Correlations between the Physicochemical and Functional Properties of Rice

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Three typical U.S. rice varieties were studied for differences after storage for 1 year at temperatures between 4 and 40 °C. In this manner, rice samples with a relatively wide range of physicochemical and functional properties were obtained. From the experimental results it became apparent that water intake by cooked grains, stickiness after cooking, and rice flour dough leavening were all correlated to the quality determining factors: the average molecular weight of denatured oryzenin (rice storage protein) and the binding of denatured oryzenin to starch. On the basis of these facts from one chosen quality, other qualities (quality-determining factors) of rice could be predicted.

INTRODUCTION

Functional properties of rice are important because they define the commercial usefulness of rice in different food products (Furia, 1972). Cooking, processing, and baking qualities of rice establish the economic values of rice grains and flours (Juliano, 1985). Most of rice is polished (milled) by about 10–20% in order to get rid of the bran outer layers which have some undesirable properties, like increased hardness and rancidity. Great part of polished rice is consumed directly after cooking but an increasingly significant part of rice is used after milling to flour as an additive to gels, puddings, ice creams, and other similar products (Furia, 1972). Rice starch is used in cosmetics.

Important qualities of rice are, for example, water absorption, swelling, fragility, hardness, and gelation properties. Cooking qualities depend on cooking time, stickiness, cohesiveness, and tenderness. Some correlations have been found between the cooking properties and the protein content (Juliano et al., 1965). The importance of –S–S– bonds for the cooking properties of rice was also emphasized (Hamaker and Griffin, 1990). Although rice does not have gluten and thus its baking properties are inferior to wheat or rye, it is often used as an additive in different baking products (Furia, 1972). Baking qualities are characterized, for example, by dough leavening.

Rice shows great varietal differences, and the functional and physicochemical properties of rice grains or flours are subject to changes during storage (Kondo and Okamura, 1937; Tani et al., 1964; Barber, 1969; Shibuya et al., 1974; Villareal et al., 1976; Perez and Juliano, 1981; Ramarathnam and Kulkarni, 1984; Juliano, 1985). These changes are very significant and are important for the rice industry.

Recently, I have described the changes of physicochemical and functional properties of rice grains during storage at different temperatures for different time intervals (Chrastil, 1990a–c) and have shown that stickiness of cooked rice grains was correlated to starch–protein interactions.

In this work, in order to obtain a set of rice samples with different physicochemical and functional properties, we studied varietal and storage differences of rice grains in a wide range of storage conditions. Three typical U.S. varieties of rice grains were stored at different temperatures for different time intervals. In this manner we have obtained 21 samples of rice grains (7 for each variety) with different physicochemical and functional properties. Some of these properties were analyzed and related to each other.

EXPERIMENTAL PROCEDURES

Materials. All chemicals were analytical reagents of the highest obtainable purity from Sigma Chemical Co., St. Louis, MO, or Aldrich Chemical Co., Milwaukee, WI.

Rice Storage. Rice was obtained from rice producers and kept at room temperature for 14 days before storage. Total time after harvest was less than 1 month. Polished (20% removed) rice grains of three U.S. varieties [Lemont (long), Mars (medium), and S-201 (short)] were stored in triplicate in closed jars at 4, 25, 30, 35, and 40 °C. The relatively large polish was removed to be sure that the polished rice was only endosperm. Before storage the samples were cleared of small debris and broken grains. After storage the jars were cooled to room temperature (25 °C) and opened. Portions of the grains before and after storage were ground to flour for dough leavening measurements.

Moisture Content. Moisture content was obtained by drying the rice grains to constant weight at 110 °C. The accuracy of this method was sufficient for our purpose.

Total Protein. Total protein was determined by the modified micro-Kjeldahl method (Meyer, 1938). The protein content was calculated by using the factor 5.95 (Juliano, 1985).

Total Starch. Starch content was determined in flour by a modified colorimetric method of Clegg (1956).

Grinding. Rice grains were ground to flour in a micromill (Technilab Instruments, Pequannock, NJ). All rice grains were ground in the same manner (10 g of grain, 3 min of grinding). The temperature of the flour after grinding was always less than 35 °C.

Water Absorption by Cooked Rice. Unbroken stored rice grains (100 g) were added to boiling water (500 mL). During cooking, the grains were mixed by a magnetic stirrer and the volume of the liquid was kept constant by adding boiling water. After 30 min the supernatant was decanted, and the cooked rice was quickly washed three times with MeOH (300 mL). The surface of the rice grains was dried with filter paper and the rice was weighed. Water absorption was expressed in percent.

