Protein–Starch Interactions in Rice Grains. Influence of Storage on Oryzenin and Starch

J. Chrastil

Southern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, New Orleans, Louisiana 70179

Influence of storage on oryzenin, starch amylose, and amylopectin has been studied in grains of two U.S. rice varieties (Lemont, long grain, and Mercury, medium grain). Although total protein and starch content did not change during storage, protein solubility decreased. Storage of rice grains, especially at higher storage temperatures, resulted in increased disulfide bonds and average molecular weight of oryzenin. On the other hand, the average molecular weight of amylose decreased, but that of amylopectin increased. Oryzenin interacted with starch by reversible binding to amylopectin and/or amylose. This binding decreased during storage, especially at higher storage temperatures, and was related to the stickiness of cooked rice.

Postharvest changes influence chemical, physicochemical, and functional properties of rice grains. Although average composition, as provided by total protein, starch, and lipids, changes very little, the physicochemical and functional properties of rice change significantly during storage (Juliano, 1985).

Total starch did not change during storage of rice grains, but after longer storage some increase in starch degradation products was observed (Kester et al., 1956; Desikachar, 1956; Tani et al., 1964; Iwasaki and Tani, 1967; Barber et al., 1968; Shoji and Kurasawa, 1981).

Rice grain protein consists mainly of the storage protein, called rice glutelin or oryzenin (over 80–90% of total protein). The rest of the proteins are albumins, globulins, and prolamins. Although some of the rice grain protein fractions have been analyzed by chromatography, electrophoresis, and/or amino acid analysis, their changes during storage have not been studied in detail (Jones and Czonka, 1927; Sawai and Morita, 1968; Tecson et al., 1971; Juliano and Boulter, 1976; Villareal and Juliano, 1978; Yamagata et al., 1982; Zhao et al., 1983).

Because little is known in detail about the storage changes of starch and proteins and their interactions, in the present investigation we have further examined some chemical and physicochemical changes and interactions of oryzenin and starch in two U.S. rice varieties during storage at different temperatures.

EXPERIMENTAL PROCEDURES

Materials. All chemicals were analytical reagents of the highest obtainable purity from Sigma Chemical Co., St. Louis, MO, or J. T. Baker Chemical Co., Phillipsburg, NJ.

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

Rice Storage. Highly polished (30% removed) rice grains (less than 1 month after harvest) of two U.S. rice varieties (Lemont, long grain, and Mercury, medium grain) were stored in triplicates in closed jars at 4 and 40 °C. At the beginning of storage and after 12 months, the grains were ground to flour for subsequent tests. These triplicates were used as a starting material for all extraction and analytical experiments.

Grinding. Rice grains were ground in a water-cooled micromill (Technilab Instruments, Pequannock, NJ) to flour (10 g of grains, 3 min of grinding). The flour was sieved, and the fractions with less than 0.01-mm particle size were used for extractions.

Total Protein. Total protein was determined by the modified micro-Kjeldahl method (Meyer, 1938; Folin and Farmer, 1912). The nitrogen content thus obtained was corrected for moisture content, and the protein content was calculated by using the factor 5.95 (Juliano, 1985).

Alkali-Extractable Protein. Flour was extracted by shaking with 0.01 M NaOH (1 + 2 w/v) for 1 h at 25 °C. The pH of the flour suspension in 0.01 M NaOH (1 + 2 w/v) was close to 7 (about 7.1). The suspension was immediately centrifuged at 40000*g* in a cold rotor (4 °C) for 15 min. The supernatant was filtered (Whatman No. 2), diluted (1 + 50 v/v), and analyzed for protein by the method of Lowry (Lowry et al., 1951), using pure egg albumin as a standard.

Starch Content in Flour. Total starch was determined by a modified colorimetric method of Clegg (1956). Ten milligrams of rice flour was weighed on a microbalance. One milliliter of 1 M NaOH and 2 mL of water were added, and the sample was heated in capped tube on a waterbath at 95 °C for 30 min with occasional mixing. After cooling, 0.1 mL of the sample was diluted by 5 mL of H_2O. Five hundred microliters of the diluted sample was heated with 2.5 mL of anthrone solution (760 mL of 96% H_2SO_4 plus 1 g of anthrone per liter) at 100 °C for 15 min. After cooling, the color was read at 630 nm vs H_2O. Five hundred microliters of glucose solution (50 mg/L) and 0.5 mL of H_2O were used as standard and blank, respectively. The standard curve was linear up to 0.8 absorbance.

