

Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses

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Abstract: Maize, wheat, amaranth, rice and soybean were screened for protein content. Alcohol-soluble (A1 and A2) and glutelin (G1 and G2) fractions were isolated and compared in terms of their amino acid and protein compositions. The average proportions of nitrogen content between total alcohol-soluble proteins (TASP) and total glutelins (TGlu) in the pseudocereals amaranth and soybean were about 1.8:26.9 and 14.9:12.3 respectively. In the cereals maize and wheat these proportions were 47.8:33.2 and 44.7:31.2 respectively. The sum of essential amino acids was 47.6 and 60.3 g per 100 g protein in amaranth and soybean respectively. The highest contents of methionine, lysine and arginine were found in the pseudocereals. The relatively high content of essential amino acids shows that pseudocereals could be used as a nutrient substitute for cereals.

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Keywords: cereals; pseudocereals; proteins; amino acids

INTRODUCTION

Allergens and enzyme inhibitors are well known in cereals. A gliadin fraction from wheat causes coeliac disease. Proteins from wheat, rice, maize and barley may give rise to allergic reactions such as atopic dermatitis and asthma.¹ These components are not found in pseudocereals and legumes such as amaranth and soybean. Therefore these plants might be used as substitutes for cereals in gluten-free diets.² Furthermore, pseudocereals contain relatively high amounts of dietary fibre, which improves lipid metabolism and takes part in the prevention of LDL-C oxidation.^{3,4} After processing, these plants can be used as flours or flakes or in biscuits and breakfast foods.^{2,5} However, protein structure is sensitive to high temperatures, changes in pH and action of proteolytic enzymes. All these factors affect proteins during various stages of food and pharmaceutical preparation as well as during action/release in the human body.

The nutritional value of pseudocereals is mainly connected to their proteins. Proteins are an important group of biomacromolecules that are involved in physiological functions.⁶ Natural vegetable proteins are useful materials owing to their safeness, high biocompatibility, nutritional value and low cost.

Finding new vegetable proteins rich in essential amino acids is important for the food and pharmaceutical industries.

Amaranth and quinoa produce significant amounts of edible grain, especially amaranth, which is described as the 'grain of the 21st century'.^{7–11} Both amaranth and quinoa are good sources of minerals and vitamins and they contain larger amounts than most of the common cereal grains.^{8,12} The protein content of amaranth is about 16%.⁷ The nutritional quality of amaranth proteins is also very high in comparison to cereals and some legumes.¹³ Amaranth has high contents of lysine, arginine, tryptophan and sulphur-containing amino acids.^{7–9} The lysine content of amaranth is twice that of wheat and three times that of maize. The nutritive value is about 75, compared with values of 44, 57 and 62 for maize, wheat and barley respectively.¹⁰ On the other hand, protein content and amino acid composition depend on genotype and growing conditions.^{8,14,15}

Alcohol-soluble prolamins represent the major storage proteins in cereals such as maize and wheat.¹⁶ In some plants, however, prolamins are not the major storage proteins. Globulins are accumulated in oats, legumes and tubers, and glutelins in rice, as their

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major storage proteins.^{16–20} It has been shown that oat and amaranth globulins and rice glutelin are highly homologous and are related to the legume 11S storage proteins.^{20–23} Most of the recent investigations have been focused on the main storage protein fractions such as prolamins in cereals and globulins in pseudocereals,^{9,24–29} with emphasis on nutritional, structural and functional properties. To the best of our knowledge, there have been no studies on the differences and identity of isolated seed protein fractions such as alcohol-soluble proteins and glutelins of amaranth, soybean, wheat, rice and maize. This paper reports the distribution of protein fractions from cereals and pseudocereals as established by solvent extraction. The characterisation is based on amino acid and protein analyses.

MATERIALS AND METHODS

Sample preparation

Whole mature seeds of amaranth (*Amaranthus hypochondriacus*), soybean, maize, rice (*Oryza sativa*) and wheat (*Triticum aestivum* L) were investigated. Seeds were ground in a mill through a 60-mesh screen. The resulting meal was defatted in a Soxhlet extractor with *n*-hexane for 10 h and then stored at 5 °C after removal of hexane.

