Cereal Chemistry

Physicochemical Properties of Protein of Developing and Mature Rice Grain[1]

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ABSTRACT

Analyses were carried out on acetone powders of the whole grain of *Oryza sativa* L. (variety IR8) during development, from a few days after flowering to maturity. Nonprotein nitrogen decreased in concentration, but the amount per grain remained relatively constant. The protein fractions per grain increased during the first 2 weeks after flowering, but only glutelin and prolamin continued to increase in amount throughout development. Albumin-globulin per grain and the concentration of urea-sodium chloride extract of the acetone powder decreased during the later stages of development. Gel filtration of the urea-salt extract on Sephadex G-100 gave three fractions corresponding to molecular weights of >2, 0.8, and 0.3×10^5. Amino acid analysis also showed the 4-day sample to have a higher lysine content than did either the 14-day sample or the mature grain. These data were interpreted on the basis of analysis of preparations of protein fractions of brown rice.

Few workers have studied the changes in protein during development of the rice grain (1,2). Recent morphological and biochemical studies have shown that seed proteins exist as discrete protein-rich bodies or aleurins (3) in the endosperm of corn (4), wheat (5), rice (6,7,8), and other grains (3). This paper reports part of an investigation of changes during development and storage of rice grain (9). Changes in protein properties were estimated by differential percolation, extraction with urea-salt solution and gel filtration, and amino acid analysis of the proteins.

Hess (10) fractionated proteins of wheat and rye endosperm into wedge protein and starch-adhering protein. In efforts to isolate native protein from rice endosperm, this flotation method was applied to ball-milled rice flour.

MATERIALS AND METHODS

Samples of developing IR8 rice grain were obtained from a previous study (7). Fresh rough rice was ground in a mortar and pestle in liquid nitrogen, and defatted with cold acetone ($-20°$C.).

Percolation

The protein of the acetone powder was extracted by a modification of the percolation method of Maes (11). Acetone powder (10.0 g.), 10 g. of finely ground pumice (200-mesh), and 100 g. of sea sand (E. Merck Ag.) were thoroughly mixed in a mortar. The percolation column (25×3.5 cm.)

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was plugged with glass wool and packed with successive layers of quartz (E. Merck Ag.), sea sand, sample pumice-sand mixture, sea sand, and quartz. The quartz and sand layers were about 1.5 cm. thick. The column was covered on top with glass wool and held in place with a few glass marbles. The column was leached successively at 25°C. with 250 ml. of 5% sodium chloride, 150 ml. of 60% ethanol, and 150 ml. of 0.4% sodium hydroxide. The salt extract was dialyzed against distilled water. Aliquots of the extracts were analyzed for nitrogen by the micro-Kjeldahl method (13). Protein content was calculated from Kjeldahl nitrogen data (N × 5.95). Albumin-globulin was calculated by difference from dialyzable nonprotein and total salt-soluble nitrogen. Total nitrogen extraction ranged from 49.4 to 61.7% of the Kjeldahl nitrogen of the acetone powders.

Urea-Salt Extraction and Gel Filtration

Acetone powder (10.0 g.) was homogenized with 40.0 ml. of $3M$ urea-$0.5M$ sodium chloride in $0.01M$ pH 7.0 phosphate buffer for 3 min. in an Omnimixer (ice bath), and the mixture was centrifuged at 17,000 r.p.m. at 4°C. for 30 min. The clear supernatant was dialyzed against the solvent at 4°C. Relative absorbance of the fresh clear extract was obtained on 0.20 ml. of the extract diluted to 3.00 ml. Protein concentration of the extracts was determined colorimetrically by the method of Lowry et al. (14); crystallized bovine plasma albumin (Armour Laboratories) was used as standard.

A quantity (4 ml.) of the dialyzed extract to which sucrose had been added was introduced onto two columns of Sephadex G-100 gel (2.5 × 32 cm.) previously equilibrated with the same solvent. Fractions (3 ml.) were collected at the rate of 30 to 35 ml./hr. Absorbance of the fractions at 280 mμ was obtained with the Beckman DU spectrophotometer. Crystalline proteins were used to calibrate the column for void volume and molecular weight estimation (15,16). Solutions of the protein fractions prepared from BPI-76 brown rice in the urea-salt solvent were also subjected to Sephadex G-100 gel filtration.