Extraction of Rice Grain Components. Oryzenin, starch, amylose, and amylopectin were prepared by the modification of several classical methods (Whistler et al., 1945; Kurzman et al., 1973; Juliano, 1985).

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 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).

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. The 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).

Preparation of Amylose. Starch (8 g) was dissolved in 800 mL of boiling water and cooled to 60 °C. After the addition of 2 g of thymol, the solution was left overnight at room temperature (25 °C). The precipitated amylose complex was centrifuged at 3000*g* for 15 min. To eliminate thymol, the pellet was washed three times with 200 mL of acetone and dried in vacuo at room temperature (25 °C). Dried amylose was dissolved in 200 mL of boiling water, and the thymol (1 g) precipitation was repeated as described above.

Preparation of Amylopectin. The supernatant from the amylose precipitation by thymol was evaporated in vacuo at room temperature almost to dryness. The thymol precipitation was repeated with 200 mL of boiling water and 0.5 g of thymol. The solution was filtered with a Millipore filter (0.5 μm), and amylopectin was precipitated by pouring the filtrate into 1 L of acetone and 50 mL of 10 M HCl. The suspension 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 Content in Oryzenin and Starch. Oryzenin, starch, amylose, or amylopectin was dissolved in 0.1 M NaOH (10 mg/mL). Oryzenin solution was diluted 1:50 and starch, amylose, and/or amylopectin solutions were used directly for protein determination by Lowry's method (Lowry et al., 1951).

Amylose Content in Oryzenin and Starch. For this analysis, oryzenin and amylopectin solutions (see above) were used undiluted, starch solution was diluted 1:4, and amylose solution was diluted 1:10. Amylose was determined by a colorimetric method (Chrastil, 1987). Two hundred microliters of oryzenin, starch, amylose, or amylopectin solutions was pipetted into 5 mL of 0.5% TCA in 10-mL test tubes. The solutions were mixed, and 0.05 mL of 0.01 N I_2/KI solution (1.27 g/L I_2 plus 3 g/L KI) was added to each tube and mixed immediately. The absorbance was read after 30 min at 25 °C (room temperature) at 620 nm vs H_2O in a Shimadzu 260 double-beam spectrophotometer. The standard was pure potato amylose, and the blank was H_2O.

Ultracentrifugation. Oryzenin, starch, amylose, and/or amylopectin solutions in 0.1 M NaOH were centrifuged at 20 °C at 200000*g*, 300000*g*, and 400000*g*, and the rate of sedimentation was determined by analyzing upper and lower layers in the centrifuge tube after 1, 2, and 6 h for protein. From that, the average sedimentation constant was calculated by conventional methods (Rickwood, 1984).

Viscosity. Viscosities of oryzenin, starch, amylose, and amylopectin solutions were measured at 25 °C with a Brookfield cone/plate digital viscosimeter Model DV-II using a 0.5-mL cone. Intrinsic viscosities (Billmeyer, 1984) were estimated from seven concentrations (0.25, 0.5, 0.75, 1.0, 2.0, 2.5, and 3.0%) of these compounds in 0.1 M NaOH by the statistical regression analysis.

Light Scattering. The unimodal average molecular weights of oryzenin and starch (1% solution in 0.1 M NaOH) were determined at 25 °C on the Coulter M4 SD submicron particle analyzer. The α and β constants of the analyzer were standardized by several protein solutions of known molecular weights (cytochrome *c*, bovine serum albumin, alcohol dehydrogenase, β-amylase, and thyroglobin).

Determination of Equilibrium Adsorption Contants. Difference spectra between the mixtures oryzenin plus starch (oryzenin plus amylose or oryzenin plus amylopectin) and oryzenin only versus starch (amylose or amylopectin) blanks were measured automatically on a programmable Shimadzu 260 double-beam spectrophotometer. Negative differential peaks were obtained at 285 nm. The peak heights were automatically read against the base line at 350 nm. To determine the adsorption equilibrium constants, the differential peaks were determined by combinations of seven concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.75, and 1.0 g/L of 0.1 M NaOH) of oryzenin and starch components in the mixtures, which resulted in 49 samples for each starch component. The equilibrium adsorption constants were calculated by statistical regression analysis of the experimental results.

