Use of scanning electron microscopy to indicate the similarities and differences in pseudocereal and cereal proteins

Shela Gorinstein,[1]*† Elke Pawelzik,[2] Efren Delgado-Licon,[2]‡ Kazutaka Yamamoto,[3] Shoichi Kobayashi,[4] Hajime Taniguchi,[5] Ratiporn Haruenkit,[6] Yong-Seo Park,[7] Soon-Teck Jung,[8] Jerzy Drzewiecki[9] & Simon Trakhtenberg[10]

1 Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University – Hadassah Medical School, PO Box 12065, Jerusalem 91120, Israel
2 Institute of Agricultural Chemistry, Georg-August-University Goettingen, D-37075 Goettingen, Germany
3 National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan
4 Iwate University, Faculty of Agriculture, Iwate, Japan
5 Chubu University, College of Bioscience and Biotechnology, Kasugai, Aichi, Japan
6 Department of Agricultural Industry, Faculty of Agricultural Technology, King Mondkut Institute of Technology, Ladkrabang, Bangkok, Thailand
7 Department of Horticultural Science, Mokpo National University, Republic of Korea
8 Department of Food Engineering, Mokpo National University, Republic of Korea
9 Plant Breeding and Acclimatization Institute, Radzikow, Poland
10 Institute of Cardiology, Kaplan Medical Center, Rehovot, Israel

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Summary Isolated and separated protein fractions from cereal and pseudocereal grains were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis and scanning electron microscopy. Prolamin, the main storage protein in cereal such as maize, showed a difference in electrophoretic patterns and fine structure in comparison with those from amaranth and soybean. In contrast glutelins from amaranth, soybean and maize showed some similarity in the distribution of protein bands and in microstructure. Amaranth and soybean were closely similar in distribution of protein fractions and their microscopic structure. As an addition to chemical analyses, microscopy helped to understand and visualize structural changes and textural differences in protein fractions. Pseudocereals can be used as a nutritive substitute of some cereals in functional foods.

Keywords Electrophoretic separation, isolated protein fractions, main storage proteins, nutritive substitution, structural changes.

Introduction

Seed storage proteins of grain crops meet the major dietary protein requirement of over half of the world population (Mandal & Mandal, 2000). However, seed proteins in general are deficient in some essential amino acids and hence are of poor nutritional quality. Therefore, intensive research is going on to isolate and characterize these proteins and their genes and to produce transgenic crop plants with modified seed protein genes, with a view to improving their nutritive value as human food and animal feed (Mandal & Mandal, 2000).

One of the most widespread groups of plant proteins is the prolaminin superfamily, which comprises cereal seed storage proteins, a range of low-molecular-mass sulphur-rich proteins (many of which are located in seeds) and some cell wall glycoproteins. This superfamily includes several

*Correspondent: Fax: 972 2 6757076;
e-mail: gorin@cc.huji.ac.il

†Shela Gorinstein is affiliated with the David R. Bloom Center for Pharmacy of the Hebrew University, Jerusalem, Israel.
‡Technological Institute of Durango, Mexico.

major types of plant allergens: nonspecific lipid transfer proteins, cereal seed inhibitors of α-amylase and/or trypsin and 2 S albumin storage proteins of dicotyledonous seeds (Shewry *et al.*, 2002).

The prolamin fractions in all wheat, barley, rye, triticale and oat samples show strong reactivity against the rabbit anti-gliadin (wheat) antibodies. The bands are extremely strong in the case of the spelt wheat samples (Matuz *et al.*, 2000). In their report Matuz *et al.* (2000) suggest that the consumption of bread and pasta made from the flour of this spelt wheat species cannot be recommended for patients suffering from gluten sensitivity. The immunoreactive zones were not found in the protein range corresponding to the prolamin fractions from maize and millet samples. No reactivity was observed against the buckwheat and amaranth samples, which are not cereals (Matuz *et al.*, 2000).

The relationship between diet and asthma is an area of controversy that has never been fully evaluated. Attempts at dietary prevention of asthma have produced conflicting results (Armentia *et al.*, 2001). Allergens from cereals that show cross-reactivity with proteins in grass pollen have recently been identified (Armentia *et al.*, 2001). An early intake of cereals in the diet during early life might cause IgE sensitization to cereals. It is not known whether such sensitization predisposes the development of allergy to pollen (Armentia *et al.*, 2001).

