warriner, Chester, UK) according to the method of Bradford (1976).

2.4. SDS–PAGE

The 7S and 11S globulin samples were analyzed 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 protein samples were reconstituted to 0.5 mg/ml in Tris–HCl buffer (0.125 M, pH 6.8) containing 4% (w/v) SDS, 20% (v/v) glycerol, 3% (w/v) dithiothreitol (DTT) and 0.001% (w/v) Bromophenol Blue. For non-reducing SDS–PAGE analysis, DTT was omitted from the sample treatment buffer. Aliquots (30 μl) containing 15 μg of protein were loaded into the gel wells and electrophoresis was carried out at constant current of 75 mA on 15% T acrylamide mini-gels. Electrophoresis of 2S albumins was carried out on 10–20% T gradient polyacrylamide gels (16 × 16 × 1.5 cm) at a constant current of 60 mA, using a Hoefer (SE 600 Series) unit. The SDS–PAGE analysis was conducted under reducing and non-reducing conditions. The protein bands were stained with Coomassie Brilliant Blue R-250 (Sigma–Aldrich Chemical Co., Poole, UK). Gels were photographed digitally by the University of Leeds Media Services. The following polypeptides were used as low-range molecular weight markers (Sigma–Aldrich Chemical Co., Poole, UK): bovine serum albumin (66,000 Da), ovalbumin (45,000 Da), glyceraldehyde-3-phosphate dehydrogenase (36,000 Da), carbonic anhydrase (29,000 Da), trypsinogen (24,000 Da), soybean trypsin inhibitor (20,000 Da), α-lactalbumin (14,200 Da) and aprotinin (6500 Da).

2.5. Native PAGE

The protein samples were reconstituted to 0.5 mg/ml in Tris–HCl buffer (0.125 M, pH 8.8) containing 20% glycerol and 0.001% (w/v) Bromophenol Blue. Aliquots (30 μl) containing 15 μg of protein were loaded onto 6% T gels and a gradient of 6–20% of T gels and electrophoresis was conducted at a constant current of 75 mA, using a Bio-Rad Miniprotean® II unit.

2.6. N-terminal protein sequencing

7S globulin samples were run on 15% acrylamide mini-gels and semi-dry electroblotted onto Fluorotrans (Pall) polyvinylidene difluoride (PVDF) membranes using 3-(cyclohexylamino)-1-propane sulphonic acid (CAPS) buffer (10 mM, pH 11.0) containing 10% methanol in distilled water as the blotting buffer. Once the blotting was completed, the membrane was stained for a few seconds in 0.005% (w/v) sulforhodamine B in 30% (v/v) methanol and 0.2% (v/v) acetic acid in distilled water. Protein bands to be sequenced were excised from the blot, washed in 1 ml of 50% (v/v) methanol/0.1% (v/v) trimethylamine, dried and placed in the sequencer cartridge. N-terminal amino acid sequencing was conducted on a Procise 494 liquid-pulse instrument (PE Applied Biosystems), with on-line narrow-bore 140C HPLC system and 610A chromatogram analysis software. The N-terminal sequencing of the 7S globulin polypeptides was carried out by Dr. Jeff Keen at the School of Biochemistry and Microbiology, University of Leeds (UK).

3. Results and discussion

3.1. General

The seed storage proteins consist of complex groups of proteins that are often difficult to purify because of their heterogeneous nature and lack of unique functionalities. In addition, the major storage globulins, namely the 7S and 11S globulins, are oligomeric proteins and exhibit dissociation/association behaviour, depending on the pH and ionic strength of the medium, which further complicates the isolation of these proteins from the source plant material. In the present investigation we have purified the 2S, 7S and 11S proteins of sesame; the 7S protein for the first time and the 2S and 11S proteins for comparative purposes. We have deliberately avoided the use of protease inhibitors, which can make assessment of the purification process clearer, because we are particularly interested in the proteins as they might be present in food. For example, stability to digestive proteases may be characteristic of allergen molecules.