Table I. Selected Chemical and Physical Properties of Stored Rice[a]

	medium grain			long grain		
	control	4 °C	40 °C	control	4 °C	40 °C
moisture	13.0	13.0	12.9	13.2	13.1	13.1
protein						
total, %	8.3	8.3	8.3	7.6	7.6	7.6
soluble, %	6.0	5.3	3.6	5.1	4.7	3.0
starch						
total, %	90.0	89.5	89.4	90.1	90.0	90.1
amylose, %	17.0	17.1	17.7	26.0	26.1	27.0
oryzenin						
S, % as -SH	0.20	0.19	0.13	0.16	0.16	0.14
S, % as -SS-	0.13	0.14	0.20	0.16	0.16	0.18
stickiness, n_{max}	9.5	9.1	5.0	3.0	2.3	0.5

[a] The results are the means from the rice sample triplicates. The variation of the triplicates of moisture, protein content, and total starch from the mean was less than ±2%. The variation of stickiness from the mean was less than ±5%. The statistical differences of amylose, cysteine, and cystine are shown in the text. Control is a postharvest rice. Rice was stored at 4 and 40 °C for 12 months.

Maximum Absorbance of Amylose–Iodine Complex. The colored reaction mixtures of amylose with iodine (see above) were scanned on a Shimadzu 260 double-beam spectrophotometer at 25 °C. Absorption peaks λ_{max} were compared to the molecular weight of amylose (standard curve was prepared from four potato amyloses with known molecular weights) by using the method of Bailey and Whelan (1961).

Cysteine and Cystine in Oryzenin. Oryzenin was dissolved in 10% formic acid, and the free -SH and -SS- bonds in oryzenin were determined by a direct method (Chrastil, 1989) without the hydrolysis of oryzenin.

Stickiness of Cooked Rice. Stickiness of cooked rice grains was determined by the rice cluster distribution curve method (Chrastil, 1989). Cooking time was 30 min (100 g of rice grains plus 500 mL of boiling water). Distribution curves were constructed from the cluster weight classes (with the range of 0.05 g) and expressed in percent of total weight. The maximum on the distribution curves was $n_{max} = -1/\ln(1 - N/N_0)$, where N_0 is the number of grains before cooking and N is the number of clusters (including single cooked grains) after cooking.

RESULTS AND DISCUSSION

Moisture Content. Moisture content in rice grains store as described above did not change significantly during storage (Lemont rice had 13.2 and 13.1% and Mercury rice had 13.0 and 12.9% water, before and after storage, respectively).

Total Protein. The amount of total protein in rice grains did not change during storage. Storage temperature (up to 40 °C) had no influence on total protein content (Table I).

Alkali-Extractable Protein. To determine the differences in protein extractability before and after storage of rice, we have chosen a slightly alkaline extraction (see Experimental Procedures). Acids (for example, lactic or formic acid) or strong alkalies (for example, 0.1 M or stronger NaOH) extracted all protein fractions by repeated extractions, and thus this would not show the solubility differences. On the other hand, in slightly alkaline medium (pH 7–7.5) the extractability of rice proteins was limited and decreased very significantly during storage, especially at higher storage temperatures (Table I). Albumins, globulins, and/or prolamins were easily extractable by 0.01

Table II. Molecular Weights of Rice Oryzenin and Starch Components[a]