On the basis of such studies (Matuz *et al.*, 2000; Armentia *et al.*, 2001; Shewry *et al.*, 2002) it is desirable to find a substitute for cereal grains for those individuals not able to consume them. The nutritional value of pseudocereals is mainly connected to their protein composition. Proteins are an important group of biomacromolecules that are involved in physiological functions (Wright, 1987). Natural vegetable proteins are useful materials because of their high biocompatibility, nutritional value and low cost. Development of new vegetable protein sources rich in essential amino acids may be important for the food and pharmaceutical industries.

Plants such as amaranth can be used (Bressani *et al.*, 1993; Dodok *et al.*, 1997) because large amounts of essential amino acids have been reported in amaranth (Fadel *et al.*, 1996). Evaluation of the nutritional value of the amaranth plant in experiments on growing rats have shown that that the heat treatment of amaranth increased the body weight gain of rats even when the animals consumed diets containing only 10% crude protein (Andrasofszky *et al.*, 1998; Chavez-Jauregui *et al.*, 2000). Extrusion produces a highly acceptable snack product based on amaranth flour (Dodok *et al.*, 1997).

Although alcohol-soluble prolamins are the major storage proteins in maize (Wall *et al.*, 1988; Gorinstein *et al.*, 1991; Hamaker *et al.*, 1995; Landry *et al.*, 2000), in some plants prolamins are not the only storage proteins. For example globulins are accumulated in soybean and amaranth, as their major storage proteins (Wright, 1987; Okita *et al.*, 1988; Gorinstein *et al.*, 1991, 2001; Katsube *et al.*, 1999).

Considerable work has been published concerning the differences and identity of electrophoretic patterns in isolated seed protein fractions of maize, soybean and amaranth (Wall *et al.*, 1988; Gorinstein *et al.*, 1991, 1999; Hamaker *et al.*, 1995; Landry *et al.*, 2000). A variety of microscopic techniques are available for studying the microstructure of cereals and in research on cereal-based products microscopy provides resolution, chemical specificity, and sensitivity rarely matched by other techniques (Autio & Salmenkallio-Marttila, 2001). Application of protein microscopy in cereals and pseudocereals has been described in some reports with an emphasis on functional properties of protein gels formed during food processing (Ker *et al.*, 1993; Yuno-Ohto *et al.*, 1994; Konishi *et al.*, 1995; Batterman-Azcona & Hamaker, 1998; Autio & Salmenkallio-Marttila, 2001; Bugusu *et al.*, 2002).

This paper reports the distribution of protein fractions in amaranth, soybean and maize as established by solvent extraction. The identity and differences based on electrophoretic patterns and microstructure of protein fractions is shown.

Materials and methods

Sample

Whole mature seeds of plants such as amaranth (*Amaranthus hypochondriacus*), soybean [*Glycine mar* (L.) Merrill] and maize (*Zea mays* L.) were investigated. Seeds were ground on a mill (Janke &

Kunkel GmbH & Co. KG –IKA, Labortechnik, Staufen, Germany) through a 60-mesh screen. The meal was defatted in a Soxhlet extractor with n-hexane for 10 h and then was stored at 5 °C after removal of hexane.

Protein extraction

Proteins were extracted stepwise according to the following methods (Gorinstein *et al*., 1991, 1999; Landry *et al*., 2000). The meal (1 g) was extracted with a solvent: sample ratio of 6 : 1 for alcohol-soluble proteins and 3 : 1 for glutelins (v/w) and vigorously shaken. The extracts were separated by centrifuging at 10 000 ***g*** for 10 min. Each step was repeated twice. The sequence of the solvents used was the following: total alcohol-soluble proteins (TASP) were extracted with 55% (v/v) isopropanol (IP), containing 4% (v/v) 2-mercaptoethanol (2-ME); total buffer-soluble proteins – glutelins (TGlu) with 0.125 M sodium borate buffer, pH 10, containing 3% (v/v) 2-ME plus 1% sodium dodecyl sulphate (SDS) (w/v). Extracts were combined, lyophilized and dissolved in sample buffer which contained 10% glycerol, 5% 2-ME, 2% SDS in 0.125 M (Tris-HCl) pH 6.8. Then the extracts were boiled for 5 min before being loaded. The nitrogen content in each fraction was determined by micro-Kjeldahl method combined with a colorimetric determination (Nkonge & Balance, 1982).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

The Laemmli (1970) method was used for sodium dodecyl sulphate polyacrylamide gel electrophoresis, using gradient (5–20% w/v) gels. The gels were 1.5 mm thick and consisted of a 2 cm stacking gel and a 10 cm running gel. 50 μg protein was applied to sample slots.

