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Isolation and Physicochemical Characterization of Seed Proteins in Rice Bean (*Vigna umbellata* (Thumb.) Ohwi and Ohashi)

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Globulins comprised the largest fraction in rice bean seed proteins and accounted for 50 to 60% of total proteins, with albumin at 17 to 26%, prolamin at 1.5 to 1.8% and glutelin at 3.2 to 4.2%.

Globulins separated into three peaks with molecular weights of approximately >700,000, for the first peak, 175,000 and 18,000 for accession (acc) 28; 138,000 and 7,000 for acc 46 and 60,000 and 3,000 for acc PROC 1. Albumins exhibited 3 major peaks and a minor one. The major peak had a molecular weight >210,000. The other peaks had: 75,000, 3,900 and 1,700 for acc 28; 144,000, 14,800 and 2,000 for acc 26 and 56,000, 7,800 and 3,400 for Acc PROC 1. Both albumins and globulins exhibited heterogeneity showing 7 to 10 bands on polycrylamide gel electrophoresis.

Key words: rice bean, *Vigna umbellata*, seed proteins, albumin, globulin, glutelin, prolamin, gel filtration, electrophoresis.

Legumes have become a popular source of dietary proteins in developing countries. In the Philippines, only mungbean, peanut, cowpea and soybean are the major economically important grain legumes. However, many more legumes are underutilized including rice bean or tapilan (*Vigna umbellata*). Mature seeds and ricebean are used in food dishes in the same way mungbean is used and its immature leaves and pods are ingredients of vegetable dishes.

In order to widen the usage of rice bean as a dietary source of protein, it is necessary to study its proteins. In this paper we report the isolation and physicochemical characterization of rice bean proteins. In a separate publication [1], we reported an evaluation of their nutritional quality.

MATERIALS AND METHODS

Source and preparation of seed samples

Dried seed samples of three accessions of rice bean-acc 28 (yellow brown), acc 46 (black) and acc PROC 1 (maroon), were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding, University of the Philippines Los Baños. The first two accessions are indigenous to the Philippines while the third one came from the People's Republic of China.

Seeds were ground in a Wiley mill to pass 60 mesh and were kept in air tight containers at 4 C until used.

Isolation, fractionation and characterization of the soluble proteins

Fractionation of proteins from the seed meals was done based on solubility differences [2]. The whole seed meals were defatted thrice with n-hexane (1:10 w:v, meal to solvent ratio) at 4 C with continuous stirring for 4 h each. The defatted meal and protein fractions were kept in air-tight containers in freezer until used.

Determination of % protein fractions

One hundred mg of the defatted meals were mixed with 2.5 mL of 5% NaCl in a vortex mixer, allowed to stand for 30 min with occasional mixing, and centrifuged at 7,000 rpm for 15 min. The clear supernates, which contained the albumins and globulins, were collected and diluted to 5.0 mL with distilled water and their protein content analyzed.

The supernatants were dialyzed against water at 4°C to precipitate the globulins. The dialysates, which contained the albumin fraction, were analyzed for protein. Extraction was done twice more with 5% NaC1. Protein content was determined on the second and third extracts of soluble proteins.

The residues from the third extraction of soluble proteins were extracted with 70% ethyl alcohol. The glutelins were extracted with 0.5 M NaOH solution from the residue.

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Preparative fractionation of albumin and globulin

Fifty g each of the defatted rice bean meals were stirred in 0.5M NaCl (20 mL: 1 g of meal) at 4°C for 1 h. The slurry was centrifuged at 12,000 x g and 4°C for 20 min in a sorvall RC 5 centrifuge.

The clear extracts were dialyzed at 4°C against distilled water until free from NaCl and precipitation of the globulins was completed. The mixtures were centrifuged at 12,000 x g and 4°C for 20 min. The precipitated globulins were washed with distilled water, collected and lyophilized.

The supernatants, and washings were combined and lyophilized to give the albumin fraction.

The lyophilized albumin and globulin fractions were kept in air-tight containers in a freezer until used for analysis.

Gel filtration of the albumin fraction

Fifty mg samples of each of the albumin fractions dissolved in 3.0 mL of 0.05M sodium phosphate buffer pH 7.0 were filtered through Whatman No. 1 filter paper, and were chromatographed on Sephadex G-200 column (1.5 x 70 cm) using the same buffer. The molecular weights of the eluted proteins were estimated using the calibration curve prepared with catalase (Mw 232,00), bovine serum albumin (Mw 69,000) cytochrome (Mw 12,400).