Stickiness. The stickiness of the rice grains after cooking was determined by the rice cluster distribution method (Chrastil, 1990a). Cooked rice and clusters of sticky rice grains, prepared as described above for water absorption by cooked rice, were spread on a glass plate. Single grains and clusters were gently separated without damaging the clusters and without using force or sharp instruments to break the clusters. The clusters and single grains were counted and the stickiness was calculated from the equation

$$n_{max} = -[\ln[1-(N/N_o)]]^{-1}$$

where N is the number of clusters + single grains after cooking and N_o is the total number of grains before cooking.

Dough Leavening. Rice flour dough was prepared by mixing and kneading flour (5 g) with commercial Fleishman's yeast (0.1 g), sucrose (0.1 g), and water (4 mL) at room temperature (25 °C). The dough was transferred into a 30-mL graduated test tube, the inner surface of which was covered by a thin layer of oil, and compressed to the bottom of the tube by a piston. The piston was removed and the test tube immersed in a water bath at 40 °C. After 30 min, the volume of the dough was read. The results were expressed in relative percent.

Preparation of Oryzenin. Rice flour (20 g) was extracted by sonication (Tekmar Sonic Disrupter, used power 20 W) in 40 mL of ether plus 40 mL of MeOH for 1 h at 0–5 °C (in ice–water bath). The extracted flour was centrifuged at 3000*g* for 15 min, and the extraction was repeated twice. After the last extraction, the defatted flour was dried in air, extracted by sonication in 100 mL of H_2O for 1 h at 0–5 °C (albumin extract) and centrifuged at 3000*g* for 15 min. This extraction was repeated three times. The flour (still wet) was then extracted by sonication in 100 mL of 5% NaCl at 0–5 °C (globulin extract) and centrifuged at 3000*g* for 15 min. This extraction was also repeated three times. Finally, the flour was extracted three times with 100 mL of 70% EtOH (prolamin fraction) and three times with 100 mL of H_2O (to wash the remaining salt and alcohol).

Oryzenin was then extracted by sonication in 100 mL of 0.025 M NaOH at 0–5 °C and centrifuged at 3000*g* for 15 min. The extraction was repeated three times. Combined supernatants were precipitated by 70% TCA (final TCA concentration about 5%) and centrifuged at 3000*g* for 15 min. The pellets were washed twice with 5% NaCl, water, and 70% EtOH and centrifuged again. Finally, the pellet was washed twice with 100 mL of acetone and dried in vacuo at room temperature (25 °C). The purity was controlled by the colorimetric methods for protein (Lowry et al., 1951) and starch (Clegg, 1956). In separate experiments the oryzenin from the same rice samples was extracted by 100 mL of 1.5% lactic acid. The extraction procedure was the same as with 0.025 M NaOH. Because of the buffering effect of protein the final pH of the alkaline extract was 7.5 and of the lactic acid extract 3.2.

Preparation of Starch. The flour after the extraction of oryzenin was sonicated in 200 mL of DMSO for 1 h at room temperature (the suspension was cooled so that the temperature did not reach 50 °C) and centrifuged at 3000*g* for 15 min. The extraction was repeated twice. Warm suspension was filtered through Whatman no. 4 filter paper and poured slowly into 1 L of EtOH with intensive mixing. Precipitated starch was centrifuged at 3000*g* for 15 min, washed three times with 200 mL of acetone, and dried in vacuo at room temperature (25 °C). Protein and starch content in the starch samples was controlled by the colorimetric methods of Lowry et al. (1951) and Clegg (1956), respectively. Amylose content in starch was determined by the method of Chrastil (1987).

Molecular Weights of Oryzenin and Starch. The average molecular weights of the purified oryzenin and/or starch were determined from intrinsic viscosities by the equations shown elsewhere (Chrastil, 1990b).

Binding of Oryzenin to Starch. The equilibrium binding constants of oryzenin on starch were determined from the differential spectra at 285 nm by the equations

$$K_{eq} = \Delta A / P^n S^m$$

or

$$K_b = K_{eq} M_P^n \cdot M_S^m$$

where K_{eq} and K_b are the apparent equilibrium binding constants (g/L or molar) and n and m are the molar binding ratios of oryzenin on starch (Chrastil, 1990b).

RESULTS AND DISCUSSION

By storage of three typical U.S. varieties of rice at different temperatures and time intervals it was possible to obtain a relatively wide range of physicochemical and functional properties.