The duration of electrophoresis was 2 h at 150 V. Gels were stained with 0.25% Coomassie Brilliant Blue R in methanol/water/acetic acid (5 : 5 : 1 v/v) and destained in the same solvent. M_W standards were used to estimate protein subunit molecular weights in kDa: phosphorylase b (94); haemoglobin, canine (67); ovalbumin (43); carbonic anhydrase (30); trypsin inhibitor, soybean (20) and lactalbumin, bovine milk (14).

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on dried protein samples, which were mounted on aluminium stubs with double sticky tape and coated with 20 nm of gold. The samples were viewed and photographed on a JEOL JSM-880 SEM (JEOL, USA, Inc., Peabody, MA, USA) at accelerating voltage of 05–30 kV. Current for scanning was 2×10^{-10} Amp (Enamuthu *et al*., 1993; Konishi *et al*., 1995; Chan *et al*., 1997).

Results and discussion

Electrophoretic separation

Total alcohol-soluble proteins (TASP) from *A. hypochondriacus* and soybean (Fig. 1a, lanes 2 and 3) showed unseparated material of about 8–14 kDa and minor bands at 20 kDa and moderate sized bands at 32 and 60 kDa. Prolamin electrophoretic patterns from maize (Fig. 1a, lane 4) showed three characteristic bands of 19 (weak),

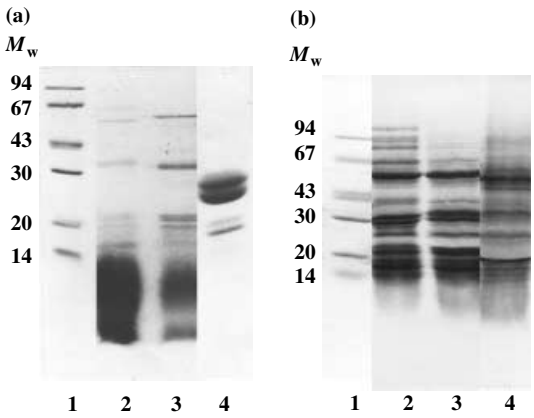

Figure 1 Sodium dodecyl sulphate polyacrylamide gel electrophoresis in 5–20% polyacrylamide gel of total alcohol-soluble proteins (TASP) in (a) lane 1, molecular marker (94, phosphorylase b; 67, haemoglobin, canine; 43, ovalbumin; 30, carbonic anhydrase ; 20, trypsin inhibitor, soybean and 14, lactalbumin, bovine milk); lane 2, amaranth; lane 3, soybean; lane 4, maize; of total buffer-soluble proteins- total glutelins (TGlu) in (b) lane 1, the same molecular marker as in (a); lane 2, amaranth; lane 3, soybean; lane 4, maize. TASP were extracted with 55% (v/v) isopropanol (IP), containing 4% (v/v) 2-mercaptoethanol (2-ME); TGlu with 0.125 M sodium borate buffer, pH 10, containing 3% (v/v) 2-ME and 1% sodium dodecyl sulphate (SDS) (w/v).

22 and 27 kDa. TASP from *A. hypochondriacus* and soybean contained 80–85% of polypeptides of 8–14 kDa. The rest of the amaranth proteins were minor fractions. The protein subunits in the region of 8–14 kDa, in which was concentrated the major unseparated material from amaranth and soybean, differ completely from those of maize. The maize prolamin, the main storage protein, did not show any electrophoretic relationship with amaranth and soybean alcohol-soluble fractions. The electrophoretic data proved that alcohol-soluble proteins did not represent the main protein fraction in cereal-like plants such as *A. hypochondriacus* and soybean (Gorinstein *et al.*, 1991, 1998; Landry *et al.*, 2000).

Main subunits of 15–22, 26–32 and 50–57 kDa were common in all glutelin fractions from amaranth, soybean and maize (Fig. 1b, lanes 2–4).