Gel filtration of the globulin fraction

Thirty mg samples of each of the globulin fractions were dissolved in 2.0 mL of 0.05M sodium phosphate buffer pH 7.0 in 0.5M NaCl and chromatographed individually on the Sepharose 4B column (1.5 x 70 cm). A calibration curve was prepared with standards (thyroglobulin Mw 669,000, catalase Mw 210,000, bovine serum albumin Mw 69,000, and cytochrome Mw 12,000).

Disc gel electrophoresis

The protein fractions were subjected to disc gel electrophoresis following the modified procedure of Davis [3] and in the presence of sodium dodecyl sulfate [4].

Protein analysis

Kjeldahl nitrogen was determined according to standard AOAC procedures [5]. Crude protein content was estimated by multiplying Kjeldahl nitrogen by a factor of 6.25.

RESULTS AND DISCUSSION

Fractionation and characterization of the seed proteins

Like most legumes, the globulins comprise the largest fraction and accounted for 56 to 60% of the total proteins (Table 1). Albumins contributed 14 to 17%, glutelins 3 to 4% and the prolamins composed the smallest fraction at 1.5 to 1.8%. The total nitrogen extracted was in the range of 76 to 83%.

Gopinathan et al [6] reported globulins to constitute 38-54% of total seed proteins in several populations of *Vigna minima* and *V. umbellata*. Similar values for the globulin fractions of Phaseolus seeds, [7, 8] mungbean and peas have been reported [9].

The contribution of the albumins obtained for rice bean were slightly higher than the reported value of 7% in cowpea [10] and 8.1% in mung bean [9]. Chickpeas, Phaseolus seeds and peas exhibited similar values as rice bean at 12.2%, 13% and 14.1% of albumins in the total protein, respectively [9].

The amount of nitrogen extracted is similar to the 74 to 82% solubilization reported by Evans and Kerr [11] and Powrie [8] and higher than the 70% obtained by Jambunathan and Mertz [12]. Differences in solubilization of

Table 1. Distribution of total protein in rice bean seeds.

Fraction	Amount of protein (g/100 g seed meal)					
	Acc 28		Acc 46		Acc PROC 1	
Albumin	2.90 ± 0.28	(17)	3.80 ± 0.42	(18)	4.45 ± 0.42	(26)
Globulin	9.51 ± 0.87	(56)	12.89 ± 0.93	(60)	9.96 ± 0.56	(58)
Prolamin	0.26 ± 0.08	(1.5)	0.38 ± 0.07	(1.8)	0.28 ± 0.05	(1.6)
Glutelin	0.71 ± 0.12	(4.2)	0.82 ± 0.09	(3.8)	0.56 ± 0.09	(3.2)
Residue	3.44	(20)	3.53	(16)	2.01	(12)

[a] Values are average of three replicates.

[b] Differences between total protein and sum of solubilized protein. Percent of total protein in parentheses.

nitrogen may be due to factors other than those inherent in the seed samples. These factors may be extraction temperature, meal-solvent ration and particle size of the sample [13, 14]. It is also reported that the prolamin and glutelin fractions tend to gel and thus cause losses in extractable nitrogen [12].

The prolamins and glutelins comprise a very small fraction of the total proteins of rice bean, much lower than those reported by Gopinathan et al [6]. Thus, most of the succeeding analyses were done on the two major protein fractions, the albumins and the globulins.

The albumins of acc 28 was light yellow while the globulins were slightly gray. The protein fractions from acc 46 and acc PROC 1 were reddish brown and dense. These two latter accessions gave albumin and globulin fractions of higher nitrogen content than acc 28, as shown in Table 2.

The globulin fractions were of higher nitrogen content than the albumins of the same variety. Traces of starch in faba beans and peas and 1.8% in mung bean albumin had been reported [9]. The protein fractions of cowpea were also found positive for glycoproteins [10].

Table 2. Protein content of albumin and globulin fractions isolated from rice bean meal (g/100 g protein extract).

Variety	Amount of Protein	
	Albumin	Globulin
Acc 28	39.32	68.82
Acc 46	53.46	76.53
Acc PROC 1	52.64	71.62

Gel filtration

Albumins. The albumins exhibited 3 major peaks and a minor one (Fig. 1). All of the first fractions were eluted near the exclusion limit of Sephadex G-200 corresponding to molecular weights greater than 210,000. The estimated molecular weights of the other fractions are 75,800, 3,900 and 1,700 for acc 28; 144,000, 14,800 and 2,000 for acc 46 and 56,000, 7,800 and 3,400 for acc PROC 1. With the exception of the third fraction of acc 46, the molecular weights of the 3rd and 4th fractions were extrapolated from the calibration curve. The molecular weights of the corresponding fractions of the albumin samples differ markedly but the elution pattern are quite similar (Fig. 1). Cowpea albumins had only 3 peaks [10] with the first fraction also coming out near the void volume.