Moisture Content. The moisture content in analyzed rice grain samples was in the limits of 12.1 ± 0.2, 12.0 ± 0.2, and 11.9 ± 0.2%, in long, medium, and short rice samples, respectively. It did not change during storage and remained in the same limits of error.

Total Protein. The amount of total protein was 7.6 ± 0.02, 8.3 ± 0.02, and 8.9 ± 0.02%, in long, medium, and short rice grain samples, respectively.

Total Starch. The amount of total starch was 90.1 ± 0.3, 89.7 ± 0.3, and 90.0 ± 0.3%, in long, medium, and short rice grain samples, respectively.

Extraction Efficiency. More than 97% of total protein and/or total starch was extracted from rice grains in all cases. All oryzenin samples were more than 98% pure proteins and starch more than 99% pure starch.

Oryzenin. Oryzenin is insoluble in water or salts and all oryzenin preparations (extractions by alkali or acid) are denatured samples. Protein association forces and/or part of the –S–S– bonds are alkali or acid labile and thus the "in vivo" oryzenin should have much higher molecular weight than the denatured oryzenin. During preparation and precipitation, only part of the reversible –S–S– bonds returns to its original configuration and the association forces are irreversibly destroyed. However, under the same extraction conditions, if the differences in the denatured oryzenin or its subunits (which are always prepared and analyzed under the drastic urea and SDS conditions) are significantly high we may assume that they represent some corresponding changes in the "in vivo" oryzenin. Otherwise we cannot study oryzenin and many similar proteins at all.

The average molecular weight of denatured oryzenin prepared under the same conditions was different in long, medium, and short rice grain varieties and increased in stored samples by up to over 100%. The average variation of the mean from triplicates was ±2 kDa (Table I). The average molecular weight of the oryzenin extracted by lactic acid was the same. The differences were smaller than ±2% and were neglected. The increase in molecular weight of oryzenin during storage was caused mainly by the increased –S–S– bonds and the changes in subunit composition (Juliano, 1985; Chrastil, 1990a–c).

Starch. The average molecular weight of starch did not change much in stored samples (see Chrastil, 1990b). The average molecular weight of starch was 2.01×10^6, 2.4×10^6, and 2.24×10^6 in long, medium, and short rice grains, respectively. The maximum change during storage was about ±5% and was neglected. The above average molecular weight values were used in the calculation of the equilibrium binding constants, K_b. Amylose content in starch was slightly higher in stored samples (see Chrastil, 1990b), but these changes were much smaller (plus 0.2–0.6, 0.1–0.5, and 0.1–0.5% amylose increase in stored samples, in long, medium, and short rice grains, respectively) than the varietal differences (26.2 ± 0.1, 17.0 ± 0.08, and 19.1 ± 0.09% amylose in starch from long, medium, and short rice, respectively). Generally, the structural changes of starch during storage could be neglected when compared to the relatively large physicochemical and structural changes of oryzenin.

Oryzenin–Starch Binding. We could not determine how much oryzenin was bound per gram (mole) of starch because we did not have a pure standard of oryzenin–starch complex in order to measure its absorbance at 285 nm. However, from the differential absorbance ΔA, obtained with different concentrations of oryzenin and starch, we could determine the $n{:}m = r_{os}$ (molar ratios of oryzenin to starch in the binding complex) and/or the apparent binding constants, K_{eq} or K_b.

The equilibrium binding of oryzenin on starch in long, medium, and short rice grain varieties was different and

Table I. Physicochemical and Functional Parameters of Rice Samples[a]