Preparation of Reference Protein Fractions

Protein fractions were prepared from BPI-76 brown rice (400 g.) previously defatted with petroleum ether. Albumin-globulin was obtained by soaking the defatted brown rice with 1 liter of $0.5M$ sodium chloride in $0.1M$ pH 7.1 tris (hydroxymethyl) aminomethane (TRIS) buffer and homogenizing at high speed in a Waring Blendor for 3 min. at 4°C. The homogenate was stirred for another 1 hr. and filtered through cheesecloth. The filtrate was centrifuged at 19,000 r.p.m. for 30 min. at 0°C., passed through a 2-cm. layer of Celite filter-aid, dialyzed against distilled water, and lyophilized. Yield: 2.1 g. Nitrogen content, 14.2% dry basis (d.b.). Moisture content was determined by loss of weight at 130°C. (13).

The residue after albumin-globulin extraction was washed thoroughly with sodium chloride-TRIS solution until the washing was negative to the biuret reaction. The residue was then extracted with 1 liter of 75% ethanol at 4°C. for 2.5 hr., and centrifuged at 2,000 r.p.m. at 0°C. for 30 min.

The ethanol extract (prolamin) was dialyzed against distilled water and lyophilized. Yield: 0.7 g. Nitrogen content, 9.98% d.b.

The residue after prolamin extraction was resuspended in 1 liter of 75% ethanol for 20 min. and centrifuged. The residue was shaken with twice the volume of 0.4% sodium hydroxide for 1 hr. at 4°C. and centrifuged at 2,000 r.p.m. at 0°C. for 30 min. The turbid supernatant was passed through a 1-cm. bed of Celite filter-aid, dialyzed against distilled water until the dialyzing water was neutral, and then lyophilized. Yield: 6.4 g. Nitrogen content, 16.8% d.b.

Pentose content of these extracts was determined by the orcinol test (17) at 675 mμ, with D(−)-ribose as standard. Prolamin did not produce the same color with the reagent, but instead had its maximum absorbance at 435 mμ.

Prolamin (20 mg.) was hydrolyzed in a sealed tube with 10 ml. of 2% sulfuric acid for 10 hr. at 120°C. The hydrolysate was neutralized with saturated barium hydroxide and its pH adjusted to 7 with barium carbonate. The supernatant liquid was separated by centrifugation, passed through a mixed-bed resin column of Amberlite IR-4B and IRC-50, and concentrated under reduced pressure below 55°C. Aliquots of this solution and of standard sugars, glucose, arabinose, xylose, and ribose, were spotted on Whatman No. 1 filter paper, developed with the solvent ethyl acetate-pyridine-water (8:2:1) for at least 24 hr., and air-dried. The chromatograms were sprayed with alkaline silver nitrate (18).

Flotation

BPI-76 milled rice (14.2% protein, wet basis) was ball-milled for 17 hr. and subjected to flotation according to Hess (10) as employed by Vones et al. (19). The rice flour (40 g.) was suspended in 80 ml. of benzene-chloroform mixture (d^{21} = 1.37) in an Omnimixer at low speed and centrifuged at room temperature at 2,100×g for 30 min. The supernatant was collected and the flotation procedure repeated four times on the residue. The combined supernatants and the residue were air-dried. Yield of fraction: 400 mg. with 52% protein, wet basis. The residue from the flotation at d^{21} = 1.37 was subjected to two more series of flotations at densities of 1.39 and 1.42. The lighter fractions obtained were 1.08 and 1.98 g., with protein contents, wet basis, of 35.6 and 23.7%, respectively. The residue after the three successive flotations (density > 1.42) was 25.8 g. with 9.87% protein, dry basis.

BPI-76 brown rice (14% protein) was similarly subjected to flotation.

Amino acid analysis was performed with a Beckman Model 120 amino acid analyzer (12,20) on the acetone powders of 4-day, 14-day, and mature grain before and after dialysis against distilled water, and on the reference protein fractions.