Protein extraction

Proteins were extracted stepwise according to the following methods.^{16,23,30} 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) under vigorous shaking. The extracts were separated by

centrifuging at $10\,000 \times g$ for 10 min. Each step was repeated twice. The sequence of solvents used was the following: alcohol-soluble subfractions: 55% (v/v) isopropanol (IP)—alcohol-soluble A1; 55% (v/v) IP containing 4% (v/v) 2-mercaptoethanol (2-ME)—alcohol-soluble A2; glutelins: 0.125 M sodium borate buffer (pH 10) containing 3% (v/v) 2-ME—glutelin G1; then with the same solvent plus 0.5% (w/v) sodium dodecyl sulphate (SDS)—glutelin G2. Total glutelins (TGlu) were extracted with 0.125 M sodium borate buffer (pH 10) containing 3% (v/v) 2-ME plus 1% (w/v) SDS. Total alcohol-soluble proteins (TASP) were extracted with 55% (v/v) IP containing 4% (v/v) 2-ME. In order to compare and to find the optimum conditions for alcohol-soluble subfractions, the extraction was also done with 70% (v/v) IP containing 4% (v/v) 2-ME—alcohol-soluble A2. The nitrogen content in each fraction was determined by the micro-Kjeldahl method combined with colorimetric determination.³¹

Amino acid analysis

Samples were hydrolysed with 6 M HCl and 3% phenol solution in an MLS-MEGA microwave system (MLS GmbH, Leutkirch, Germany) for 20 min at 160 °C. The power was set at 1000 W for the first 5 min and 500 W for the remaining 15 min. The vacuum-dried samples were then dissolved in 100 µl of 20 mM HCl and filtered through a 0.45 µm filter. Derivatisation was done with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate.³² The sample was injected into a Multi-Pump Gradient Waters (Dr Ing Herbert Knauer GmbH, Berlin, Germany) HPLC system with a B1184742 vertex Knauer column (150 mm