rice	stored at °C	MW, kDa	r_{os}	WC, %	ST: n_{max}	DL, %
long	C	188 ± 0.5	1.55 ± 1	134 ± 0.5	3.9 ± 2	57 ± 1
	4	191 ± 0.6	1.43 ± 1	135 ± 0.3	3.4 ± 2	59 ± 0.5
	25	204 ± 0.3	1.20 ± 2	137 ± 0.4	1.4 ± 2	61 ± 0.6
	30	208 ± 0.4	1.12 ± 2	137 ± 0.6	0.9 ± 3	63 ± 0.9
	35	211 ± 0.5	1.07 ± 1	137 ± 0.5	0.6 ± 2	64 ± 1
	40	216 ± 0.5	0.99 ± 2	138 ± 0.4	0.01 ± 5	65 ± 0.7
	40[b]	218 ± 0.4	0.88 ± 1	139 ± 0.6	<0.01 ± 10	66 ± 0.6
medium	C	119 ± 0.4	2.70 ± 2	124 ± 0.3	12.1 ± 2	45 ± 0.5
	4	120 ± 0.3	2.60 ± 2	126 ± 0.6	11.3 ± 2	47 ± 0.7
	25	155 ± 0.4	2.06 ± 1	130 ± 0.3	7.5 ± 1	53 ± 0.6
	30	163 ± 0.4	1.92 ± 1	131 ± 0.4	6.5 ± 3	54 ± 1
	35	167 ± 0.5	1.85 ± 1	132 ± 0.4	6.0 ± 2	55 ± 0.9
	40	176 ± 0.4	1.70 ± 2	134 ± 0.6	5.0 ± 4	57 ± 0.7
	40[b]	193 ± 0.3	1.48 ± 1	135 ± 0.3	3.4 ± 2	61 ± 0.5
short	C	158 ± 0.3	2.01 ± 1	132 ± 0.3	7.2 ± 1	51 ± 0.9
	4	162 ± 0.2	1.94 ± 2	133 ± 0.2	6.7 ± 2	52 ± 1
	25	181 ± 0.4	1.53 ± 1	135 ± 0.3	3.8 ± 4	57 ± 0.5
	30	192 ± 0.5	1.42 ± 2	135 ± 0.4	3.0 ± 3	59 ± 0.7
	35	200 ± 0.5	1.34 ± 2	135 ± 0.3	2.4 ± 1	62 ± 0.5
	40	201 ± 0.4	1.26 ± 1	137 ± 0.5	1.8 ± 4	64 ± 0.8
	40[b]	212 ± 0.4	1.05 ± 1	137 ± 0.2	0.4 ± 3	67 ± 1

[a] The rice grains were stored for 12 months. The results are from triplicates with standard deviation of the mean in percent rounded to one digit. C = control (postharvest rice); MW = average molecular weight of oryzenin; r_{os} = n:m = equilibrium binding ration of oryzenin on starch; WC = water intake by cooked grains; ST = stickiness of cooked grains; DL = dough leavening. [b] Rice was stored for 18 months.

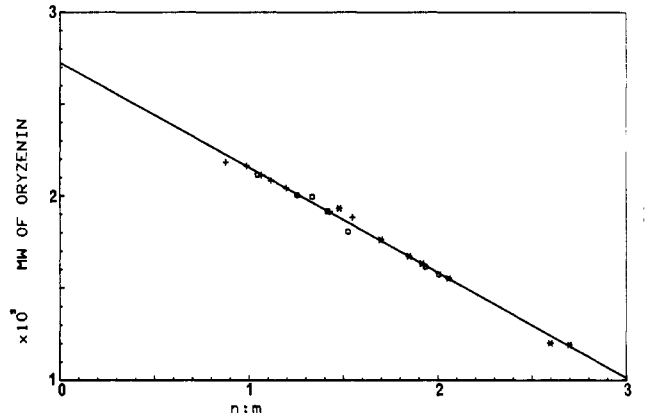

Figure 1. Correlation between equilibrium binding ratio n:m of oryzenin on starch and average molecular weight (MW) of oryzenin in different varieties of rice: + = long; * = medium; O = short. Lines were calculated by regression analysis.

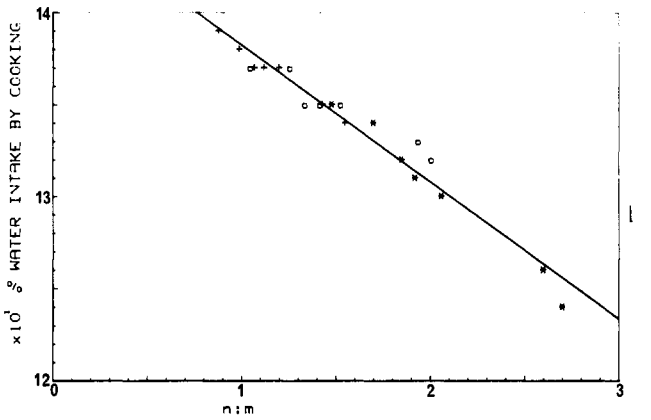

Figure 2. Correlation between equilibrium binding ratio n:m of oryzenin on starch and water intake by cooking: + = long; * = medium; O = short. Line was calculated by regression analysis.