Two-dimensional gel electrophoresis analysis revealed the occurrence of some glutelins as the major proteins in the seed (Seguranieto *et al.*, 1992). Based on our experimental and literature data it can be concluded that the globulin fraction is not the only main storage protein, but that the glutelin fraction can also be suggested as one of the main proteins in the pseudocereals and legumes (Gorinstein *et al.*, 1998, 1999; Katsube *et al.*, 1999).

Upon electrophoresis, prolamins were made up of fewer and less abundant components in amaranth and soybean. It was shown the prolamin-like and prolamin fractions were completely different (Fig. 1a), but the glutelin fractions showed some similarity in the same plants (Fig. 1b).

Microstructure

It is known that protein structure and distribution vary in the kernels of the various cereals and between varieties of cereals and pseudocereals (Gorinstein *et al.*, 1991; Hamaker *et al.*, 1995; Fadel *et al.*, 1996; Autio & Salmenkallio-Marttila, 2001). Cereal-based foods are derived from grains that have a well-organized microstructure (Batterman-Azcona & Hamaker, 1998; Autio & Salmenkallio-Marttila, 2001). The microstructure determines the appearance and texture of protein fractions and the stability of the final product. Therefore it was important to reveal the similarities and the differences between identically extracted protein fractions from pseudocereal (amaranth), legume (soybean) and cereal (maize) in order to find useful substitutes for cereal.

The TASP have different morphology as observed by SEM (Fig. 2). However, the fine structures of TASP from *A. hypochondriacus* and soybean extracted by the same solvent system were similar (Fig. 2a–d). These results are in agreement with others who have shown that amaranthus seed protein bodies are morphologically similar to types of legumes (Konishi *et al.*, 1995).

Maize (Fig. 2e and f) differed from the pictures shown for amaranth and soybean, which consisted of large particles irregular in shape with a rough surface texture. The morphology of maize prolamins (Fig. 2e and f) differs from the other proteins described. Maize powder contains small spherical particles of regular shape with a smooth surface. Similar morphological features were observed for recombinant human deoxyribonuclease powder, which was obtained in the same way as in our experiments (Chan *et al.*, 1997). It is known that the processing, such as milling, causes microstructural changes in proteins and influences the fine microstructure. Therefore the samples were ground and then extracted under the same experimental conditions.

In our previous research different solvent systems were used to extract the alcohol-soluble proteins (Gorinstein *et al.*, 1991). The most optimal extraction was achieved with 55% IP contained 4–5% of reductant (2-ME). Therefore in this research the samples of plants that were investigated were prepared in this way. Our results are in agreement with those in the literature, showing that this efficient procedure extracts higher amounts of prolamin than the conventional method. Proteins extracted with 55% 2-propanol plus reductant were made up of α-, β-, γ- and δ-zeins (Landry *et al.*, 2000), using other solvents for extraction gave a preparation where prolamins were found as a minor fraction.

The effect of 2-ME on the morphology of TASP (Fig. 2) is in correspondence with other reports (Bugusu *et al.*, 2002), where density and the size of particles decreased as the result of destruction of disulfide bonds. These results are in accordance