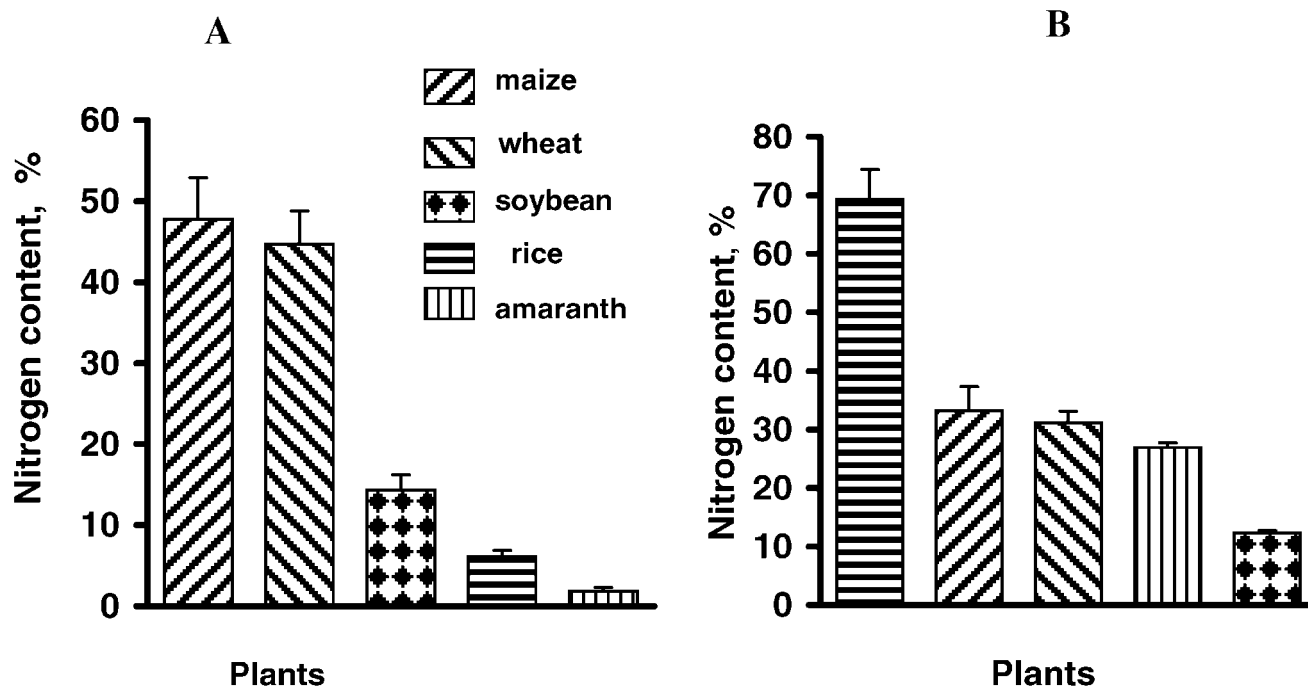


Figure 1. Nitrogen contents of (A) alcohol-soluble and (B) glutelin fractions from maize, wheat, soybean, rice and amaranth. Mean \pm standard deviation (vertical lines).

length \times 4.6 mm id, spherimage-80=DS 2–5 μ m). A Millennium 2010 chromatography manager system (Waters Corporation, Milford, MA, USA) was used to evaluate the amino acids. The scanning fluorescence detector was used at an excitation wavelength of 250 nm and an emission wavelength of 395 nm. The gradient programme consisted of 40% acetate phosphate buffer and 60% acetonitrile.

The values of tyrosine and cysteine were added and presented as a total value. Results are given as g per 100 g protein.

Statistics

To verify the statistical significance of the measured parameter means, their 95% confidence intervals of three times analysed samples \pm SD were defined; p values <0.05 were considered significant.

RESULTS AND DISCUSSION

Protein content

The nitrogen contents of the total alcohol-soluble protein (TASP) and total glutelin (TGlu) fractions from maize, wheat, amaranth, rice and soybean are presented in Fig 1. The amount of TASP was higher using extraction with 55% IP than 70% IP containing 4% (v/v) 2-ME. The same results were obtained when the glutelins (Fig 1) were extracted with a higher concentration of SDS.²³ The average proportions of nitrogen content between TASP and TGlu in the pseudocereals amaranth and soybean were about 1.8:26.9 and 14.9:12.3 respectively. In the cereals maize and wheat these proportions were 47.8:33.2 and 44.7:31.2 respectively. Based on these data, it can be concluded that not only the globulin fraction is a major storage protein,³³ but also the glutelin fraction can be

suggested as one of the major proteins in pseudocereals.

Alcohol-soluble proteins (prolamin-like) contained about 2% of the total nitrogen in pseudocereals and 15% in legumes. It can be concluded that the prolamin-like fraction of amaranth is not a storage protein. The apparent paucity of nitrogen in the alcohol-soluble protein fraction of amaranth can be explained by the fact that this fraction is a minor one in comparison with cereal plants.^{16,21,22} Maize, wheat and other cereal prolamins contain about 45% of the total nitrogen.^{16,23} As described above, the prolamins of all plants were extracted with a solvent (55% IP+5% 2-ME)/sample ratio of 6:1, as used in previous studies.¹⁶ The optimum procedure of extraction was with a (55% IP+5% 2-ME)/sample ratio of 6:1. The extraction depends on the milling of the seed, the sequence in which the solvents are used, the temperature and the reducing agent. Therefore only some data shown in Fig 1 corresponded with other results.^{16,21–24}

Amino acid analysis

The results of the amino acid analysis of the amaranth and soybean fractions are shown in Figs 2–4.

The sum of essential amino acids was 47.6 and 60.3 g per 100 g protein in amaranth and soybean respectively, ie the essential amino acid concentration was higher in soybean than in amaranth. Amaranth total protein showed significantly higher ($p < 0.05$) Glu, Gly and Met concentrations than soybean, whilst the non-essential amino acid Tyr+Cys and the essential amino acids Ile, Leu and Phe were significantly higher ($p < 0.05$) in soybean than in amaranth (Fig 2). Both amaranth and soybean could supply preschool child and adult requirements of Ile, Leu,