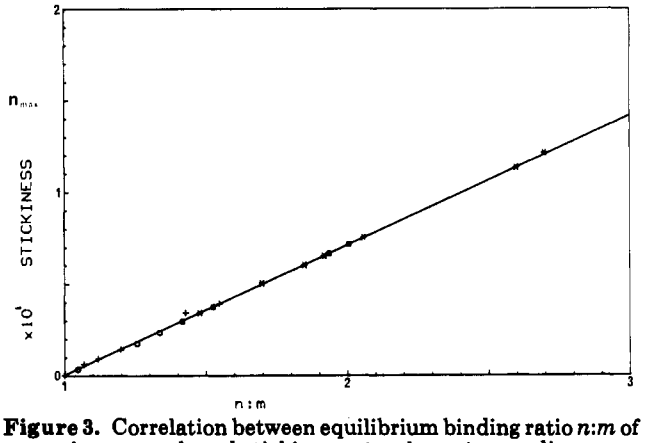

Figure 3. Correlation between equilibrium binding ratio n:m of oryzenin on starch and stickiness: + = long; * = medium; O = short. Line was calculated by regression analysis.

decreased in stored samples. The average variation of the mean from triplicates, r_{os}, was ±0.03 (Table I). The results with oryzenin extracted by lactic acid were the same with only small differences (±2%) which were neglected.

Water Absorption by Cooked Grains. The water intake by long, medium, and short cooked grains was different and increased in stored samples. The average variation of the mean from triplicates was ±0.4% (Table I).

Stickiness. The stickiness of long, medium, and short grains after cooking was different and decreased in stored samples. The average variation of the mean from triplicates was ±0.1 (n_{max}) (Table I).

Dough Leavening. The dough leavening from long, medium, and short grains was different and increased in stored samples. The average variation of the mean from triplicates was ±1% (Table I).

Correlations. From the mathematical analysis of the experimental results it was apparent that the physicochemical and functional properties of rice grains or flours were directly or indirectly correlated (Figures 1–4). No correlations were found between the physicochemical and functional properties and the total protein, starch, or amylose contents.

General correlations were found between average molecular weights of oryzenin, equilibrium oryzenin–starch binding constants, water intake by cooked grains, stickiness of cooked rice grains, and dough leavening.

For example, the equilibrium binding ratio, r_{os}, showed a descending linear correlation to the average molecular

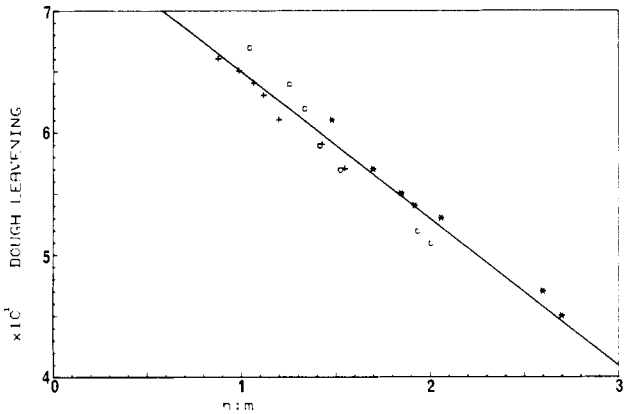

Figure 4. Correlation between equilibrium binding ratio *n:m* of oryzenin on starch and dough leavening: + = long; * = medium; o = short. Line was calculated by regression analysis.

weight of oryzenin (MW) [MW = 272508 − 57080.8 × r_{os} with the correlation coefficient r = 0.996 (Figure 1)], a descending linear correlation to the water intake by cooking (WC, %) [WC = 145.69 − 7.446 × r_{os} with the correlation coefficient r = 0.979 (Figure 2)], an ascending linear correlation to the stickiness of cooked grains (ST, n_{max}) [ST = −10.406 + 8.283 × r_{os} with the correlation coefficient r = 0.999 (Figure 3)], and a descending linear correlation to the dough leavening (DL, %) [DL = 79.98 − 12.012 × r_{os} with the correlation coefficient r = 0.977 (Figure 4)]. Because of the above correlations, mathematically, there must also exist linear correlations obtained by cross-linking between these physicochemical and/or functional properties (2 vs 3, 2 vs 4, and 3 vs 4).

These correlations do not tell us what is the determining or limiting factor, but from our previous work (Chrastil, 1990a,b,c), we may assume that the main determining factors for these physicochemical and functional properties of rice are the molecular weight of oryzenin and the equilibrium binding of oryzenin on starch.

The oryzenin–starch interactions and the determinations of molecular weights of oryzenin were effected with denatured protein in alkaline pH, because of solubility problems. However, as it is apparent from the studies of Dahle (1971) it may be assumed that in the natural system at pH 6 or below the protein–starch interactions should be much stronger.

