NOTE

Interference of Phytate with Extraction of Protein from Brown Rice Using 5*M* Acetic Acid

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ABSTRACT

Patterns of 5*M* acetic acid extracts of milled and brown rices, obtained by aluminum-lactate (AL) polyacrylamide gel electrophoresis (PAGE) and reversed-phase high-performance liquid chromatography, showed the presence of major glutelin proteins in milled rice that were absent in brown rice. Phytate in the bran fraction was shown to selectively bind glutelin in 5*M* acetic acid, since adding 1% sodium phytate to milled rice before extraction by acetic acid resulted in loss of some of the PAGE bands. Extraction of brown rice by 0.1*M* sodium acetate buffer pH 5 to remove unbound phytate before extraction of protein by acetic acid resulted in an AL-PAGE pattern similar to that of milled rice.

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In the study of aluminum-lactate (AL) polyacrylamide gel electrophoresis (PAGE) and reversed-phase high-performance liquid chromatography (HPLC) of 5*M* acetic acid extracts of sister line IR cultivars IR28-IR29, IR32-IR38-IR40, IR36-IR42, and IR52-IR54, the AL-PAGE bands at 54 and 100 mm (IRRI 1990) and the HPLC peaks at 26.5–27.5 min (A. Hussain, *unpublished data,* 1990) appeared in milled rice but were absent in brown rice. The absence of some protein bands or peaks in brown rice on milling could be explained by the loss of protein-rich embryo and bran layers, but milled rice remains the major (90%) fraction of brown rice.

Saio and Kubo (1963) reported the formation of a phytate-glutelin complex during the storage of brown rice; however, such anomalous protein behavior is not observed on extraction with dilute (0.1*N*) alkali, since up to 98% protein extraction is attained (Cagampang et al 1966). De Meester (1972) reported that free phytate in rice is optimum at pH 4–5. The association of magnesium and potassium ions with soluble phytate decreases with a decrease in pH from 7 to 3 (Champagne et al 1986). We studied the possible role of phytate in the anomalous protein pattern of brown rice protein extracts with 5*M* acetic acid.

MATERIALS AND METHODS

Rice Samples

Samples of brown and milled rices were obtained from the Plant Breeding, Genetics and Biochemistry Division of the International Rice Research Institute (IRRI). Brown rice was obtained by dehulling rough rice with a dehuller (THU 35A, Satake Engineering Co. Ltd., Tokyo) and milling it with a Satake TM-05 pearling machine or a micro sample mill (Pearlest, Kett Electric Laboratory, Tokyo).

Samples of IR24 and IR36 brown rice were milled to obtain 4–5, 6–7, and 10–11% weight removal of bran polish. Protein fractions of ether-defatted IR24 brown rice flour (2 g) were prepared by three prolamin extractions by 15 ml of 60% 1-propanol, followed by three albumin-globulin extractions by 0.7*M* sodium chloride (Huebner et al 1990). Glutelin was then extracted from the residual flour with either 5*M* acetic acid or 0.1*N* sodium hydroxide. Distilled water was used in preparing all aqueous solvents. The protein fractions were characterized by AL-PAGE.

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AL-PAGE and HPLC

Brown and milled rices were ground through a Udy cyclone mill with a 1-mm sieve, and 0.5 g of each ground sample was extracted for 2 hr with 2 ml of 5*M* acetic acid. The protein extract was stored at −20°C and prepared for AL-PAGE according to the method of Hussain et al (1989). At IRRI, AL-PAGE was performed using a gel mixture containing 10 g of acrylamide, 0.5 g of bisacrylamide, 0.1 g of ascorbic acid, 1.5 mg of ferrous sulfate, 20 ml of glycerol, 85 ml of 0.25% aluminum lactate-lactic acid to pH 3.1, 50 μl of N,N,N′,N′-tetramethylethylenediamine, and 75 μl of ammonium persulfate solution (100 mg/ml).

Sodium phytate (5 mg) was added to 0.5 g of milled rice before performing acetic acid extraction of the milled rice flour. In another experiment, 0.5 g of brown rice flour was preextracted with 2.0 ml of 0.1*M* sodium acetate buffer (pH 5) to remove unbound phytate. The residue was then extracted with 2.0 ml of 5*M* acetic acid. In another experiment, the 0.1*M* sodium acetate buffer extract was heated at 100°C for 2 min, cooled, readded to the brown rice, and made up to 5*M* with glacial acetic acid. The sodium acetate and acetic acid extracts were run on AL-PAGE.