RESULTS AND DISCUSSION

Percolation of rice proteins of developing grain indicated significant changes in the quantity of protein fractions (Fig. 1). Nonprotein nitrogen remained practically constant in amount per grain. It was a major fraction (about 15%) of the total nitrogen in the 4-day grain but dropped to less than 3% of the total nitrogen in the mature grain.
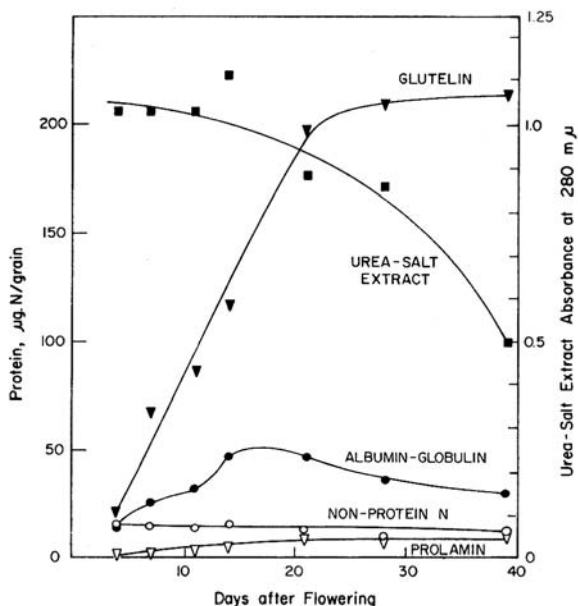
Fig. 1. Changes in protein fractions and absorbance of $3M$ urea-$0.5M$ sodium chloride extract of rough rice during grain development.

Protein nitrogen of the grain did not change considerably in concentration during development, but it increased fivefold in amount per grain (7). Albumin-globulin (salt-soluble proteins) increased in amount per grain during the first 2 weeks of ripening, reached an optimum level between day 14 and day 21, and decreased progressively toward maturity. Prolamin showed a sixfold increase per grain during development. The major protein change was the rapid increase of glutelin in the grain between day 4 and day 21. Maximum contents of prolamin and glutelin were attained shortly before maturity. Presumably, the synthesis of albumin-globulin terminated earlier than that of glutelin and prolamin in the developing grain.

Kester *et al.* (21) similarly found that the salt-soluble and nonprotein nitrogen contents of milled rice dropped to 9–11% and less than 2% of total nitrogen, respectively, during maturation. Tadokoro and Abe (1) noted that during the last 20 days of grain development of Japanese rice, glutelin showed little change, whereas albumin gradually decreased and globulin and prolamin increased gradually in amounts per grain. This period was later than the period of rapid protein synthesis in the grain between day 4 and day 21 which we observed and may explain the minor changes in protein fractions which they reported. Similar early synthesis of salt-soluble protein and its later decrease and later synthesis of glutelin and prolamin have been reported for the developing grain of barley (22), corn (23), and wheat (24).

The particle size of the powdered sample has been shown to be a critical factor in determining yields by leaching extraction (12). Although the recoveries of 49–62% may be improved by further grinding of the acetone

powder, this modification would considerably reduce the already slow per-colation rate. Besides, previous experiments have shown that such an increase in protein extraction yields was mainly in the quantity of glutelin (12).

The solubility of proteins in the developing rice grain in $3M$ urea-$0.5M$ sodium chloride in pH 7.0 phosphate buffer was similarly studied. Urea has been employed to improve the solubility of cereal glutelins and prolamins in neutral solvents (25,26), and it would be interesting to determine whether or not the solubility in this solvent will parallel that of the albumin-globulin fraction.

Absorbance at 280 mμ of the urea-salt extract of acetone powder de-creased abruptly during the later stages of grain development (Fig. 1). This coincided with the drop of albumin-globulin concentration in the rice grain. When expressed in relative absorbance per grain basis rather than per unit weight, the urea-salt-extractable protein gave a curve during development similar to that of albumin-globulin. The solubilities of rice albumin-globulin, prolamin, and glutelin preparations in this solvent were 80, 23, and 22%, respectively. Presumably, the salt-urea-soluble proteins are mainly albumin-globulin, together with lower-molecular-weight (MW) fractions of prolamin and glutelin. However, it should be noted (Fig. 1) that the major drop in the concentration of urea-salt-soluble proteins occurred after maximum glutelin synthesis had occurred in the developing grain. A similar decrease in urea-dispersibility of wheat proteins during maturation was reported re-cently (27); the results indicated a gradual increase in MW and complexity of the synthesized proteins. Similar changes in molecular size of the proteins in the developing rice grain will explain their decreased solubility in urea-salt and salt solutions. Daftary and Pomeranz (26) found only 32% of rice protein dispersible with $3M$ urea.