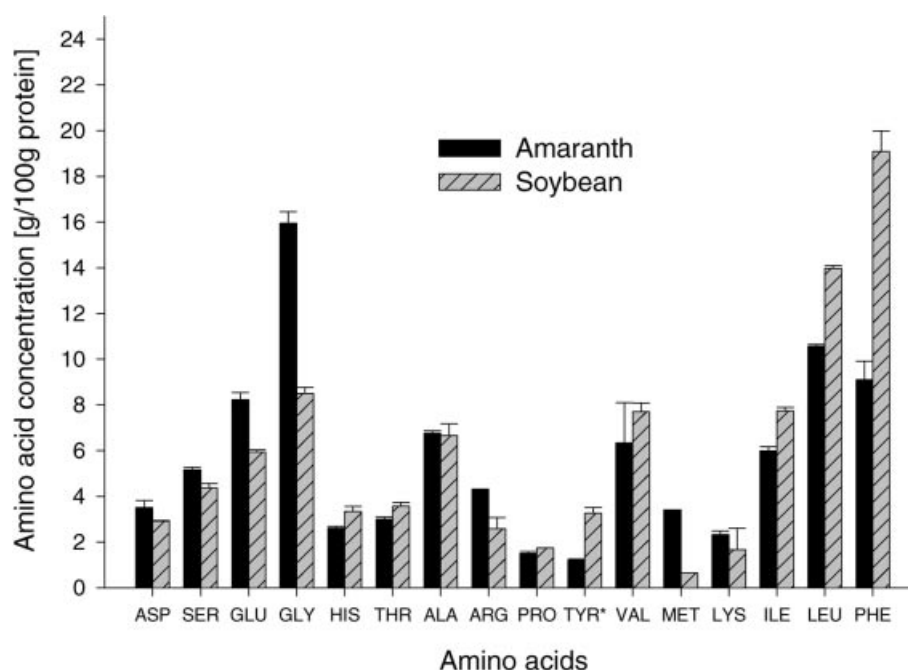


Figure 2. Amino acid compositions of amaranth and soybean proteins. Bars indicate standard error. * = Tyr + Cys.

Phe, Val and His, as well as Met in amaranth and Thr in soybean. The requirement of Lys for adults but not for preschool children could be supplied by amaranth and soybean protein. Low concentrations of Cys eluted most of the time along with Tyr; for this reason they are presented together as Tyr in Figs 2–4.

Alcohol-soluble proteins A1 from amaranth and soybean showed nearly the same amounts of essential amino acids such as valine, isoleucine, leucine and methionine. Phenylalanine was as much as 2.5 times higher in soybean than in amaranth. Amaranth prolamin-like A1 had significantly higher ($p < 0.05$) Ser and Thr concentrations than soybean (Fig 3). Glu, Gly, His, Ala, Tyr, Met and Lys in soybean A1 were higher ($p < 0.05$) than in amaranth A1 (Fig 3).

In prolamin-like A2 fractions from amaranth and soybean, valine, isoleucine, leucine and phenylalanine were similar. The amount of lysine was relatively high

in A1 and A2 in comparison with cereals. Amaranth and soybean prolamin-like A2 fractions had zero and very low Met concentrations respectively, but the difference was not significant (Fig 3).

Glutelin fractions G1 and G2 from amaranth and soybean showed even more identical amino acid compositions in terms of valine, leucine, lysine and phenylalanine, in accordance with others.^{21,22,33}

Ala and Met concentrations were higher ($p < 0.05$) for amaranth than for soybean in the glutelin G1 fraction, whilst Lys and Ile were higher ($p < 0.05$) for amaranth than for soybean in the glutelin G2 fraction (Fig 4). Glutelins G2 showed high concentrations of Leu for amaranth and Phe for amaranth and soybean (Fig 4). The protein content and amino acid composition of our results are in accordance with others.^{21,22,33} It was shown that, in comparison with wheat (13.5–14.5%), maize (10.6–13.8%), barley (10–14.9%) and