3.2. Purification of the sesame 11S globulin

The major sesame storage protein, i.e., the 11S globulin, was purified by means of gel filtration chromatography. SDS–PAGE analysis of samples treated with reducing agents revealed that the isolated 11S globulin was composed of two acidic polypeptides (34.5 and 30.6 kDa) and a single basic polypeptide (20.2 kDa) (Fig. 1). The SDS–PAGE patterns of the 11S globulin samples treated with and without with DTT were very different. Such differences between the gels run under reducing and non-reducing conditions indicated the presence of disulphide bonds within the 11S protein structure. A typical image of the sesame 11S globulin run under non-reducing conditions is shown in Fig. 1. The 11S globulin was composed of four major protein bands, of 36.0, 41.0, 46.6 and 51.8 kDa, when analysed by SDS–PAGE in the absence of reducing agents. Such polypeptides corresponded to the intermediary subunits of the 11S globulin. The isolated protein was homogeneous, judged by ion-exchange chromatography on DEAE-Sephacel and native PAGE (data not shown). The identity of the protein was confirmed by means of

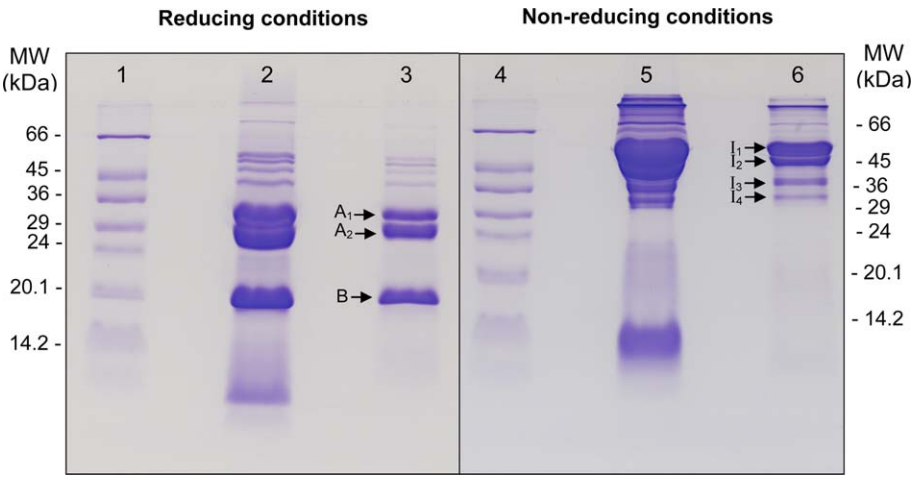

Fig. 1. SDS–PAGE of sesame 11S globulin samples under reducing and non-reducing conditions. Samples were run on 15% acrylamide gels. Lanes 1 and 4: low-range molecular weight markers; Lanes 2 and 5: water-insoluble 35% ammonium sulphate fraction loaded onto the Sephacryl S-200 HR column; Lanes 3 and 6: purified 11S globulin after re-chromatography of the 11S-rich peak eluted from Sephacryl S-200 HR on Sephacryl S-300 HR. Samples shown in lanes 1, 2 and 3 were treated with DTT. Samples shown in lanes 4, 5 and 6 were run without DTT. A_1 and A_2, acidic polypeptides; B, basic polypeptide; I_1, I_2, I_3 and I_4, intermediary subunits.

immunoblotting with polyclonal anti-sesame 11S globulin antibodies (Fig. 2).

3.3. Purification of the sesame 2S albumin

The purification of the sesame 2S albumin was accomplished by means of gel filtration chromatography. SDS–PAGE analysis under non-reducing conditions indicated that the sesame 2S albumin was composed of a single polypeptide with an apparent molecular mass of 13 kDa (Fig. 3). The polypeptide could be cleaved into a large and small subunit when treated with reducing agents such as DTT. The identity of the protein was confirmed by immunoblotting with an anti-sesame 2S albumin polyclonal antibody (Fig. 3).

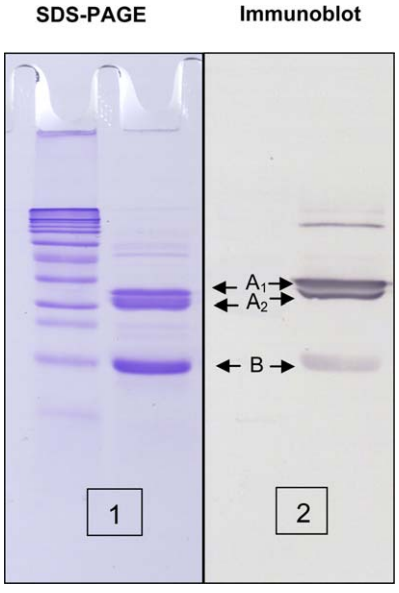

Fig. 2. SDS–PAGE and immunoblotting of the 11S globulin from sesame: 1, SDS–PAGE of the purified 11S globulin under reducing conditions on a 15% acrylamide gel; 2, immunoblot of the 11S globulin probed with an anti-sesame 11S polyclonal antibody. A_1 and A_2, acidic polypeptides; B, basic polypeptides.