Gel filtration on Sephadex G-100 of all urea-salt extracts of developing grain gave three distinct peaks, corresponding to mean MW's of over 2.0, 0.8, and 0.3×10^5 (9). The first peak corresponded to the void volume (Fig. 2). Recovery based on total absorbance ranged from 89 to 95% of the sample. The amounts of the three fractions generally followed the de-crease in urea-salt-soluble proteins during the later stages of maturity. Al-though in terms of total absorbance of the fractions the middle peak was slightly less than the two other peaks during day 4 and day 14 after flower-ing, total absorbance of the first and third peaks decreased faster toward maturity than the middle peak. This second peak became sharper during maturation. Mean total absorbance ratio of three peaks was approximately 1:1:1. Repetition of gel filtration after 3 days' standing of the extract at 4°C. resulted in a decrease in the total absorbance of the first peak with a cor-responding increase in the third one. All the fractions showed a positive test for carbohydrates with phenol-sulfuric acid (28). Preliminary amino acid assays showed that the second protein fraction has a lower lysine content than the first and third fractions.

Gel filtration of protein-solubility fractions in urea-salt solution showed distinct differences in their patterns (Fig. 3). Albumin-globulin was sepa-rated into the three major fractions, the first peak lesser in total absorbance

than the others. In contrast, albumin-globulin from bran gave only the first and third peaks and only a trace of the second peak. Since extracts from both
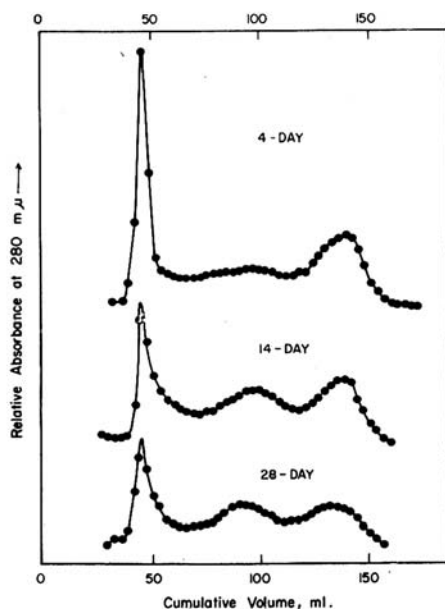

Fig. 2. Elution curves in Sephadex G-100 (2.5 × 32 cm.) of 3M urea-0.5M sodium chloride extract of rough rice of developing rice grain.
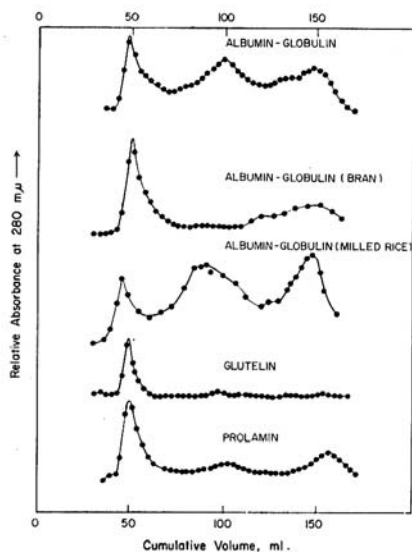

Fig. 3. Elution curves in Sephadex G-100 (2.5 × 32 cm.) of brown rice protein fractions. Solvent: 3M urea-0.5M sodium chloride in 0.01M pH 7.0 phosphate buffer.

brown and milled rice had the middle peak, this indicates a difference, at least in MW distribution, between the albumin-globulin extract of the bran (aleurone layer plus germ) and that of the starchy endosperm. With starchy endosperm development, the greater sharpness of the middle fraction shown in Fig. 2 may be due largely to albumin-globulin of the starchy endosperm (milled rice).

Glutelin gave mainly the first high-MW peak on gel filtration. Subsequent gel filtration on Sephadex G-200 showed that it has a MW of at least 260,000. Starch and polyacrylamide-urea gel electrophoreses also did not result in migration of rice glutelin from the sample well (9), presumably owing to its high MW and molecular sieving in these gels. Prolamin was fractionated by gel filtration mainly into the first and third peaks.