rice	[η], mP	$M(\eta)$, $\times 10^5$	S	M(s), $\times 10^5$	λ, nm	$M(\lambda)$, $\times 10^5$	M(u), $\times 10^5$
oryzenin							
medium-grain rice							
control	0.094	1.03	6.52	1.19			1.2
4 °C	0.095	1.12	6.58	1.20			1.3
40 °C	0.102	2.01	9.12	2.06			2.0
long-grain rice							
control	0.096	1.22	6.98	1.33			1.2
4 °C	0.098	1.44	7.08	1.36			1.1
40 °C	0.102	2.01	9.08	2.05			2.1
amylose							
medium-grain rice							
control	0.356	1.24	4.80	1.15	612	1.20	
4 °C	0.352	1.18	4.86	1.18	611	1.16	
40 °C	0.341	1.03	4.50	1.02	608	1.04	
long-grain rice							
control	0.375	1.55	5.40	1.45	619	1.53	
4 °C	0.370	1.46	5.45	1.47	618	1.48	
40 °C	0.346	1.10	4.66	1.09	609	1.08	
amylopectin							
medium-grain rice							
control	0.750	29.9	26.06	31.0			
4 °C	0.756	31.0	26.64	31.1			
40 °C	0.762	32.0	27.15	32.3			
long-grain rice							
control	0.720	25.2	23.70	24.8			
4 °C	0.723	25.6	23.80	25.0			
40 °C	0.757	31.2	26.67	31.2			
starch							
medium-grain rice							
control	0.718	24.9	23.95	23.3			25
4 °C	0.721	25.3	23.97	25.4			24
40 °C	0.735	27.5	24.52	26.5			26
long-grain rice							
control	0.680	19.7	21.70	21.0			20
4 °C	0.685	20.3	21.76	21.1			20
40 °C	0.703	22.7	22.12	21.7			21

[a] Medium- and long-grain polished rice was stored at 4 and 40 °C. Control is a postharvest rice. The average molecular weights were determined from intrinsic viscosity [$M(\eta)$] in millipoise, from sedimentation constants [M(s)] in Swedberg units, from absorption maxima [$M(\lambda)$] (only amylose), and from light scattering [M(u)] as unimodal average molecular weight (only oryzenin and starch). The results are means from rice sample triplicates. The statistical differences are shown in the text.

M NaOH, and the decreased extractability was caused mainly by decreased solubility of oryzenin which constituted over 90% of the total rice protein (not shown here). This is understandable because the molecular weight of oryzenin almost doubled during storage (Table II).

Total Starch. The amount of total starch in rice grains did not change during storage. Storage temperature (up to 40 °C) had no influence on total starch content (Table I).

Extraction Efficiency. All oryzenin, starch, amylose, and amylopectin preparations were analyzed for protein, starch, and amylose (see Experimental Procedures). More than 97% of total protein and/or total starch was extracted from rice grains. Oryzenin preparations were over 98% pure protein, and the purity of starch, amylose, and/or amylopectin was better than 99%.

Amylose in Starch. Amylose content in starch from stored rice grains increased during storage, especially at higher storage temperatures (Table I). The increase was small but significant. For example, the amylose content determined as triplicates of triplicate medium-grain rice samples (see Experimental Procedures) before storage was 17.0, 17.1, 16.7, 17.3, 16.9, 17.2, 17.0, 16.8, and 17.0 and after storage at 40 °C was 17.7, 18.0, 17.6, 17.7, 17.5, 17.5, 18.0, 17.6, and 17.7. This resulted in statistical P values of 1.000.

Similarly, the amylose content determined as triplicates of triplicate long-grain rice samples before storage was 25.7, 26.3, 26.0, 26.2, 25.8, 26.1, 25.9, 26.0, and 26.0 and after storage at 40 °C was 27.3, 26.7, 27.0, 27.3, 26.7, 27.1, 26.9, 27.0, and 27.0 with statistical P values of 1.000. These changes could not be caused by differences in extraction or amylose and/or amylopectin purity because the extraction of starch was always better than 97–98% and the purity of amylose and amylopectin (determined colorimetrically) was always better than 98%. Thus, even if just amylose would remain nonextracted before storage and amylopectin after storage (which is highly improbable), it would still be an apparent amylose increase.

Cysteine and Cystine in Oryzenin. Part of the –SH groups of oryzenin were oxidized to –SS– bridges during storage. The oxidation was significant but not complete. For example, approximately 40% of total cysteine was in oxidized form after harvest and about 60% after storage for 12 months at 40 °C (Table I). Although the total cysteine plus cystine content in oryzenin prepared from different postharvest or stored rice varieties was quite constant (0.3–0.4% S), the ratio of –SH to –SS– in different varieties before and after storage varied significantly (from 1.6 to 0.5). In two varieties studied in this work cystine in oryzenin increased during storage, but the relative extent of this increase was not directly related to the storage time, storage temperature, and/or rice variety.

