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 Technologia 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%-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).

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Table I. Solvent Systems Used To Extract Alcohol-Soluble Proteins from *A. hypochondriacus*

procedure	alcohol content in water or solvent, % v/v	2-ME, % v/v	ratios solvent/solid, v/w	amount of protein extracted, %
1[a]	55 2-PrOH	0	10:1	1.2
1[a']	55 2-PrOH	0.6	10:1	1.6
1[a'']	55 2-PrOH	5.0	10:1	2.1
2[b]	55 2-PrOH	0	6:1	1.0
2[b']	55 2-PrOH	2.0	6:1	1.9
2[b'']	55 2-PrOH	5.0	6:1	2.0
3[c]	60 1-PrOH/10 mM Tris-HCl/1 mM EDTA	0	20:1	0.9
4[d]	60 2-PrOH	0	10:1	1.1
4[d']	60 2-PrOH	0.1	10:1	1.2
5	60 2-PrOH	0	6:1	1.0
5'	60 2-PrOH	2.0	6:1	1.7
6[e]	70 2-PrOH	0	10:1	0.9
6[e']	70 2-PrOH	0.6	10:1	1.4
6[e'']	70 2-PrOH	1.0	10:1	1.6
6[e''']	70 2-PrOH	2.0	10:1	1.8
6[e'''']	70 2-PrOH	5.0	10:1	1.9
7[f]	45 EtOH	0	10:1	0.7
7[f']	45 EtOH	5.0	10:1	1.2
8[g]	70 EtOH	0	4:1	0.6
8[g']	70 EtOH	5.0	4:1	0.9
9	70 EtOH	0	5:1	0.6
9'	70 EtOH	5.0	5:1	1.2
10	70 EtOH	0	6:1	0.6
10'	70 EtOH	1.0	6:1	0.9
10''	70 EtOH	5.0	6:1	1.1
11[h]	70 EtOH	0	7:1	0.6
11[h']	70 EtOH	2.0	7:1	0.9
12	70 EtOH	0	20:1	0.6

[a'] Landry and Moureaux (1970, 1980). [b'] Bietz (1982). [c] Yamagata et al. (1982). [d'] Paulis and Wall (1979). [e'] Landry and Moureaux (1970). [f] Kim et al. (1978). [g] Padhye and Salunkhe (1977). [h'] Okita et al. (1988).

RESULTS AND DISCUSSION

Procedures in Table I were used to extract alcohol-soluble Amaranth proteins. Quantitative data of protein yield with different solvent systems show that the procedures of Bietz (1982), Landry and Moureaux (1970, 1980) and Paulis and Wall (1978) for the extraction of cereal prolamins were not effective for the extraction of alcohol-soluble Amaranth seed proteins. The methods of Kim et al. (1978), Okita et al. (1988), Padhye and Salunkhe (1977, 1979), and Yamagata et al. (1982) for the extraction of prolamins from rice and oats were not efficient for Amaranth alcohol-soluble proteins.

Gorinstein et al. (1991) demonstrated that 70% 2-PrOH/0.6% 2-ME extracted about 2% of the total nitrogen. These data are in agreement with those of Konishi et al. (1985). The maximum amount of alcohol-soluble proteins was extracted with 55% 2-PrOH/5% 2-ME (10:1 v/w) (Table I, line 3). Alcohol-soluble Amaranth proteins probably contain intermolecular disulfide bands as zein proteins in corn (Paulis, 1981). This explains why increased amounts of reducing agents gave maximum extraction.

However, Correa et al. (1986) showed that prolamin extracted from Amaranth by 70% EtOH according to the Padhye and Salunkhe (1977) method is about 11% of total proteins. In this study electrophoretic patterns of Amaranth proteins extracted by 55% 2-PrOH and 70% EtOH with varying amounts of 2-ME were compared. Patterns of proteins extracted with 70% 2-PrOH/0.6% 2-ME (Landry and Moureaux, 1970) were diffuse and weak (not shown) compared to those of alcohol-soluble proteins extracted by 55% 2-PrOH/5% 2-ME (sample:solvent = 1:6). These data agree with those Yamagata et al. (1982), showing that extraction with 60% 1-PrOH was at least twice as effective as 70% EtOH for rice prolamins. These

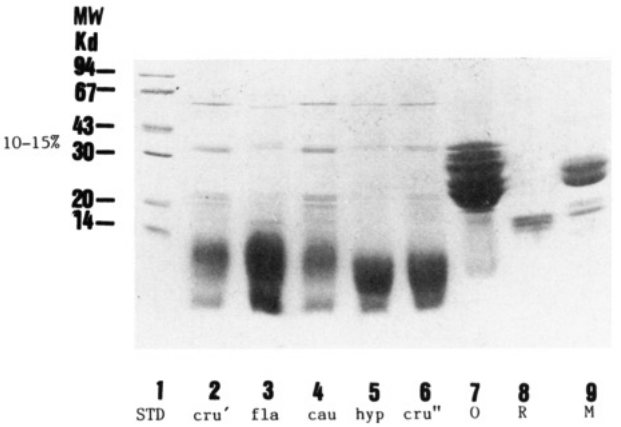

Figure 1. SDS–PAGE of alcohol-soluble proteins from different species of *Amaranth* seeds and cereals in gradient (10–15%) PAAG. Proteins were extracted from the meal directly with 70% EtOH/2% 2-ME (7:1 v/w). 1, Standard; 2–9, *A. cruentus* (cru'), *A. flavus*, *A. caudatus*, *A. hypochondriacus*, *A. cruentus* (cru''), oats, rice, and normal maize, respectively.

alcohol-soluble proteins were of about 14 kDa with minor subunits of 20, 30, and 65 kDa. But alcohol-soluble proteins extracted with the same solvent system using a ratio of sample to solvent of 1:10 and precipitated by acetone showed the main subunit of nonseparated proteins in the region of 10–14 kDa and a very sharp line of about 20 kDa.