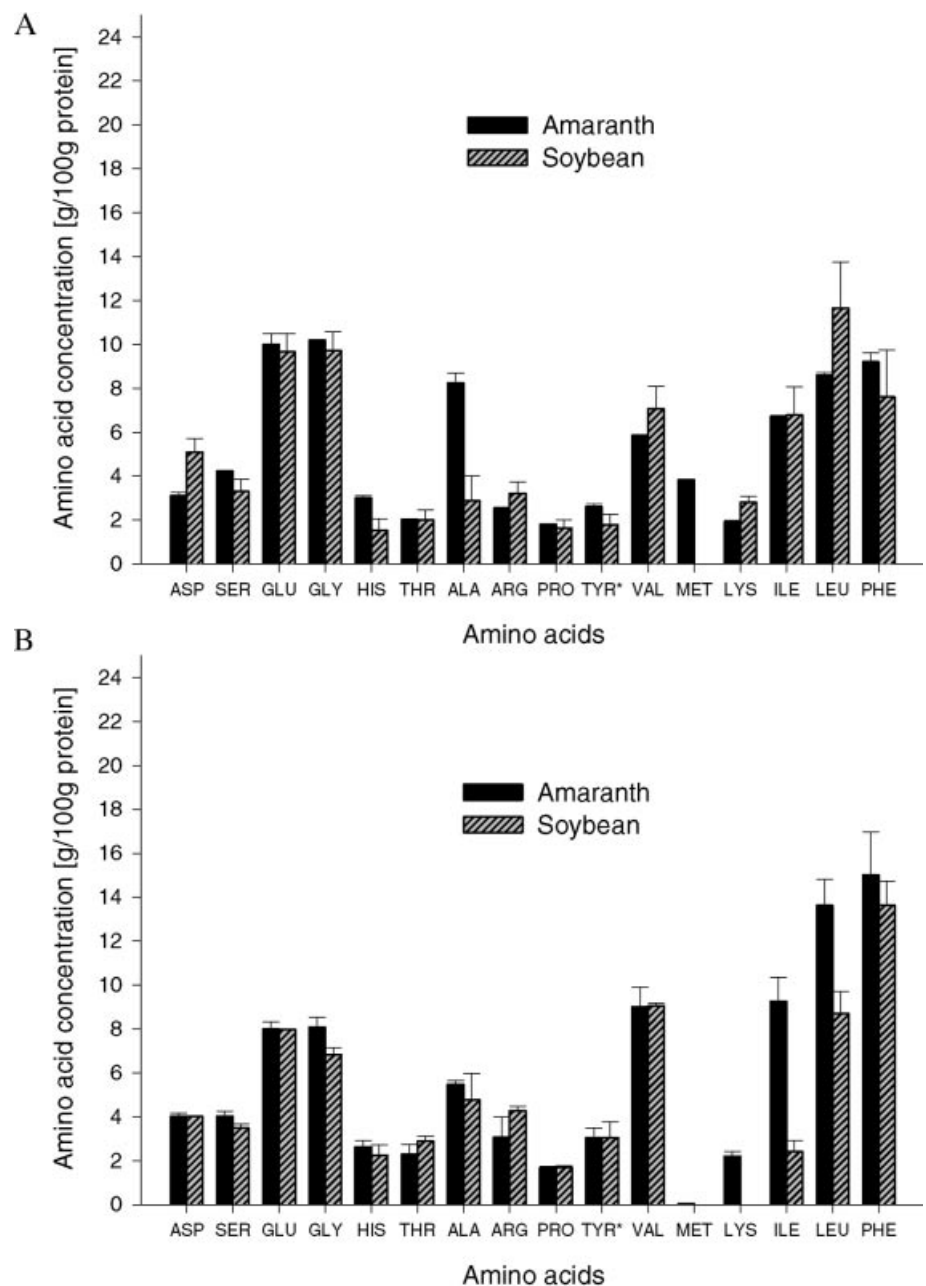


Figure 3. Amino acid compositions of amaranth and soybean (A) prolamin-like A1 and (B) prolamin-like A2 fractions. Bars indicate standard error. * = TYR + CYS.