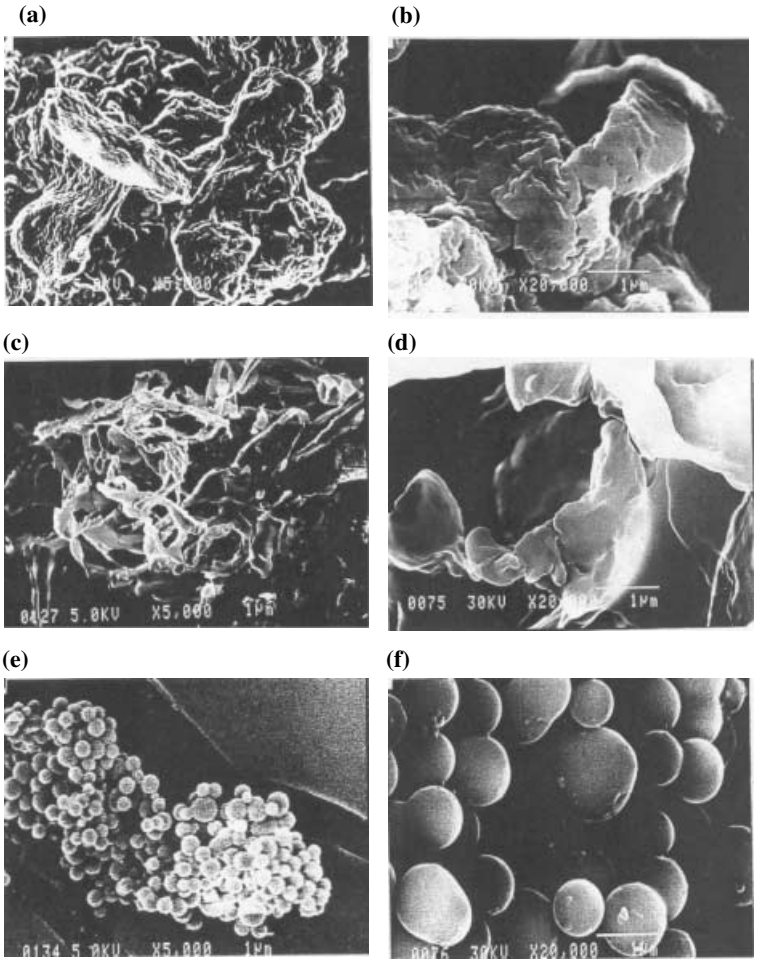

Figure 2 Microstructure of total alcohol-soluble proteins (TASP) from amaranth, soybean and maize: (a) and (b), amaranth; (c) and (d), soybean; (e) and (f), maize (prolamins); (a, c and c), magnification ×5000; (b, d and f), magnification ×20 000.

with the data on gels formed from plant proteins that demonstrated reduction in gel hardness upon action of 2-ME (Ker *et al*., 1993; Yuno-Ohto *et al*., 1994). Total glutelins (TGlu) extracted with 2-ME from *A. hypochondriacus* (Fig. 3a and b), soybean (Fig. 3c and d) and maize (Fig. 3e and f) demonstrate similarity in morphology (Konishi *et al*., 1995), containing large plates of irregular form with a smooth surface. Saline-soluble glycinins and insoluble glutelins are the major storage proteins in soybean and amaranth (Katsube *et al*., 1999).

Electrophoretic and functional properties indicated a significant correlation between soluble protein fractions from soybean and amaranth. The protein fractions shared some common electrophoretic bands as well as a similar amino acid composition (Gorinstein *et al*., 2001). In this report we have shown that the similarity in pseudocereal and legume was found also in other protein fractions (alcohol- and buffer-soluble). True glutelins from maize were extracted in the same way as for legume and pseudocereal (Landry *et al*., 2000). Our results are in accordance with others that amaranth is a valuable resource, containing a higher level of protein of better or similar quality than cereals like maize and may play an important role in human diets (Bressani *et al*., 1993; Yanez *et al*., 1994; Dodok *et al*., 1997; Gorinstein *et al*., 2001).

Conclusion

Electrophoresis of the different protein classes in polyacrylamide gels on sodium dodecyl sulfate-containing media was used to separate and char-