3.4. Purification of the sesame 7S globulin

3.4.1. General

The sesame 7S globulin was purified from the water-soluble 80% ammonium sulphate fraction by anion-exchange chromatography on Q Sepharose Fast Flow. A typical elution profile, using Q Sepharose Fast Flow at pH 8.0, is shown in Fig. 4. The elution profile was reproducible and, when monitored at 280 nm, indicated the presence of four peaks. The first two peaks, which were eluted at the washing stage, were mainly composed of low molecular weight 2S albumins. The third peak, which eluted from the column with a sodium chloride concentration of 0.20 M, corresponded to sesame 7S globulin. Finally, the last peak eluted with an increased molarity of sodium chloride of 0.36 M and contained several high molecular weight polypeptides.

3.4.2. SDS–PAGE analysis

The purified 7S globulin was characterised by SDS–PAGE in the presence and absence of a reducing agent. SDS gel electrophoresis under reducing conditions revealed that the 7S globulin was composed of at least eight polypeptides with molecular weights ranging from 12.4 to 65.5 kDa (Fig. 5). In order to elucidate the nature of the bond forces between the polypeptides, the isolated 7S globulin was subjected to SDS–PAGE in the absence of DTT (Fig. 5). The same polypeptide pattern emerged for the 7S globulin samples treated with and without reducing

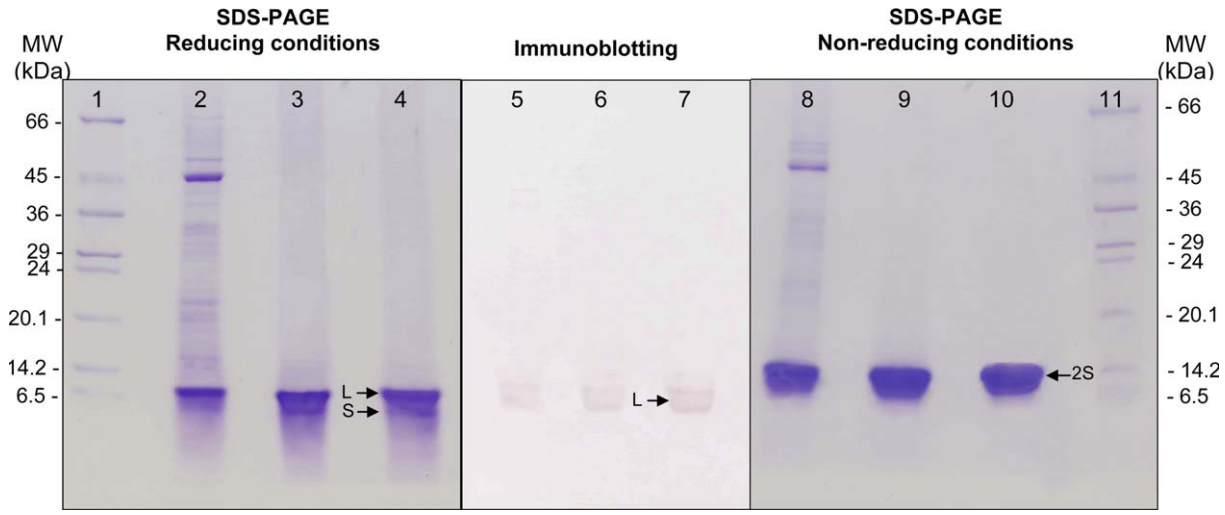

Fig. 3. SDS–PAGE and immunoblotting of sesame 2S albumin samples. SDS–PAGE was run on gradient 10–20% acrylamide gels. Lanes 1 and 11: low-range molecular weight markers; Lanes 2 and 8: water-soluble 80% ammonium sulphate fraction loaded on to the Sephadex G-100 column; Lanes 3 and 9: 2S albumin-rich fraction eluted from Sephadex G-100; Lanes 4 and 10: 2S albumin sample after re-chromatography on Sephadex G-50. Samples shown in lanes 1, 2, 3 and 4 were treated with DTT. Samples shown in lanes 8, 9, 10 and 11 were run in the absence of DTT. Lanes 5, 6 and 7 correspond to the immunoblot of the reduced 2S albumin samples probed with an anti-sesame 2S albumin polyclonal antibody. L, large subunit; S, small subunit; 2S, 2S albumin under non-reducing conditions.