Globulins. The globulins separated into three fractions each, the first one in all of the samples eluting at the void volume of Sepharose 4B (Fig. 2) with molecular weight greater than 700,000. The approximate molecular weights of the other fractions are 175,000 and 18,600 for acc 28; 138,000 and 7,000 for acc 46 and 60,000 and 3,000 for acc PROC 1. The molecular weights of the third fraction of acc 46 and acc PROC 1 were extrapolated from the calibration curve for Sepharose 4B. As in the albumins, the molecular weights of the fractions differ but the elution patterns of the globulins were very similar. A similar run on cowpea gave 3 protein peaks with molecular weights from greater than 700,000 to 27,000 daltons [10].

Polyacrylamide gel electrophoresis

Under native conditions. The albumins exhibited 9 to 10 bands of varying intensities (Fig. 3A). The major

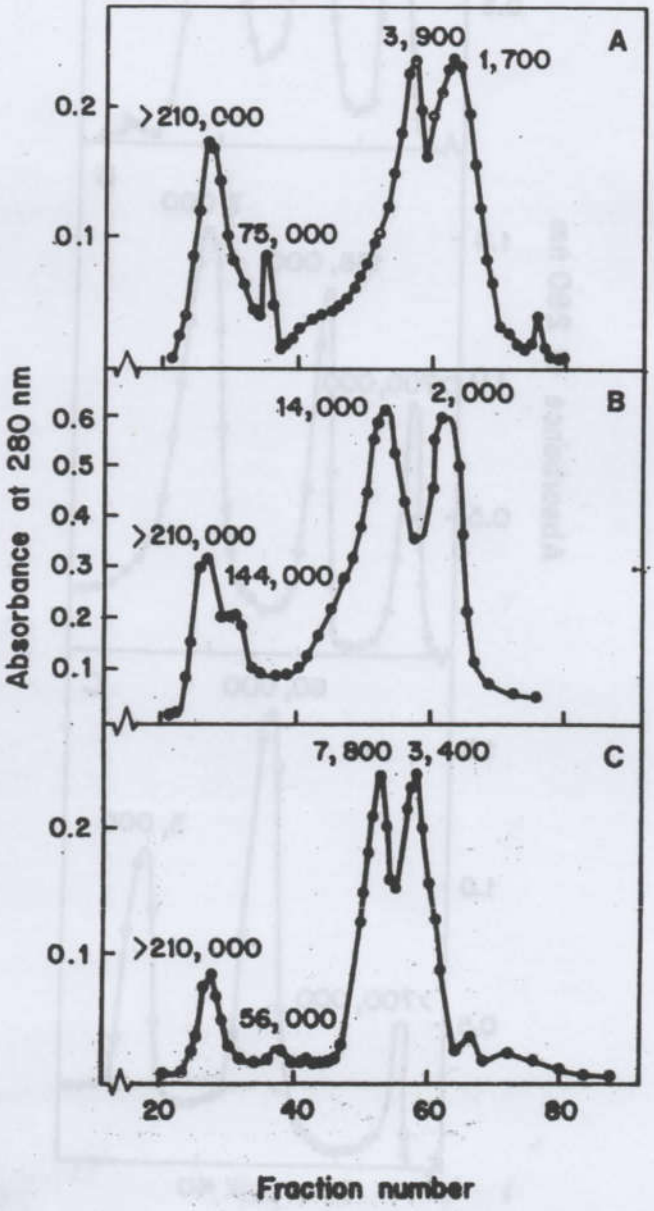

Fig. 1. Elution patterns of the albumin fractions of acc 28 (A), acc 46 (B) and acc PROC 1 (C) in sephadex G-200, 0.05M phosphate buffer pH 7.0.

subunits for the three varieties had similar mobilities from Rf 0.17 to 0.27. The samples gave two to three major subunits which may be of similar charge densities. The fast moving subunits of the albumins were lightly stained or diffused. Similarities can also be observed among the fast moving subunits such as the wide diffused band near midgel and the three lightly stained narrow bands from Rf 0.8 to 1.0.