RESULTS AND DISCUSSION

Brown rice protein extracted with 5*M* acetic acid and loaded directly into the gel showed the presence of a major band at 100 mm, but when the extract was freeze-dried before AL-PAGE was performed, this major band migrated to 120 mm. This suggests protein denaturation during dehydration and redissolving. To avoid this denaturation, the extracts were stored at −20°C instead of being freeze-dried. The 72-mm protein band missing in the brown rice electrophoregram was observed as a distinct band in the corresponding 4–5% milled sample of both rices (Fig. 1). Among the milled samples, the intensity of this band increased with the degree of milling. Acetic acid (5*M*) extracted only 12.2% of brown rice protein but 38.0% of milled rice protein. Thus, the factor complexing with these proteins is present in the bran fraction, but the protein being complexed is in the endosperm (milled rice).

Adding sodium phytate to milled rice resulted in the disappearance of the 54- and 72-mm AL-PAGE bands and loss of intensity in the 100-mm band, making the milled rice patterns similar to those of brown rice (Fig. 1). Extracting phytate from brown rice with 0.1*M* sodium acetate (pH 5) before extraction with 5*M* acetic acid resulted in the presence of the 54- and 72-mm bands in the acetic acid protein extract. The electrophoregram of the 0.1*M* sodium acetate extract showed faint, diffused bands corresponding to albumin, globulin, and glutelin. These bands also were present in the subsequent acetic acid extract. Sodium acetate extracted

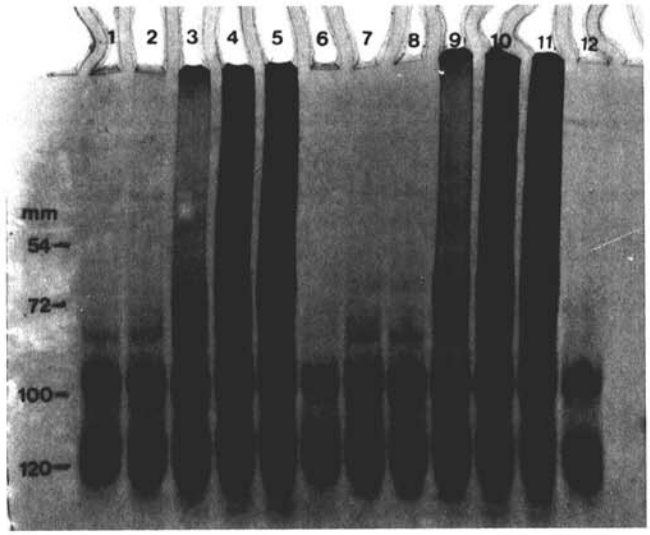

Fig. 1. Effect of degree of milling (percent of bran removed from brown rice) and phytate addition on the polyacrylamide gel electrophoretic patterns of 5*M* acetic acid extracts of IR24 and IR36 rices. 1–6, IR24; 7–12, IR36; 1 and 7, brown rice; 2 and 8, defatted brown rice; 3 and 9, 4–5% milled rice; 4 and 10, 6–7% milled rice; 5 and 11, 10–11% milled rice; 6 and 12, 10–11% milled rice plus phytate.

6.0% of brown rice protein, and the subsequent acetic acid extraction removed 23.8%, for a total of 29.8% protein extraction. The factor in the brown rice causing low protein solubility was not destroyed by heating to 100°C. Boiling the 0.1*M* sodium acetate extract, readding it to the brown rice flour, and making up to 5*M* with glacial acetic acid resulted in an AL-PAGE pattern of the extract identical to that of the direct 5*M* acetic acid extract.

AL-PAGE confirmed that the brown rice proteins extracted by 5*M* acetic acid were mainly albumins and globulins, which corresponded to the 100- and 120-mm bands (Hussain et al 1989). Prolamin showed much trailing, with the major band at about 120 mm. The protein that was selectively bound to phytate and rendered insoluble was mainly glutelin, which has major bands at 72 and 100 mm and is very soluble at pH below 3 but not at pH 5 (Takeuchi et al 1965). This would explain the greater protein extractability by 5*M* acetic acid of milled rice versus that of brown rice and the beneficial effect of 0.1*M* sodium acetate pH 5 preextraction of phytate (under conditions where glutelin is not soluble) on the solubility of brown rice protein in 5*M* acetic acid. Thus, the complexing of bran phytate and milled rice glutelin can be related to the loss of the protein bands (54, 72, and 100 mm) from AL-PAGE electrophoregrams and of the peaks (26.5–27.5 min) of HPLC profiles of 5*M* acetic acid extract of brown rice protein (Hussain et al 1989).

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