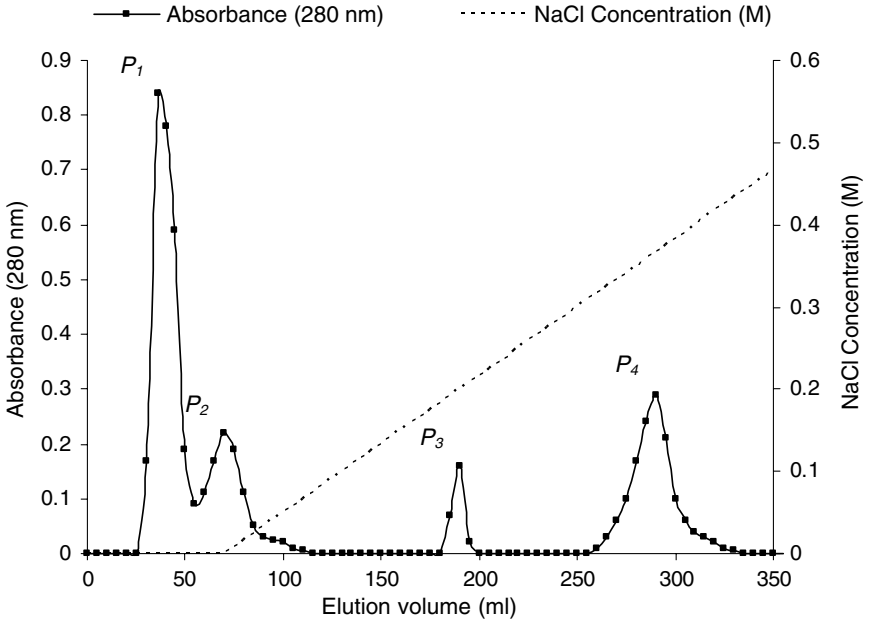

Fig. 4. Elution profile for Q Sepharose Fast Flow fractionation of the water-soluble 80% ammonium sulphate protein fraction. Protein (1 mg/ml) was dissolved in elution buffer (1.8 ml) and applied to the column. Unbound proteins were eluted with 70 ml of phosphate buffer (0.1 M, pH 8.0) at 0.5 ml min^{-1}. Bound proteins were eluted with a 300 ml salt gradient from 0 to 0.5 M sodium chloride in phosphate buffer (0.1 M, pH 8.0). Fractions (5 ml) were collected.

agent. Therefore, results indicated that the sesame 7S globulin did not have disulphide bonds holding the polypeptides together. These findings further supported the classification of the purified protein as a 7S globulin, since 7S globulins are classically recognised as being devoid of disulphide bonds (Shewry, Napier, & Tatham, 1995).

The existence of a large number of polypeptides is not unique to the sesame 7S globulin. Indeed, 7S globulins from several species have also been found to possess a complex polypeptide composition over an extensive range of molecular weights. Gatehouse, Croy, Morton, Tyler, and Boulter (1981) observed that the mature pea 7S globulin consisted of eight polypeptides, with molecular weights ranging from 12.5 to 50.0 kDa. The mature vicilin from broad bean was also composed of a similar range of polypeptides to its pea counterpart and at least five polypep-