The ultraviolet spectrum of albumin-globulin in urea-salt solution showed a higher absorbance at 260 than at 280 mμ, whereas prolamin and glutelin had higher absorbance at 280 than at 260 mμ.

Aminograms of whole acetone powders and dialyzed homogenates provide information on the relative composition of free and protein amino acids in the developing grain. The greatest differences were shown in the 4-day grain, where nonprotein nitrogen was a major fraction of the total nitrogen, and the least differences in the 39-day grain, when nonprotein nitrogen was less than 3% of total nitrogen (Table I). Free amino acids had higher proline but lower tyrosine and arginine contents than protein in all samples, based on their total nitrogen. Taira and Taira (2) by microbiological assay similarly found a higher proline content in free amino acids than in protein

TABLE I

CHANGES IN THE AMINO ACID CONTENT OF IR8 GRAIN DURING DEVELOPMENT[a]

AMINO ACID	TOTAL NITROGEN			PROTEIN[b]			STANDARD ERROR	LSD (5%)
	Day 4[c]	Day 14	Day 39	Day 4	Day 14	Day 39		
	g.	g.	g.	g.	g.	g.		
Alanine	7.39	6.53	6.70	7.23	6.38	6.70	0.20	
Arginine	6.02	7.46	8.07	6.99	8.54	8.52	0.19**	0.66
Aspartic acid	12.6	10.6	10.6	12.2	10.3	10.8	0.32*	1.1
Glutamic acid	18.0	22.0	21.8	17.2	21.2	21.4	0.49**	1.7
Glycine	5.94	5.22	5.26	5.68	5.26	5.41	0.13*	0.45
Histidine	2.28	2.46	2.56	2.46	2.58	2.71	0.10	
Isoleucine	4.68	4.26	4.16	4.63	4.08	3.96	0.23	
Leucine	8.62	8.66	8.02	8.58	7.86	8.02	0.30	
Lysine	6.50	4.18	4.26	6.67	4.10	4.54	0.14**	0.49
Methionine	1.82	2.18	2.33	1.98	1.56	2.39	0.30	
Phenylalanine	5.30	5.58	5.19	4.95	5.07	4.90	0.25	
Proline	5.36	5.45	5.17	4.90	4.31	4.66	0.36	
Serine	5.30	5.66	5.51	5.05	5.14	5.28	0.24	
Threonine	4.80	4.16	4.20	4.71	4.24	4.20	0.16	
Tyrosine	2.06	3.18	2.54	3.40	4.39	4.13	0.20**	0.71
Valine	6.22	6.43	6.26	6.55	6.48	6.20	0.47	
Ammonia	2.38	2.31	2.28	2.16	2.41	2.02	0.10	
Total	105.27	106.32	104.91	105.34	103.90	105.79		

[a] Calculated to 16.8 g. of nitrogen and adjusted to 95% nitrogen recovery of acid hydrolysate. Only trace amounts of cystine were recovered.
[b] Dialyzed acetone powder.
[c] Days after flowering.

of rice during development, but the changes for tyrosine and arginine were not consistent. The 4-day grain had higher lysine and lower glutamic acid levels both in total nitrogen and true protein than the 14-day and mature grains, which had similar aminograms (Table I). Taira and Taira (2) similarly reported the same relative lysine and glutamic acid contents of protein in the early-milky grain as compared to those of the late-milky and fully ripened grain.

Amino acid analysis of protein fractions of BPI-76 brown rice showed marked differences in some of the amino acids (Table II). Albumin-globulin

TABLE II

AMINO ACID COMPOSITION OF BPI-76 BROWN RICE PROTEIN AND ITS FRACTIONS[a]