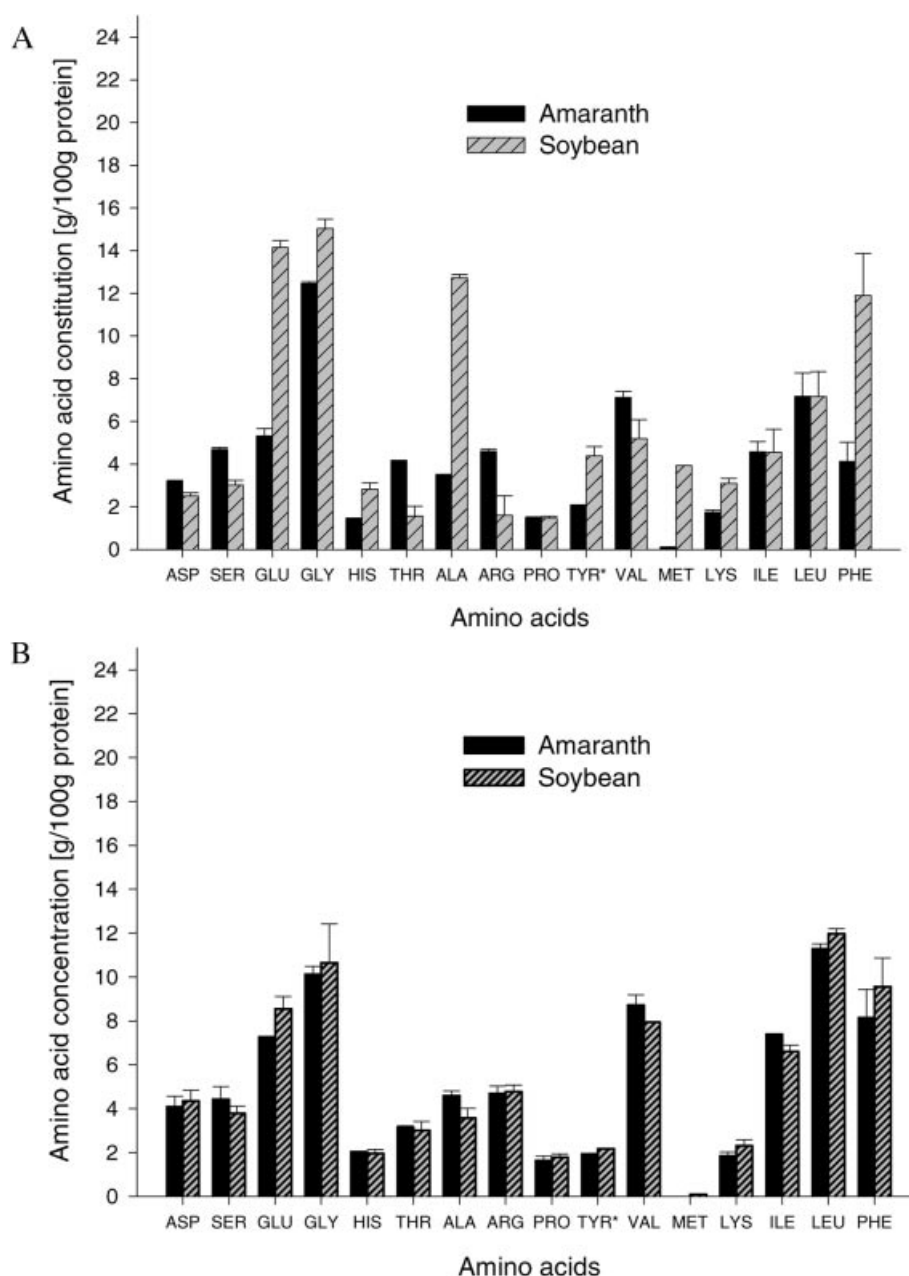


Figure 4. Amino acid compositions of amaranth and soybean (A) glutelin G1 and (B) glutelin G2 fractions. Bars indicate standard error. * = TYR + CYS.

oats (12.4–12.9%), amaranth had a high protein content (15.4%) with a favourable amino acid composition.^{34–36} The highest methionine, lysine and arginine were found in pseudocereals.³⁶ Pseudocereals and soybean have a high nutritional value due to the balanced amino acid composition of their proteins. These plants are highly nutritive^{34,35} and composed presumably of easily digestible albumins and globulins (about 50%) and glutelins (about 31%). Our results suggest that the poor methionine and lysine contents of cereals could be supplemented by adding amaranth and soybean to the diet.

CONCLUSION

This paper presents the extraction and characterisation of the protein fractions from cereals and pseudocereals. The plants were evaluated for their protein

fractions in terms of average proportions and relative amounts. Apparently, a correlation between amino acid composition and nutritional value of proteins exists, and a close identity between amaranth and soybean was found. Based on its rich protein and amino acid compositions, amaranth could be a nutritive substitute for cereals and improve value in different diets.

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