

Alcohol-Soluble and Total Proteins from Amaranth Seeds and Their Comparison with Other Cereals

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The extractibility of alcohol-soluble proteins from different species of Amaranth seeds was studied as a function of water/ethanol (EtOH) and water/2-propanol (2-PrOH) mixtures at concentrations of 45-70%, respectively, with varying amounts of reducing agent 2-mercaptoethanol (2-ME) from 0 to 5%. Most alcohol-soluble proteins were extracted with 55% 2-PrOH/5% 2-ME. On the basis of the results of different extraction methods and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), alcohol-soluble proteins of Amaranth contain 80-85% polypeptides of 10-14 kDa and 7% 20-kDa polypeptides, the rest being minor fractions. Only slight differences were observed in subunits of four Amaranth species. Prolamins and total proteins extracted from oats, rice, maize, and sorghum did not show any electrophoretic relationship with Amaranth alcohol-soluble fractions and total proteins.

INTRODUCTION

The major storage proteins in most cereal grains are the alcohol-water-soluble prolamins, which are extracted by alcoholic solution with or without the addition of reducing agents (Esen, 1986; Gorinstein et al., 1983; Kreis et al., 1985; Landry and Moureaux, 1970, 1980).

Prolamins are the major storage proteins in wheat, barley, rye, maize, and sorghum (Bietz, 1982; Kreis et al., 1985). Oats and rice have globulins and glutelins, respectively, as major proteins, but small amounts of prolamins are also present (Juliano, 1972; Kim et al., 1978; Padhye and Salunkhe, 1979; Peterson, 1978; Villareal and Juliano, 1978; Yamagata et al., 1982).

Amaranth, a member of the Amaranthaceae family, is an important new source of food and feed (Betschart et al., 1981; Correa et al., 1986; Konishi et al., 1985). The amount of alcohol-soluble proteins in Amaranth is low as in oats and rice (Kim et al., 1978; Konishi et al., 1985; Padhye and Salunkhe, 1979). Although alcohol-soluble proteins were found in Amaranth species (Correa et al., 1986; Gorinstein et al., 1991; Konishi et al., 1985), they have not been studied as well as other protein fractions. Albumins, globulins, and glutelins contain most of the total nitrogen, and globulins are the major storage proteins in Amaranth (Konishi et al., 1985). Only about 2% of total nitrogen was found in the alcohol-soluble fraction of different species of Amaranth (Gorinstein et al., 1991; Konishi et al., 1985).

We present here a paper on alcohol-soluble and total Amaranth proteins. We demonstrate different extraction methods for alcohol-soluble Amaranth seed proteins and compare the proteins with prolamins of other cereals such as maize, sorghum, oats, and rice.

MATERIALS AND METHODS

Sample Preparation. Whole mature seeds of Amaranth [*Amaranthus (A.) cruentus* (cru'), *A. flavus* (fla), *A. caudatus* (cau), *A. hypochondriacus* (hyp), and *A. cruentus* (cru'')] were investigated. Cru', fla, cau, and hyp were grown in Brazil and were given to us by Dr. Alrindo Moreira Sales, Instituto de Tecnologia de Alimentos, Campinas, Brazil. Cru'' was grown in Mexico and was donated by Dr. A. Sanchez-Marroquin, National

Institute of Agricultural Research, Mexico. For comparison of prolamins we used sorghum with high tannin content, normal maize, oats, and rice. These cereals were stocked at the Plant Breeding Laboratory, Sementes, Agroceres, Brazil.

Amaranth and other seeds were ground on a mill with a 60-mesh screen and defatted in a Soxhlet extractor with *n*-hexane for 10 h. The meal was stored at 5 °C after removal of hexane. Extractability of alcohol-soluble proteins from Amaranth seeds was studied at 20 °C as a function of the alcohol content (45-70%), aqueous EtOH or 2-PrOH mixtures with 2-ME varying from 0 to 5%. Sixteen solvent systems were used (Table I), lines 1-6 and 8-16 respectively. Alcohol-soluble proteins were extracted according to the methods of Bietz (1982), Kim et al. (1978), Landry and Moureaux (1970, 1980), Okita et al. (1988), Padhye and Salunkhe (1977), Paulis (1981), Paulis and Wall (1979), and Yamagata et al. (1982) with changes in extraction time, concentration of reducing agent, and proportion of solvent to solid. Extraction of alcohol-soluble proteins was also done with other solvent systems (Table I, lines 7 and 17-28). Solvents described in Table I, lines 26 and 28, extracted alcohol-soluble proteins directly.

Extracts were combined, lyophilized, and dissolved in sample buffer which contained 10% glycerol, 5% 2-ME, and 2% SDS in 0.125 M Tris-HCl, pH 6.8. Then the extracts were boiled for 5 min before being loaded. Proteins were then precipitated with acetone (1:2 volumes) at -20 °C overnight, and the precipitate was dissolved in the same sample buffer.

Total proteins were extracted directly from whole meal with 0.125 M Tris-borate/5% SDS/2% 2-ME buffer, pH 6.8 (24:1 V/W). Samples were boiled for 5 min, cooled to room temperature, and centrifuged (Bietz and Sharma, 1983; Okita et al., 1988).

Alcohol-soluble proteins obtained by different extraction methods, as well as total proteins, were analyzed by SDS-PAGE according to the method of Laemmli (1970). The gels were 1.5 mm thick and consisted of a 2-cm stacking gel and a 10-cm running gel. The 5-20% and 10-15% acrylamide gradients were made from stock solutions of 0 and 30% acrylamide in 0.8% Bis and 0.1% SDS in 0.375 M Tris-HCl, pH 8.8. Fifty micrograms of protein was applied to sample slots. Electrophoresis was carried out at 100 V for 4 h. Gels were stained for 2 h with 0.25% Coomassie Brilliant Blue R (BDH Limited, Poole, England) in methanol/water/acetic acid (5:5:1 v/v) and destained in the same solvent. Molecular weight standards (Sigma Chemical Co.) were used to estimate protein subunit molecular weights according to the method of Plikaytis et al. (1986).