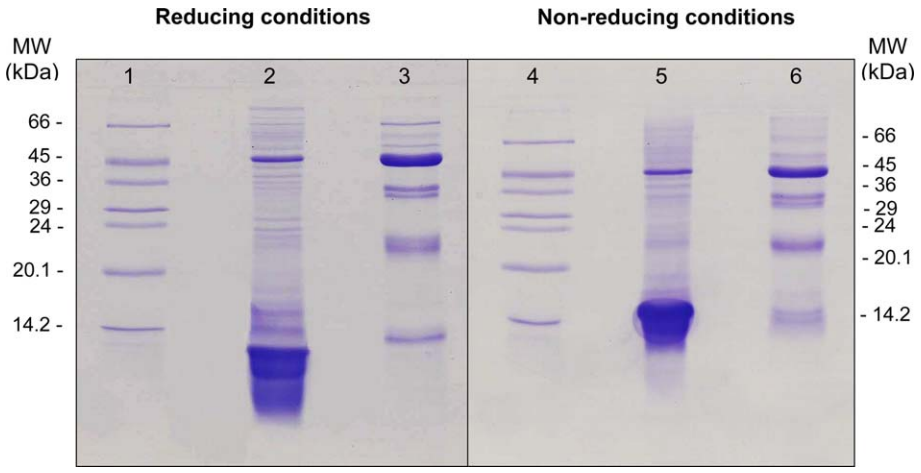

Fig. 5. SDS–PAGE of sesame 7S globulin under reducing and non-reducing conditions. Samples were run on 15% acrylamide gels. Lanes 1 and 4: low range molecular weight protein standards; Lanes 2 and 5: water-soluble 80% ammonium sulphate fraction loaded onto the Q Sepharose Fast Flow anion-exchange column; Lanes 3 and 6: sesame 7S globulin purified by Q Sepharose Fast Flow. Samples shown in lanes 1, 2 and 3 were treated with DTT. Samples shown in lanes 4, 5 and 6 were run in the absence of DTT.

tides could be observed on SDS gels (Scholz, Manteuffel, Müntz, & Rudolph, 1983). Marcone (1999) reported the existence of a 7S globulin-like storage protein in amaranth seeds which consisted of eight polypeptides with molecular weights ranging from 15.6 to 90.1 kDa. In contrast, the 7S globulins from some other species, such as soybean (Thanh & Shibasaki, 1976) and French bean (Bollini & Chrispeels, 1978; Hall, McLeester, & Bliss, 1977) were shown to be composed of three major subunits in the range 40–60 kDa. Hence, 7S globulins could be classified into two groups on the basis of whether the subunits undergo post-translational proteolysis (pea, broad bean) or do not (soybean, French bean). The 7S globulins of soybean and *Phaseolus vulgaris* differed from pea and *Vicia faba* in that subunits were not proteolytically cleaved, but were more extensively glycosylated. Although there are no available data about the glycosylation of sesame 7S globulin, the SDS–PAGE banding pattern of the purified protein seems to indicate that the 7S globulin of sesame has more resemblance to the group composed of the 7S globulins of pea and broad bean.

The purified 7S globulin was subjected to SDS–PAGE alongside its 11S globulin counterpart with the aim of comparing the polypeptide composition of the two storage proteins. Fig. 6 and Table 1 showed that no polypeptides were shared in common between both types of proteins, indicating that 7S and 11S globulins were very distinct proteins within the sesame seed on a purely structural basis. The most likely impurity to be present in the 7S globulin preparation is the similar legumin-like storage protein, i.e., 11S globulin. Nevertheless, as shown in Fig. 6, the characteristic polypeptides of the 11S globulin were absent from the 7S globulin sample analysed by SDS–PAGE. Hence, the lack of 11S globulin contamination could also be interpreted as further evidence of purity of the isolated 7S globulin.

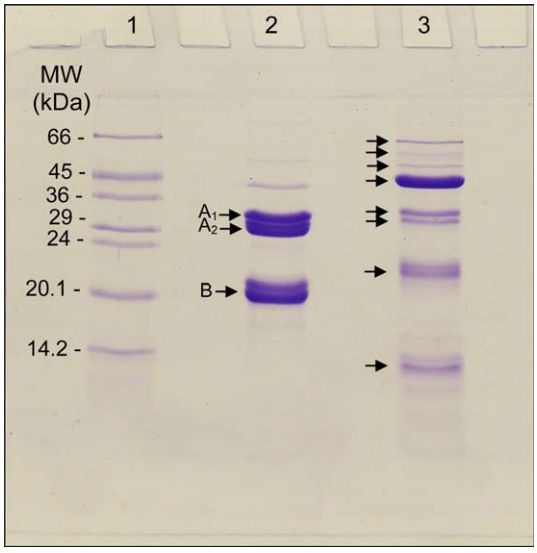

Fig. 6. SDS–PAGE of sesame 11S and 7S globulin under reducing conditions. Lane 1: low-range molecular weight markers; Lane 2: sesame 11S globulin; Lane 3: sesame 7S globulin. A_1 and A_2, acidic polypeptides; B, basic polypeptides; the main subunits of the 7S globulin are indicated by arrows.

