# Evaluation of Four *Amaranthus* Species through Protein Electrophoretic Patterns and Their Amino Acid Composition

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Amaranth seed proteins of four species, *Amaranthus cruentus*, *A. flavus*, *A. caudatus*, and *A. hypochondriacus*, were fractionated as albumins and globulins, alcohol-soluble proteins A1 and A2, and glutelins G2 and G3. Their average values were 61.3, 1.4, and 24.1, respectively. The main protein subunits have molecular masses of between 10 and 45 kDa. Variations, found for some minor bands, were also detected by amino acid analysis. Albumins, globulins, and glutelin G3 have much higher lysine contents than the alcohol-soluble and glutelin G2 protein fractions. Globulins were only intermediate in comparative contents of methionine and cystine.

## INTRODUCTION

The seed varieties of the Amaranthaceae family originated from Central and South America and the vegetable ones from southeast Asia (Saunders and Becker, 1984; Stone and Lorenz, 1984). The main species are *Amaranthus cruentus* and *A. hypochondriacus* (Konishi et al., 1985b; Teutonico and Knorr, 1985). Amaranth had been cultivated as minor group crops in Latin America: *A. caudatus* in Argentine, Peru, and Brazil; *A. hypochondriacus* in Mexico (Bressani et al., 1987; Kauffman and Haas, 1983; Teutonico and Knorr, 1985).

The amaranth seed containing starch, proteins, amino acids, lipids, minerals, and vitamins has potential in the future for food and feed resources (Konishi et al., 1985a,b; Sugimoto et al., 1981; Teutonico and Knorr, 1985). There is a great deal of work on its chemical composition, protein and amino acid content, and nutritional value, including feeding tests (Afolabi and Oke, 1981; Becker et al., 1981; Connor et al., 1980; Correa et al., 1986; Pandey and Pal, 1985; Pant, 1985). In addition, Konishi et al. (1985a) and Abdi and Sahib (1976) have characterized globulin isolated from seed of *A. hypochondriacus*. Correa et al. (1986) showed some results on amino acids composition of proteins in *Amaranthus* grain.

To our knowledge there are no data available in the literature about the features of diverse protein fractions isolated from main species of Amaranth seeds. This paper deals with the relative amounts, molecular polymorphism, and amino acid composition of the protein fractions present in Amaranth seeds.

## MATERIALS AND METHODS

**Sample Preparation.** Whole mature seeds of *A. cruentus* (purple), *A. cruentus* (yellow), *A. flavus* (black), *A. caudatus* (yellow-brown), and *A. hypochondriacus* (yellow) were used in this study.

Seeds of *Amaranthus* were ground on a mill through a 32-mesh screen. The flour was defatted with cold acetone (10 mL/g) for 48 h at −20 °C and then air-dried. The defatted flour was milled through a 60-mesh screen and stored at 4 °C until use.

Amaranth protein fractions were isolated with the same sequence of solvents developed by Landry and Moureaux (1970). Some modifications in duration of extraction were applied. The meal (1 g) was extracted with a solvent/sample ratio of 10/1 (v/w) and vigorously shaken. The extracts were isolated by centrifuging at 10000*g* for 10 min. Each step was repeated twice.

The solvent sequence and the isolated proteins were the following: 0.5 M NaCl, water [albumins (Alb) and globulins (Glo)]; 70% (v/v) 2-propanol [alcohol-soluble proteins A1 (ASP A1)]; 70% 2-propanol containing 0.6% (v/v) 2-mercaptoethanol (2-ME) [alcohol-soluble proteins A2 (ASP A2)]; 0.01 M sodium carbonate buffer (pH 10) containing 0.6% (v/v) 2-ME [glutelin G2 (Glu G2)] and then the same solvent plus 0.5% (w/v) sodium dodecyl sulfate (SDS) [glutelin G3 (Glu G3)].

Globulin was separated from albumin by dialysis against water at 4 °C for 72 h.

The nitrogen content in each fraction was determined by micro-Kjeldahl method, combined with a colorimetric determination (Nkonge and Ballance, 1982). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the procedure of Laemmli (1970), using both homogeneous (15% w/v) and gradient (10–20% w/v) gels. The amount of protein applied to sample slots was 50 μg. Gels were stained with 0.25% Coomassie Brilliant Blue R250 in 2-propanol/water/acetic acid (1/8/1 v/v/v) and destained in the same solvent. Standards obtained from Sigma Chemical Co. of 14, 20, 24, 29, 36, 45, and 60 kDa were used for the molecular weight estimation of protein subunits.

Freeze-dried samples were hydrolyzed with 6 N HCl in sealed tubes for 20 h at 110 °C with and without previous oxidation with performic acid. The vacuum-dried hydrolysate was analyzed and applied on a Beckman 120 C automatic amino acid analyzer (Spackman et al., 1958). For tryptophan determination samples were hydrolyzed with 4 N LiOH for 24 h at 110 °C followed by treatment with 6 N HCl for 22 h at the same temperature. Tryptophan levels were also obtained by Spies W method (Gorinstein et al., 1988; Spies, 1967, 1968). Mean values of eight determinations are reported in this study. All statistical data were determined by Duncan's (1955) multiple range test.

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