For example, cysteine in oryzenin from triplicate medium-grain rice samples before storage was 0.20, 0.18, and 0.22 (% S) and after storage at 40 °C was 0.13, 0.13, and 0.13. On the other hand, cystine in the same samples was 0.13, 0.14, and 0.12 and 0.19, 0.20, and 0.21 before and after storage, respectively. The cysteine content in oryzenin from long-grain rice before and after storage was 0.16,

0.16, and 0.16 and 0.14, 0.13, and 0.15 and cystine content 0.15, 0.16, and 0.16 and 0.18, 0.17, and 0.19, before and after storage, respectively. The statistical P values were 1.000 in both cases.

Average Molecular Weight of Oryzenin. Although, in the literature, there exist several empirical formulas relating the molecular weights to the sedimentation constants or intrinsic viscosities to different classes of compounds including some proteins (Rickwood, 1984), we have derived our own formula by using over 500 tabulated sedimentation constants of different proteins (Rauen, 1956; Brandrup, 1966; Sober, 1970) for the determination of an approximate relationship between the sedimentation constant (S in svedbergs) and the molecular weight. Tabulated proteins that have very little structural similarity to the rice grain storage proteins, like metalloproteins, histones, mucoproteins, snake venoms, toxins, elastins, fibrins, enzymes, and growth hormones, were excluded from the calculation. By statistical regression analysis of the tabulated experimental values the power function

$$M_S = 5395S^{1.648} \quad (1)$$

was found. The relationship between molecular weights and intrinsic viscosities was determined by a similar procedure as shown above. At first, the relationship was found between the tabulated diffusion constants (corrected for 25 °C) and molecular weights of proteins. The result of the statistical regression analysis was $M_D = 1.043 \times 10^{-12} D^{-2.71}$. Then this equation was transformed into the intrinsic viscosity ($[\eta]$ in millipoise) and molecular weight relationship by using the Stoke's formulas and our viscosity measurements of 30 protein standards (not shown here). The final equation was

$$M_\eta = 2.685 \times 10^{13}[\eta]^{8.197} \quad (2)$$

Equations 1 and 2 were used for the calculation of the average molecular weight of oryzenin from experimental sedimentation constants and intrinsic viscosities.

The molecular weights of dextrans, starches, and amyloses used as standards were determined by the manufacturer and designated on the commercial samples. The molecular weight of rice starch was determined on starches prepared from rice grains, and the molecular weight of amylose and/or amylopectin was determined on amylose and/or amylopectin isolated and purified from the rice starch as described under Experimental Procedures.

As is apparent from Table II, the molecular weight of oryzenin at higher storage temperatures almost doubled. Similar results were also obtained from the light-scattering data. The difference between the molecular weights of postharvest oryzenin and the oryzenin from rice stored at low temperatures (4 °C) was small. Thus, at higher storage temperatures, oryzenin associated to larger molecules, which might be one of the main causes of decreasing protein solubilities and extractabilities during storage.

Average Molecular Weight of Starch and Its Components. At first, the sedimentation constants (at 20 °C) and the intrinsic viscosities (at 25 °C) of 28 dextrans, starches, and amyloses (with known molecular weights from 10^4 to 5×10^6) were determined in 0.1 M NaOH. These standard curves were analyzed by statistical regression analysis (the correlation coefficients were better than 0.99) and expressed in the forms

$$M_S = 5643S^{1.923} \quad (3)$$

from sedimentation constant, and

$$M_\eta = 1.023 \times 10^7[\eta]^{4.271} \quad (4)$$

from intrinsic viscosity.

These equations were used for the determination of molecular weights of starch, amylose, and amylopectin. The molecular weight of starch and amylopectin increased during storage, especially at higher storage temperatures (Table II). For example, the molecular weight of amylopectin from long-grain rice triplicates, determined from viscosity, was before storage 25.0, 25.2, and 25.4×10^5 and after storage at 40 °C 31.3, 31.0, 31.2×10^5. Thus, the statistical P value was 1.000.

On the other hand, the molecular weight of amylose decreased slightly but significantly. The molecular weight of amylose from the same rice samples as above was before storage 1.56, 1.53, and 1.57×10^5 and after storage at 40 °C 1.10, 1.09, and 1.12×10^5. The statistical P value was 1.000. The statistical differences in other cases (not shown here) were equally significant (with $P > 0.999$).