Table 1
SDS–PAGE molecular weight distribution of the polypeptides of sesame 11S globulin and 7S globulin

Sesame 11S globulin (kDa)	Sesame 7S globulin (kDa)
52.0	65.5
49.5	59.2
47.2	50.3
44.0	**45.1**
34.5	**34.7**
30.5	**32.8**
20.3	**23.3**
19.2	**12.4**

The values presented in bold correspond to major polypeptides.

3.4.3. Native PAGE analysis

The 7S globulin isolated from the seeds was examined by non-denaturing gel electrophoresis to verify the homogeneity of the protein. The purified 7S globulin samples migrated as a single diffuse band and no other protein bands could be visualised on the gels, which contrasted with the protein band profile of the water-soluble, 80% ammonium sulphate fraction, composed of a number of bands with different electrophoretic mobilities (Fig. 7).

A similar pattern was observed when samples were run on 6–20% acrylamide gradient gels (Fig. 7). Close examination of the gradient gel revealed that, in addition to the main band, a minor protein band of higher relative electrophoretic mobility was also present. The presence of such a band may be attributed to an alkaline pH-induced dissociation phenomenon. Furthermore, several other storage proteins, such as the 11S globulins of peanut, buckwheat, alfalfa, caraway, cumin, amaranth and soybean, have also been reported to undergo alkaline-induced dissociation under the conditions used for native PAGE (Marcone & Yada, 1991; Marcone, Bondi, & Yada, 1994; Marcone, Kakuda, & Yada, 1998). In addition, non-denaturing PAGE analysis of the 7S globulins of pea, faba beans and peanut also revealed the presence of additional protein bands with higher electrophoretic mobilities besides the main band (Freitas, Ferreira, & Teixeira, 2000). As has been discussed previously, the main forces holding the subunits of the 7S globulin molecule together were non-covalent, weak secondary forces, such as hydrogen bonding, hydrophobic and electrostatic forces. Therefore, it is probable that the lack of covalent bonds supporting the overall 7S molecule could contribute to the dissociation of the subunits in an alkaline environment. In this respect, it is possible that the protein band of higher electrophoretic mobility observed by native PAGE corresponds to a monomer dissociated from the main trimeric 7S globulin molecule.

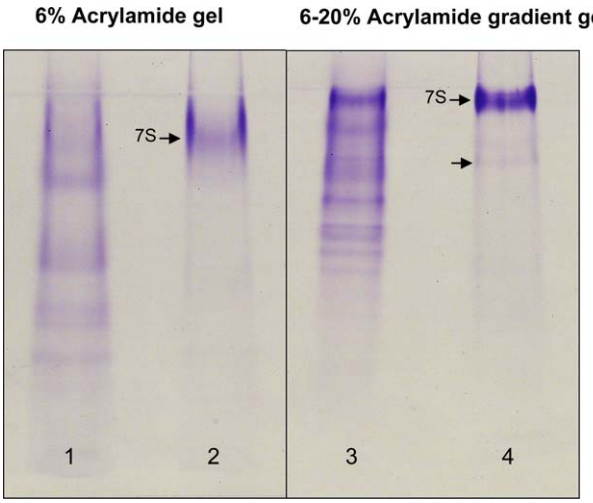

Fig. 7. Native PAGE of sesame 7S globulin samples on 6% and gradient 6–20% acrylamide gels. Lanes 1 and 3: water-soluble 80% ammonium sulphate fraction loaded onto the Q Sepharose Fast Flow anion-exchange column; Lanes 2 and 4: sesame 7S globulin samples purified by Q Sepharose Fast Flow. Samples shown in lanes 1 and 2 were run on 6% acrylamide gels under non-denaturing conditions. Samples shown in lanes 3 and 4 were run on gradient 6–20% acrylamide gels under non-denaturing conditions. The arrow indicates the high electrophoretic mobility protein band.

3.4.4. N-terminal sequencing

The four major polypeptide bands of the isolated 7S globulin were subjected to N-terminal amino acid sequencing (Fig. 8) and compared with the amino acid sequence deduced from a cDNA clone which encoded a sesame 7S globulin precursor.