Alcohol-soluble proteins that were extracted by 70% EtOH/2% 2-ME (Figure 1) had the same polypeptide composition in the main protein fraction of about 10–14 kDa with some minor subunits of 20, 30, and 65 kDa. The amount of these alcohol-soluble proteins was less than of those extracted with 55% 2-PrOH/5% 2-ME, but the separation of 70% EtOH/2% 2-ME proteins gave better resolution under the same conditions of electrophoresis. On the basis of the results obtained from different solvent systems, the alcohol-soluble proteins in Amaranth constituted 80–85% of nonseparated proteins of 10–14 kDa, 7% of 20 kDa, and the rest of minor subunits. *A. cruentus* (cru'), *A. flavus*, and *A. caudatus* showed similar protein patterns and differed from *A. hypochrondiacus* and *A. cruentus* (cru''). Prolamins, extracted from oats, rice, and normal maize by 70% EtOH/2% 2-ME, are shown in Figure 1, lines 7–9.

Only avenin demonstrated the same protein subunit in the region of 32 kDa as all varieties of Amaranth in their minor fraction. Rice prolamin (Figure 1, line 7) had molecular sizes of about 12–17 kDa, which corresponds with data of Kim and Okita (1988).

The highest subunit of 17 kDa relates only in the molecular weight of the minor fraction of *A. flavus*, *A. caudatus*, oats, and normal maize. Comparison of all Amaranth varieties with oats, rice, sorghum, and normal maize (Figure 1) showed very little relationship between protein subunits.

The SDS–PAGE was performed also on total protein extracts of different species of Amaranth, oats, rice, normal maize, and sorghum with high tannin content (Figure 2). All protein subunits of different species of Amaranth (Figure 2A, lines 2–6; Figure 2B, lines 2–6) were similar in main subunits, expect *A. flavus*, which showed a prominent band at 67 kDa. At this range it was a similar size between lines 3, 5–7, and 9 (Figure 2B). Similar sizes between Amaranth species and oats were detected in the region of 20–25 kDa.

A similar size was shown between Amaranth species and rice in the region of 14–16 kDa, at 20, 35, and 43 kDa for Amaranth and sorghum, and at 18 and 23 kDa for

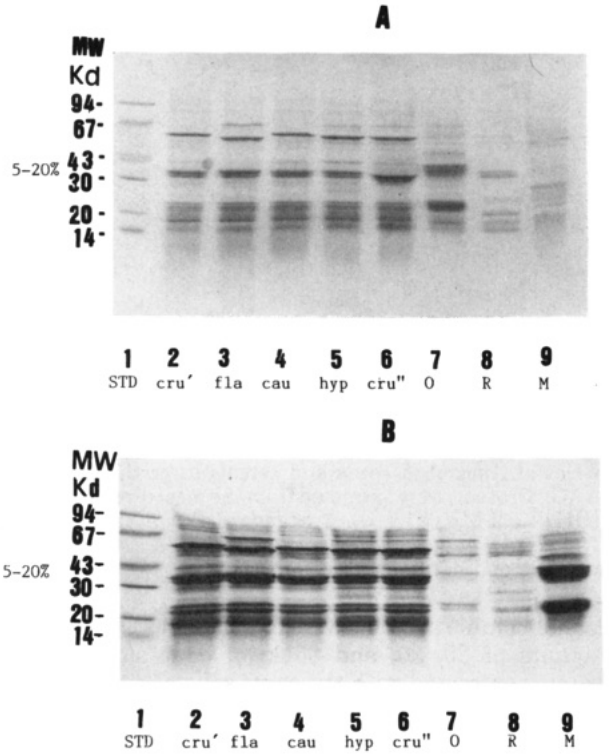

Figure 2. SDS–PAGE of total proteins from different species of *Amaranth* seeds and cereals in gradient (5–20%) PAAG. (A) 1, Standard; 2–9, *A. cruentus* (cru'), *A. flavus*, *A. caudatus*, *A. hypochondriacus*, *A. cruentus* (cru''), oats, rice, and normal maize, respectively. (B) 1, Standard; 2–9, *A. cruentus* (cru'), *A. flavus*, *A. caudatus*, *A. hypochondriacus*, *A. cruentus* (cru''), oats, normal maize, and high-tannin sorghum, respectively.

maize. Some similar patterns between Amaranth and other cereals were also observed. Electrophoretic subunits of extracted total proteins of Amaranth and other cereals verified more common patterns than alcohol-soluble fractions (Figure 1). Although Amaranth flour contains about 16% protein, its quality cannot be related to the low levels of alcohol-soluble proteins. The same relationship was found for oats and rice (Kim et al., 1978; Kim and Okita, 1988). According to Kumamaru et al. (1988), Okita et al. (1988), Peterson (1978), and Villareal and Juliano (1978), prolamins in oats and rice are at very low amounts and are synthesized during endosperm development and deposited in a specific type of protein body. In *Amaranthus* (Betschert et al., 1981; Konishi et al., 1985; Saunders and Becker, 1984) 65% of the protein is distributed in the germ and seed coat and 35% in the starch perisperm.

Albumins, globulins, and glutelins, as the major fractions, located in the protein body and the alcohol-soluble proteins of Amaranth, probably, derived from the perisperm. These membrane proteins showed nonseparated subunits in the region of 10–14 kDa and different solubility in alcohol in comparison with oats, rice, maize, and sorghum.

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