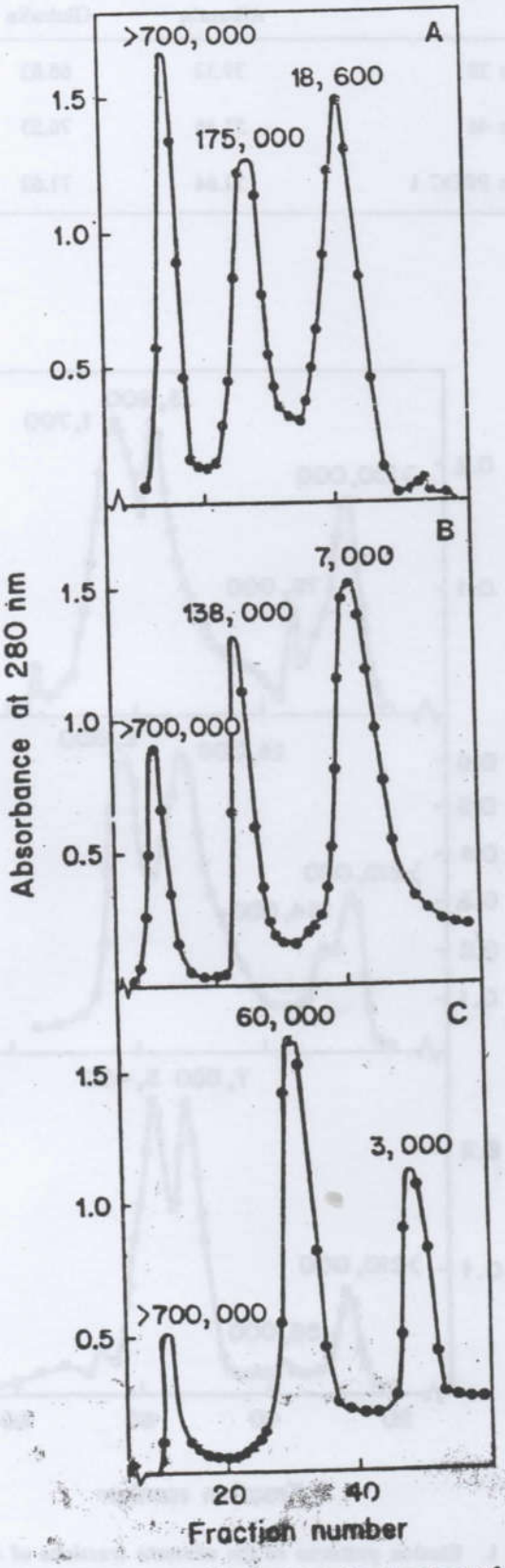

Fig. 2. Elution patterns of the globulin fractions of acc 28 (A), acc 46 (B) and acc PROC 1 (C) in Sepharose 4B, 0.05M phosphate buffer pH 7.0 in 0.5M NaCl.

The globulins gave 7 to 8 bands, with the major subunits appearing as a wide, intense band around midgel and a narrower zone at Rf 0.2 to 0.4 (Fig. 3B). Again, the major subunits for each of the varieties appeared to be quite similar. The disc gel pattern of *Vicia faba* also showed major bands at Rf 0.1 to 0.3 [15].

Electrophoretic patterns of albumins and globulins of other legumes had varying number of protein zones for each legume. Hu and Esen [16] isolated soybean albumins and globulins which gave rise to 50 to 60 major subunits. Albumins of *Phaseolus vulgaris* gave at least 68 bands while the globulins exhibited an extremely diffused banding pattern [15].

Under denaturing conditions. The major polypeptides of the albumin and globulin fractions were determined by SDS polyacrylamide gel electrophoresis (Fig. 3C & D). The albumins gave three to five fine well-stained bands corresponding to polypeptides with molecular weights over 66,000. The samples gave two major polypeptides each but the apparent molecular weights of these major units differed from one sample to another. For acc 28, the major units had molecular weights of 23,000 and 50,000 daltons. The polypeptides of acc 46 were in the range of 55,000 and 37,000 and those of acc PROC 1 had molecular weights of 29,000 and 36,000. There were several lightly stained bands and each electrophoretogram was characterized by a diffused band at Rf 0.4 to 0.8.

The globulins exhibited four to five very closely spaced bands representing polypeptides with molecular weights over 66,000. Major subunits occurred in a narrower range of molecular weights than the albumins, from 70,000 to 45,000.

The heterogeneity observed in the protein fractions has also been seen in other legume species [9, 14, 15] and in rice [17].