The N-terminal sequencing of the bands 1, 2 and 3 from Fig. 8, gave data indicating consistently rich glutamate (E) and arginine (R) contents. The potential sequence permutations for the first three bands were the following: band 1: P/E/G/S-R/E-R/E/K-E-E/R-E-E-E-E/Q-E, band 2: E-R-T/E-E/R-E/R-E-E-E/Q-E-E and band 3: E/G/R-R-R-E/R-E/R-E-E-E. In contrast to the first three bands, band 4 gave data suggesting the following sequence: G-E-T-K/R-G-T/Q-I-N-I/Q-V.

The N-terminal sequencing of the main components, revealed that the 12.4, 23.3, 32.8, 34.7 and 45.1 kDa polypeptides contained amino acid sequences that matched two regions of the amino acid sequence deduced from a cDNA clone which encoded the sesame 7S globulin precursor shown in Fig. 9 (Tai et al., 2001). Although the high glutamate contents of the sequenced bands 1, 2 and 3 complicated the reliable interpretation of a sequence, a similar amino acid region could be located in the sesame 7S globulin precursor sequence, starting at the glutamate residue number 181 (E_{181}), EREEEQEEQG (Fig. 9). In contrast, the N-terminal sequence G-E-T-K/R-G-T/Q-I-N-I/Q-V, obtained for the band 4 was homologous to the amino acid

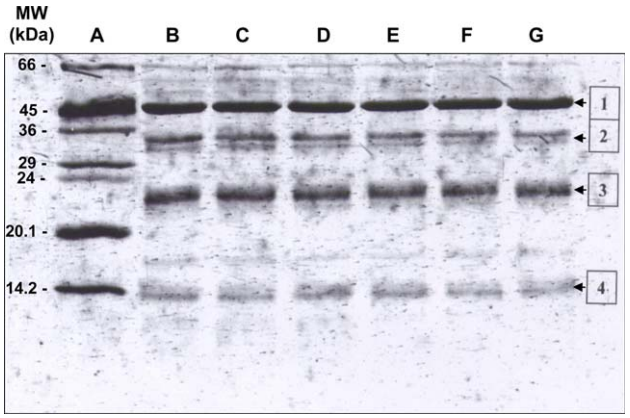

Fig. 8. 7S globulin blotted onto a PVDF membrane and stained with sulphorhodamine B. A: low-range molecular weight markers; B, C, D, E, F and G: sesame 7S globulin samples. Band 1: corresponds to the 45.1 kDa polypeptide; Band 2: corresponds to the duplet of 34.7 and 32.8 kDa polypeptides; Band 3: corresponds to the 23.3 kDa polypeptide; Band 4: corresponds to the 12.4 kDa polypeptide.

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1   mscggrlclv lfalllasav vaseskdpel kqckhqckaq qqiskeqkea ciqackeyir
61  qkhqgehgrg ggdileeevw nrkspierlr ecsrgceqqh geqreeclrr cqeeyqrekg
121 rqdddnptdp ekqyqqcrlq crrqgegggf srehcerrre ekyreqqgre ggrgemyegr
181 ereeeqeeqg rgripyvfed qhfitgfrtq hgrmrvlqkf tdrsellrgi enyrvailea
241 epqtfivpnh wdaesvvfva kgrgtislvr qdrreslnik qgdilkinag ttaylinrdn
301 nerlvlakll qpvstpgefe lffgaggenp esffksfsde ileaafntrr drlqrifgqq
361 rqgvivkase eqvramsrhe eggiwpfgge skgtiniyqq rpthsnqygq lhevdasqyr
421 qlrdldltvs lanitqgamt aphynskatk ialvvdgegy femacphmsr srgsyqgetr
481 grpsyqrvas rltrgtvvii paghpfvava ssnqnlqvlc fevnannnek fplagrrnvm
541 nqlereakel afgmpareve evsrsqqeef ffkgprqqqq grada
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Fig. 9. Deduced amino acid sequence for the sesame 7S globulin precursor (Tai et al., 2001). Accession no. Q9AUD0 (Swiss-Plot database), 7S globulin (*Sesamum indicum*). The underlined amino acids are homologous to the N-terminal sequences of the purified 7S globulin polypeptides.

region of the 7S globulin sequence starting from G_{389}, GESKGTINIY.