In cereals, storage protein can be found either in specialized membrane-bound protein bodies or packed in starchy endosperm cells as part of a continuous protein matrix (Lorenz and Kulp, 1991). During the gelatinization (cooking) process, water disrupts the protein matrix, part of the protein bodies, and the starch granules. Amylose diffuses out of the granules which eventually collapse and are held in a matrix of amylose. This enables the starch–protein contact and the formation of a protein–starch matrix (Moore and Carter, 1974; Remsen and Clark, 1978). The detailed relationship of the denatured oryzenin to its natural counterpart will be studied in our future work.

LITERATURE CITED

Barber, S. Basic Studies on Aging of Milled Rice and Application to Discriminating Quality Factors. *U.S.D.A. Foreign Res. Program* **1969**, *E25-AMS*, 1–189.

Chrastil, J. Improved Colorimetric Determination of Amylose in Starches and Flours. *Carbohydr. Res.* **1987**, *159*, 154–158.

Chrastil, J. Chemical and Physicochemical Changes of Rice During Storage at Different Temperatures. *J. Cereal Sci.* **1990a**, *11*, 71–85.

Chrastil, J. Protein-Starch Interactions in Rice Grains. Influence of Storage on Oryzenin and Starch. *J. Agric. Food Chem.* **1990b**, *38*, 1804–1809.

Chrastil, J. Influence of Storage on Enzymes in Rice Grains. *J. Agric. Food Chem.* **1990c**, *38*, 1198–1202.

Clegg, K. M. The Application of the Anthrone Reagent to the Estimation of Starch in Cereals. *J. Sci. Food Agric.* **1956**, *7*, 40–44.

Dahle, L. K. Wheat protein-starch interactions. I. Some starch binding effects of wheat-flour proteins. *Cereal Chem.* **1971**, *48*, 706–714.

Furia, T. E., Ed. *Handbook of Food Additives*; CRC Press: Cleveland, OH, 1972; pp 305–344.

Hamaker, B. R.; Griffin, V. K. Changing the Viscoelastic Properties of Cooked Rice Trough Protein Disruption. *Cereal Chem.* **1990**, *67*, 261–264.

Juliano, B. O. Rice. *Chemistry and Technology*; AACC: St. Paul, MN, 1985.

Juliano, B. O.; Onate, L. U.; del Mundo, A. M. Relation of Starch Composition, Protein Content, and Gelatinization Temperature to Cooking and Eating Qualities of Milled Rice. *Food Technol.* **1965**, *19*, 1006–1011.

Kondo, M.; Okamura, T. Storage of Rice. Comparative Study of Unhulled Rice and Hulled Rice in Regard to the Changes of its Qualities During Long Storage in Straw Bags. *Ber. Ohara Inst. Arg. Biol. Okayama Univ.* **1937**, *7*, 483–490.

Lorenz, K. J.; Kulp, K. *Handbook of Cereal Science and Technology*; Marcel Dekker: New York, 1991.

Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

Meyer, H. *Analyse der Organischen Verbindungen*; Springer Verlag: Wien, 1938.

Moore, W. E.; Carter, J. L. Protein Carbohydrate Interactions at Elevated Temperatures. *J. Texture Stud.* **1974**, *5*, 77–88.

Perez, C. M.; Juliano, B. O. Texture Changes and Storage of Rice. *J. Texture Stud.* **1981**, *12*, 321–333.

Ramarathnam, N.; Kulkarni, P. R. Effect of Storage on the Physicochemical Properties of Rice Starch and the Cooking Quality of Rice Grain. *Z. Ernahrungswiss.* **1984**, *23*, 143–150.

Remsen, C. H.; Clark, J. P. A Viscosity Model for a Cooking Dough. *J. Food Process. Eng.* **1978**, *2*, 39–64.

Shibuya, N.; Iwasaki, T.; Yanase, H.; Chikubu, S. Studies on Deterioration of Rice During Storage. *J. Jpn. Soc. Food Sci. Technol.* **1974**, *21*, 597–603.

Tani, T.; Chikubu, S.; Iwasaki, T. Changes in Chemical Properties if Rice During Storage at Controlled Temperature. *J. Jpn. Soc. Food Nutr.* **1964**, *16*, 436–442.

Villareal, R. M.; Ressurection, A. P.; Suzuki, L. B.; Juliano, B. O. Changes in Physicochemical Properties of Rice During Storage. *Staerke* **1976**, *28*, 88–94.

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