AMINO ACID	BROWN RICE	ALBUMIN-GLOBULIN	PROLAMIN	GLUTELIN	ADHERING PROTEIN	STANDARD ERROR[b]	LSD (5%)
	g.	g.	g.	g.	g.		
Alanine	6.46	6.87	6.52	5.41	6.24	0.15*	0.60
Arginine	7.18	10.9	6.76	9.46	8.71	0.30**	1.18
Aspartic acid	10.2	8.72	6.70	9.23	10.1	0.65	
Cystine	1.17	3.32	1.86	1.02	0.86	0.09**	0.36
Glutamic acid	22.5	17.2	29.1	23.0	22.4	0.39**	1.53
Glycine	5.09	6.36	3.02	4.74	4.80	0.11**	0.42
Histidine	2.24	2.73	1.64	2.52	2.42	0.54**	0.18
Isoleucine	4.62	3.25	5.06	3.90	4.05	0.09**	0.35
Leucine	9.08	6.62	12.4	8.14	7.49	0.20**	0.77
Lysine	3.22	4.83	0.32	3.07	3.68	0.06**	0.24
Methionine	1.91	2.42	0.75	1.62	1.51	0.06**	0.24
Phenylalanine	5.60	3.80	6.30	5.62	5.41	0.08*	0.34
Proline	4.97	5.77	5.76	5.05	4.51	0.23	
Serine	5.27	5.19	6.44	5.60	5.40	0.06**	0.25
Threonine	3.72	3.96	2.51	3.63	3.54	0.12**	0.47
Tryptophan	1.35	1.46	0.86	1.57		0.02**	0.07
Tyrosine	3.78	4.23	8.78	5.52	4.44	0.15**	0.58
Valine	6.41	5.70	5.30	5.14	5.61	0.18*	0.74
Ammonia	2.59	1.36	2.87	2.29	2.53	0.13**	0.52
Total	106.01	104.69	112.95	106.53	103.70		
Protein content, % d. b.	14.2	74.9	59.4	100	9.87		

[a] Calculated to 16.8 g. of nitrogen and adjusted to 95% nitrogen recovery of acid hydrolysate.
[b] Based on data for brown rice, albumin-globulin, prolamin, and glutelin.

had higher contents of lysine, sulfur-containing amino acids, arginine, glycine, histidine, and threonine, and lower contents of glutamic acid, isoleucine, leucine, phenylalanine, serine, tyrosine, and ammonia than glutelin and prolamin. Glutelin had contents of arginine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, and ammonia in between albumin-globulin and prolamin. Among the fractions, prolamin had the lowest content of lysine, tryptophan, arginine, aspartic acid, glycine, histidine, methionine, and threonine and the highest contents of glutamic acid, isoleucine, leucine, phenylalanine, serine, tyrosine, and ammonia. Of the three fractions, glutelin had the closest aminogram to that of the whole protein of BPI-76 brown rice. Identical lysine and glutamic acid contents of 3.2 and 22.5%, respectively, were obtained from actual analysis (Table II) and by calculation from the albumin-globulin/prolamin/glutelin ratio of 11.2/3.4/85.4 and the aminogram of these fractions.

The changes in opposite directions of lysine and glutamic acid contents of the grain during development (Table I) may be explained partly on the basis of the aminogram of the protein fractions. Between day 4 and day 14, albumin-globulin, glutelin, and prolamin increased in amount per grain, accompanied by an increase in glutamic acid and a drop in lysine content of protein. Between day 14 and maturity, glutelin increased twofold, whereas albumin-globulin decreased and prolamin increased in amount per grain. These changes, however, did not result in an appreciable change in the glutamic acid and lysine contents of protein. Although it has not been established whether the aminogram of these fractions remains constant during grain development, the changes in these two amino acids were consistent with the rapid increase in glutelin and prolamin after the fourth day from flowering. The similar lysine content of the grain protein at day 14 and at maturity could be partly explained if the drop in albumin-globulin content were due to insolubilization rather than to conversion into another protein of lower lysine content. The simultaneous increase in MW and complexity of the other protein fractions, glutelin and prolamin, may also account for part of the insolubilization of the protein in urea-salt solution during the later stages of development. The same trend in lysine and glutamic acid levels of milled rice protein has been reported for eight varieties of milled rice as the result of an increase in protein content (12). Here again, the increase in protein content of the variety was due to greater contents of glutelin and prolamin, with practically no change in albumin and globulin contents.