One of the main causes of heterogeneity and multiplicity of a few kinds of subunits is the occurrence of genetic variants arising in several minor bands [9]. Other causes are post-translational, among which is the occurrence of several degrees of oligomerization resulting in a series of sedimentation coefficients. There is also a possible interchange of some of the subunits, the contingent variation in size and the nature of the bound carbohydrate groups [18].

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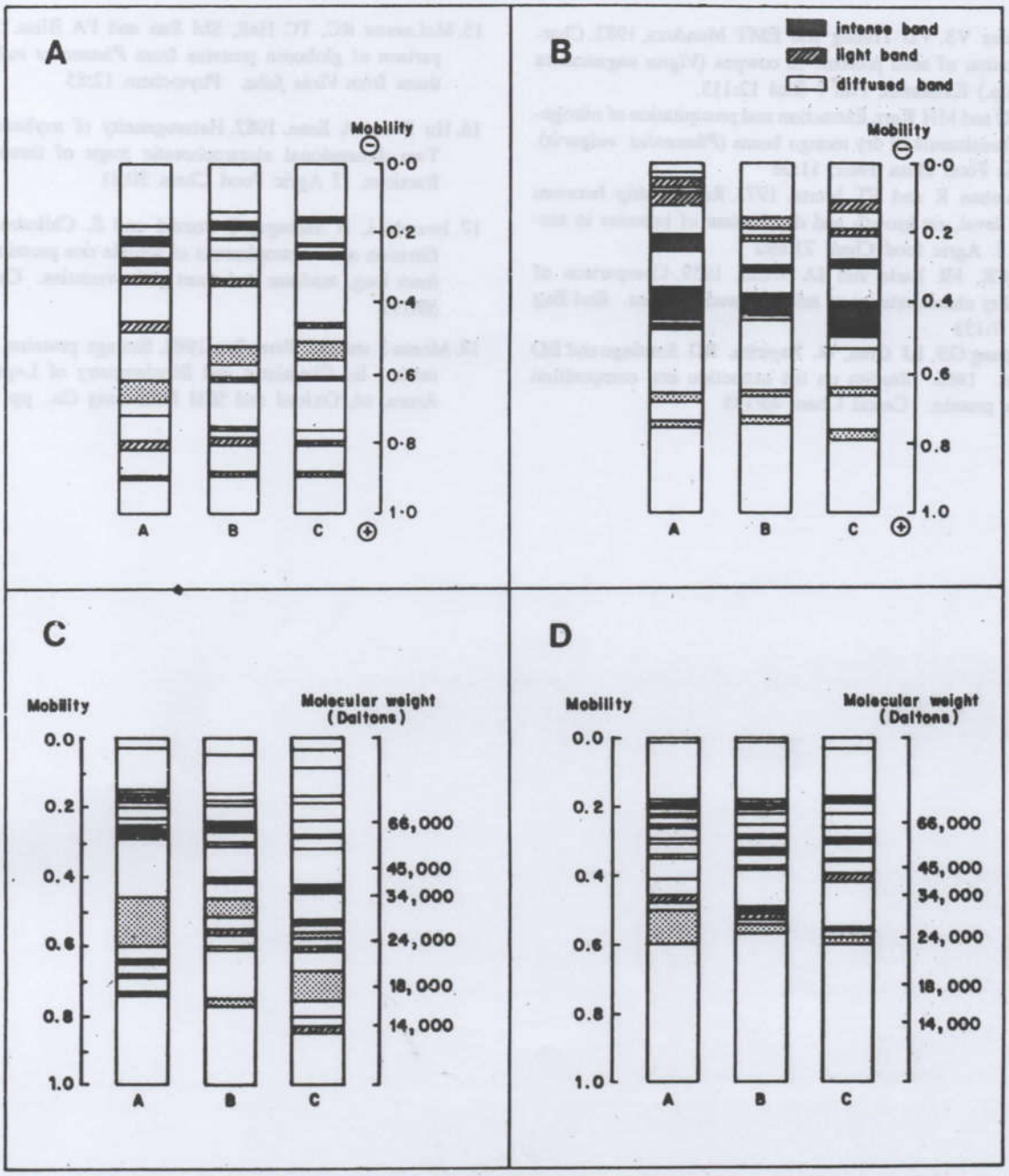

Fig. 3. Polyacrylamide gel electrophoregrams of albumins [A] and globulins [B], and SDS-polyacrylamide gel electrophoregrams of albumins [C] and globulins [D]; samples: acc 28 (A), acc (B) and acc PROCE 1 (C).

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