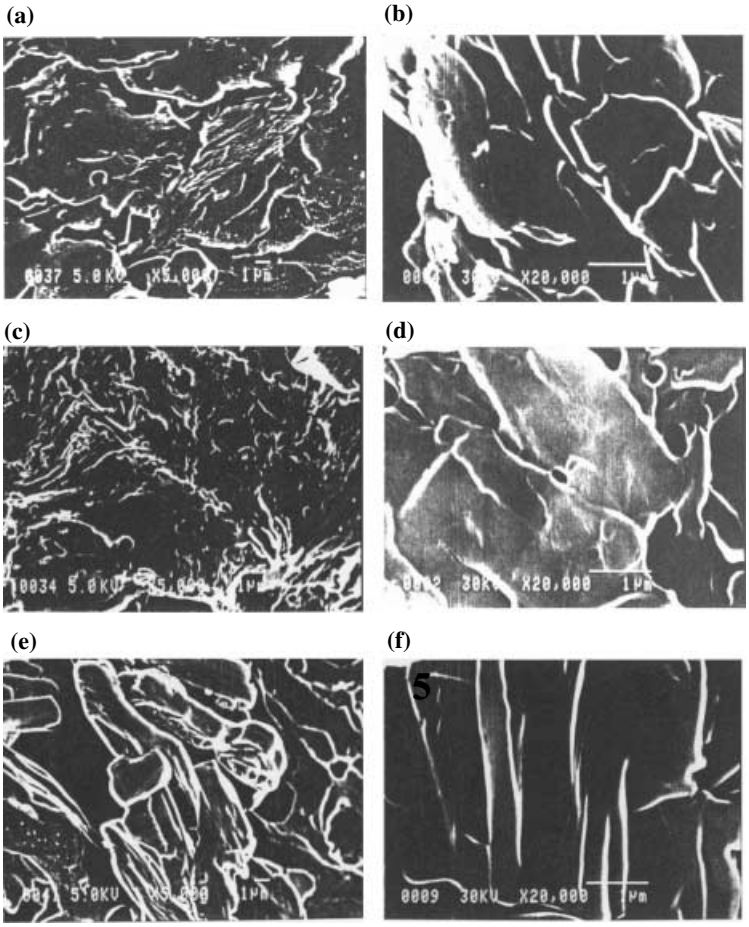

Figure 3 Microstructure of total glutelin (TGlu) from amaranth, soybean and maize: (a) and (b), amaranth; (c) and (d), soybean; (e) and (f), maize; (a, c and e), magnification ×5000; (b, d and f), magnification ×20 000.

acterize individual proteins. SEM data are consistent with the results of electrophoresis and can be used in addition to other analytical methods for analysis and comparison of different plant proteins. Apparently, correlation between electrophoretic patterns and microstructure of proteins exists and a close identity between amaranth and soybean was found. Based on glutelin electrophoretic patterns it can be postulated that the amaranth plant can be used as a substitute for several cereals or as a part of a mixture rich in food nutrients.

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References

Andrasofszky, E., Szocz, Z., Ferete, S. & Jelenits, K. (1998). Evaluation of the nutritional value of the amaranth plant – I. Raw and heat-treated grain tested in experiments on growing rats. *Acta Veterinaria Hungarica*, **46**, 47–59.

Armentia, A., Banuelos, C., Arranz, M.L. *et al.* (2001). Early introduction of cereals into children's diets as a risk factor for grass pollen asthma. *Clinical Experimental Allergy*, **31**, 1250–1255.

Autio, K. & Salmenkallio-Marttila, M. (2001). Light microscopic investigations of cereal grains, doughs and breads. *Lebensmittel-Wissenschaft und-Technologie-Food Science and Technology*, **34**, 18–22.

Batterman-Azcona, S.J. & Hamaker, B.R. (1998). Changes occurring in protein body structure and alpha-zein during cornflake processing. *Cereal Chemistry*, **75**, 217–221.

Bressani, R., Demartell, E.C.M. & Degodinez, C.M. (1993). Protein-quality evaluation of amaranth in adult humans. *Plant Foods Human Nutrition*, **43**, 123–143.

Bugusu, B.A., Rajwa, B. & Hamaker, B.R. (2002). Interaction of maize zein with wheat gluten in composite

dough and bread as determined by confocal laser scanning microscopy. *Scanning*, **24**, 1–5.

Chan, H.K., Clark, A., Gonda, I., Mumenthaler, M. & Hsu, Ch. (1997). Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. *Pharmacological Research*, **14**, 431–437.

Chavez-Jauregui, R.N., Silva, M.E.M.P. & Areas, J.A.G. (2000). Extrusion cooking process for amaranth (*Amaranthus caudatus* L.). *Journal of Food Science*, **65**, 1009–1115.

Dodok, L., Modhir, A.A., Buchtova, V., Halasova, G. & Polacek, I. (1997). Importance and utilization of amaranth in food industry. 2. Composition of amino acids and fatty acids. *Nahrung*, **41**, 108–110.

Enamuthu, J., Crites, S.G. & Swanson, B.G. (1993). Microstructure of black, green and red gram. *Food Structure*, **12**, 155–162.

Fadel, J.G., Pond, W.G., Harrold, R.L., Calvert, C.C. & Lewis, B.A. (1996). Nutritive value of three amaranth grains fed either processed or raw to growing rats. *Canadian Journal of Animal Science*, **76**, 253–257.

Gorinstein, S., Arnao De Nue, I. & Arruda, P. (1991). Alcohol-soluble and total proteins from amaranth seeds and their comparison with other cereals. *Journal of Agricultural and Food Chemistry*, **39**, 848–850.