It is interesting to note that this period after the early-milky (4-day) stage corresponded to the initial synthesis of protein bodies as shown by histological study (6,7), and to the rapid increase in glutelin and prolamin in the grain. Glutelin must then be the main protein constituent of the protein bodies, which is storage protein. Hence, the particulate protein would have a lower lysine content than albumin-globulin. Recently, Mitsuda et al. (6) reported that 83% of the protein in rice protein bodies is alkali-soluble and 11% is salt-soluble. In corn, the particulate protein was shown by Duvick (4) to be zein (prolamin), whereas it was gluten (prolamin and glutelin) in nature for wheat (29). Cytoplasmic protein in all these cases was higher in lysine content than the particulate protein.

Alkali-extract of BPI-76 brown rice of 9.1, 12.2, and 16.3% protein levels gave essentially the same aminogram, reflecting the constancy of amino acid composition of the glutelin of brown rice of a variety regardless of protein content. Presumably, the various fractions are relatively constant in composition since, regardless of variety, aminograms of glutelin and globulin of 12 samples of milled rice were similar (30). Aminogram data of the fractions were similar to those obtained by Lindner et al. (31) and Taira (32), but their lysine content of glutelin was slightly higher.

Rice prolamin is relatively low in proline but high in glutamic acid content compared with other cereal prolamins (33).

Rice glutelin's ammonia content amounted to only 60 mole % of that of aspartic and glutamic acids combined, and indicated that these two acids are not entirely in amide form, asparagine, and glutamine, in this protein. The

low cystine content of glutelin obtained may be due in part to cystine sulfur decomposition to hydrogen sulfide in alkali, as reported by Concon (34) for corn glutelin.

The protein fractions contained traces of ribonucleic acids and carbohydrates. Pentose contents of albumin-globulin and glutelin were 13.0 and 7.7 mg. %, respectively. The glutelin was relatively pure among the three preparations (Table II). In the case of prolamin, paper-chromatographic analysis showed that its major sugar component was glucose (8). The presence of carbohydrate in rice prolamin preparation has also been reported by Houston and Pence (35). Kondo and Morita (36) similarly reported the presence of nucleic acids in rice glutelin preparations.

Although Mitsuda et al. (6) have isolated rice protein bodies by discontinuous sucrose density gradient ultracentrifugation, nonaqueous isolation methods offer the advantage of avoiding solubilization during protein extraction. Protein solubilization might produce artifacts caused by interactions between grain constituents. However, fractionation of ball-milled rice endosperm flour by nonaqueous flotation into wedge protein and starch-adhering protein was inefficient. Only a 1% yield of wedge protein with 52% wet basis protein content was obtained. By contrast, Hess (10) reported 80% purity of wedge protein from wheat flour. In addition, amino acid analysis of the starch-adhering protein residue after flotation at $d^{21} = 1.42$ gave an aminogram with the same glutamic acid but a slightly higher lysine content compared to that of the starting flour (Table II). Röhrlich[3] found that adhering protein of wheat had a higher lysine and a lower glutamic acid content than wedge protein. Endosperm protein isolated from wheat flour by air-classification followed by flotation had a lower lysine and a higher glutamic acid content than the parent flour (37). Vones et al. (19) found that the wedge protein of rye had higher prolamin and glutelin fractions than adhering protein. It is interesting that Silaev and Lieh (38) also found that the aminograms of wedge and adhering proteins of five rice varieties were identical.

Similarly, fractionation was poor in ball-milling of brown rice, although recovery of the fraction with density below 1.37 was greater.

The poor fractionation obtained by flotation of rice flour as compared to wheat flour is consistent with results of another physical separation method—air-classification. Whereas high-protein fractionation was obtained from fine wheat flours, very little protein fractionation was attained with rice flour (39). One contributing factor mentioned was the small size of rice starch granules compared with those of wheat, which would affect the efficiency of the operation. Houston et al. (40) recently reported appreciably greater protein fractionation of rice flour by the use of a finer-particle material. Wolf et al. (41) observed that production of free starch and free protein in wheat flour is primarily a matter of breaking down the association of protein with starch granules above 10 μ in diameter. Only minor quantities of free protein are liberated with the freeing of small starch granules. Microscopic examination of the ball-milled rice sample indicated that the starch

granules were already discrete and no longer birefringent under polarized light. It is evident that because of the smaller starch granules of rice, more drastic methods than those employed for wheat may be required to physically separate wedge protein of rice from starch granules. Niederauer[1] also obtained similar poor fractionation of milled rice by flotation.

Acknowledgment

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