Thus, the major polypeptide band of 45.1 kDa (band 1), the duplet comprised of the 34.7 and 32.8 kDa polypeptides (band 2) and the 23.3 kDa polypeptide (band 3), had the same N-terminal amino acid sequence, which was homologous to the region EREEEQEEQG of the sesame 7S globulin sequence. The differences in molecular weight of the three bands probably arose from different cleavage sites from the C-terminus. The arginine-glutamate (R/E) N-terminal cleavage site of the first three polypeptides may have arisen from the action of papain-like proteases with specificity for peptide bonds with arginine in the P_1 position. In this respect, there is evidence of the existence of cysteine proteinases in sesame seeds (Hemalatha & Siva Prasad, 2003). However, the presence of trypsin-like serine proteases has also been reported in sesame by Hemalatha and Siva Prasad (2003), and, therefore, the possibility of the action of a trypsin-like enzyme, resulting in the Arg-Glu cleavage, cannot be ruled out. In contrast, the 12.4 kDa band had a different N-terminal sequence, which was homologous to the region GESKGTINIY of the sesame 7S globulin sequence.

Therefore, such results suggested that the main 45 kDa polypeptide and the other polypeptides of lower molecular weight corresponded to the sesame 7S globulin and not to contaminant proteins. Beyer et al. (2002) reported that a 45 kDa polypeptide was the major allergen for a group of twenty individuals with sesame allergy. Furthermore, the authors have also identified the 45 kDa polypeptide (*Ses i* 3) as the sesame 7S globulin by means of internal amino acid sequencing.

It is not clear whether the 7S globulin polypeptides, with molecular weights below 45 kDa, were products of limited proteolysis during seed maturation or occurred due to proteolytic degradation during the extraction and purification procedure. It should be noted that, although no protease inhibitors were added during the extraction and purification of sesame 7S globulin, the entire process was carried out at 4 °C and for the shortest time possible, to minimise proteolysis by exogenous proteases and, therefore, the possibility of the existence of the polypeptides observed by SDS–PAGE within mature sesame seeds could not be ruled out.

Hence, it seems possible that the low molecular weight polypeptides of the sesame 7S globulin arose partially from a post-translational proteolytic cleavage during seed maturation and partially from proteolytic degradation during the extraction of the protein. According to Gatehouse et al. (1981) and Chrispeels et al. (1982), the low molecular weight polypeptides found in deposited, i.e., isolated, pea 7S globulin originated from post-synthetic proteolysis of some of the 50 kDa subunits after their assembly into the vicilin holoprotein molecule. Two potential sites for post-translational proteolytic cleavage were identified within the 50 kDa precursor molecule of *Pisum sativum* (Gatehouse, Lycett, Delauney, Croy, & Boulter, 1983; Spencer, Chandler, Higgins, Inglis, & Rubira, 1983). Millerd (1975) reported that no evidence for proteolytic degradation of pea 7S globulin during extraction was found in comparison with extractions carried out in the presence of protease inhibitors or under dissociating conditions. In contrast, the 7S globulin from broad bean was shown to undergo cleavage during prolonged treatment at room temperature. The cleavage could be inhibited by protease inhibitors, such as 1 μM leupeptin (Scholz et al., 1983). Nevertheless, the authors also reported that small amounts of low molecular weight polypeptides were visible on SDS gels, even after short-time extraction at 5 °C, and that therefore, such polypeptides were likely to exist within the mature seed. It was concluded that there were probably two processes contributing to the cleavage of broad bean vicilin, one occurring soon after translation and produced by endogenous proteases, and another 'artifactual' one promoted by the same, or possibly exogenous, proteases occurring during extraction and purification of the protein.

Sesame seed proteins, and the 2S and 7S proteins in particular, have been reported to be allergenic in sensitised individuals. Further study of such proteins is required in order to establish the molecular basis of the interactions occurring during food processing, digestion and absorption, and with various elements of the immune system. Purification of the proteins is an essential part of such a process and may lead to improved understanding of why

some food proteins – but not others – are capable of acting as allergens in some individuals.

Acknowledgements

We are grateful to the Education, University and Research Department of the Basque Government for a research grant (BFI98.106/AK) to Dr. E. Orruño. The authors thank Professor Jason T.C. Tzen from the Graduate Institute of Agricultural Biotechnology at the National Chung Hsing University (Taiwan) for his generous donation of anti-sesame 2S antibody, and Gary Brett and Steve Chambers (Institute of Food Research, Norwich, UK) for the anti-sesame 11S globulin antibodies.

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