Gorinstein, S., Zemzer, M., Fliess, A. *et al.* (1998). Computational analyses of the amino acid residue sequences of amaranth and some other proteins. *Bioscience Biotechnology Biochemistry*, **62**, 1845–1851.

Gorinstein, S., Jaramillo, N.O., Medina, O.J., Rodrigues, W.A., Tosello, G.A. & Parades-López, O. (1999). Evaluation of some cereals, plants and tubers through protein composition. *Journal of Protein Chemistry*, **18**, 687–693.

Gorinstein, S., Delgado-Licon, E., Pawelzik, E. *et al.* (2001). Characterization of soluble amaranth and soybean proteins based on fluorescence, hydrophobicity, electrophoresis, amino acid analysis, circular dichroism, and differential scanning calorimetry measurements. *Journal of Agricultural and Food Chemistry*, **49**, 5595–5601.

Hamaker, B.R., Mohamed, A.A., Habben, J.E., Huang, C.P. & Larkins, B.A. (1995). Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. *Cereal Chemistry*, **72**, 583–588.

Katsube, T., Kurisaka, N., Ogawa, M. *et al.* (1999). Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiology*, **120**, 1063–1073.

Ker, Yi.-Ch., Chen, R.H. & Wu, Ch.Sh. (1993). Relationships of secondary structure, microstructure and mechanical properties of heat-induced gel of soy 11S globulin. *Bioscience Biotechnology Biochemistry*, **57**, 536–541.

Konishi, Y., Nakamara, R., Sugimoto, Y. & Fujimori, K. (1995). Transmission electron microscopy of protein bodies in the *Amaranthus cruentus* seed embryo. *Bioscience Biotechnology Biochemistry*, **59**, 2140–2142.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

Landry J., Delhaye S. & Damerval, C. (2000). Improved method for isolating and quantitating alpha-amino nitrogen as nonprotein, true protein, salt-soluble proteins, zeins, and true glutelins in maize endosperm. *Cereal Chemistry*, **77**, 620–626.

Mandal, S. & Mandal, R.K. (2000). Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. *Current Science*, **79**, 576–589.

Matuz, J., Poka, R., Boldizsar, I., Szerdahelyi, E. & Hajos, G. (2000). Structure and potential allergenic character of cereal proteins. II. Potential allergens in cereal samples. *Cereal Research Communication*, **28**, 433–442.

Nkonge, C. & Balance, G.M. (1982). A sensitive colorimetric procedure for nitrogen determination micro-Kjeldahl digests. *Journal of Agricultural and Food Chemistry*, **30**, 416–420.

Okita, T.W., Krishnan, H.B. & Kim, W.T. (1988). Immunological relationships among the major seed proteins of cereals. *Plant Science*, **57**, 103–111.

Seguranieto, M., Vazquezsanchez, N., Rubiovelazquez, H., Olguinmartinez, L.E., Rodrigueznester, C.E. & Herreraestrella L. (1992). Characterization of amaranth (*Amaranthus hypochondriacus* L.) seed proteins. *Journal of Agricultural and Food Chemistry*, **40**, 1553–1558.

Shewry, P.R., Beaudoin, F., Jenkins, J., Griffiths-Jones, S. & Mills, E.N.C. (2002). Plant protein families and their relationships to food allergy. *Biochemical Society Transactions*, **30**, 906–910.

Wall, J.S., Cooker, L.A. & Bietz, J.A. (1988). Structure and origin of maize endosperm alcohol-insoluble glutelin. *Journal of Agricultural and Food Chemistry*, **36**, 722–728.

Wright D.J. (1987). The seed globulins. In: *Developments in Food Proteins-5, Applied Science*, Chapter 3 (edited by B.J.F. Hudson). Elsevier.

Yanez, E., Zacarias, I., Granger, D., Vasquez, M. & Esteve, A.M. (1994). Chemical and nutritional characterization of amaranthus (amaranthus-cruentus). *Archivos Latinoamericanas de Nutricion*, **44**, 57–62.

Yuno-Ohto, N., Maeda, H., Okada, M. & Ohta, H. (1994). Heat-induced gels of rice globulin: comparison of gel properties with soybean and sesame globulins. *Journal of Food Science*, **59**, 366–370.