This was confirmed also by the changes of maximum absorbance of the amylose–iodine complex during storage. The empirical correlation between λ_{max} and the molecular weight of amylose was determined by measuring λ_{max} of five amylose standards with known molecular weights. For the molecular weights from 10^4 to 10^6 the correlation was (correlation coefficient 0.97)

$$M_\lambda = 2.603 \times 10^{-55}\lambda_{max}^{21.41} \quad (5)$$

There was another possibility to check these results. The molecular weights and contents of the starch components must be related by the equation MW starch = ((100 − % amylose)·MW amylopectin + % amylose·MW amylose))/100. Thus, by substituting the average values, for example, for long-grain rice, in this equation we get $2.0 \times 10^6 = ((100 - 26) \times (2.5 \times 10^6) + 26 \times (1.51 \times 10^5))/100 = 1.9 \times 10^6$ before storage and $2.2 \times 10^6 = ((100 - 27) \times (3.12 \times 10^6) + 27 \times (1.09 \times 10^6))/100 = 2.3 \times 10^6$ after storage at 40 °C. The agreement was good in all cases (not shown here), and it was another proof that these changes occurred and were not caused by some experimental errors.

The specific activities of most enzymes in rice grains are not depleted, and some are even enhanced by storage (Chrastil, 1990); it is possible that some of them may participate in chemical and physicochemical changes occurring in stored rice (even at low moisture content). Thus, some enzymic debranching during storage cannot be completely excluded. This would explain the small but statistically significant changes of amylose (decrease) and amylopectin (increase).

Binding of Oryzenin to Starch Components. From the differential spectra at 285 nm (Figure 1) obtained with different mixtures of oryzenin, starch, amylose, or amylopectin the reversible equilibrium binding constants in 0.1 M NaOH were calculated. If n moles of protein (oryzenin) are adsorbed reversibly on m moles of starch (amylose or amylopectin) and the adsorption is relatively small, when compared to the initial concentration of the components, then we can write

$$nP + mS \leftrightarrow P_nS_m \quad (6)$$

where P is the protein (oryzenin), S is the starch or starch component (amylose or amylopectin) and P_nS_m is the oryzenin–polysaccharide complex concentration, respectively. By assuming that the concentration of the complex P_nS_m is directly proportional to the absorbance ΔA of the complex at 285 nm, we get

$$nP + mS \leftrightarrow k'\Delta A \quad (7)$$

where k' is the proportionality constant.

When P and S are expressed in grams per liter, the molecular weights will disappear in constant k', which is

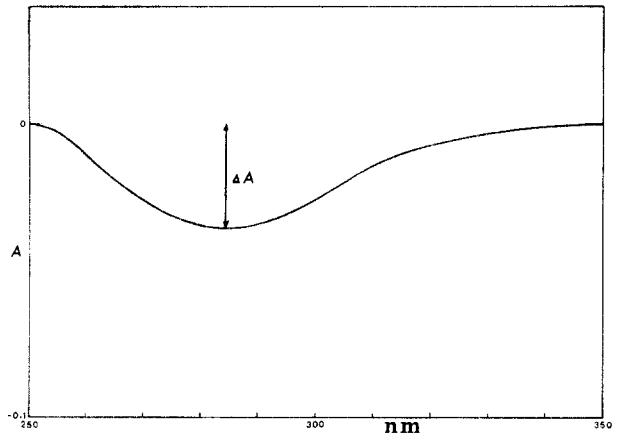

Figure 1. Example of differential absorption spectrum of oryzenin plus starch read against oryzenin alone and a starch blank. Long-grain rice was stored for 12 months at 4 °C. The curve represents the mixture of 0.3 g/L oryzenin plus 0.3 g/L starch in 0.1 M NaOH.

proportional to the equilibrium constant K_{eq} and we may write

$$\Delta A/P^nS^m = K_{eq} \quad (8)$$

The adsorption on starch was an additive property of an adsorption on amylose and amylopectin because the additive equation

$$\Delta A_3 = K_{eq1}P^{n1}[0.01\%_1S]^{m1} + K_{eq2}P^{n2}[0.01\%_2S]^{m2} \quad (9)$$

where indices 1–3 represent amylose, amylopectin, and starch, respectively, agreed very well with experimental results (not shown here).

All constants of eq 8 (n, m, and K_{eq}) were calculated by the statistical regression analysis from experimental results. Correlation coefficients of these computer calculations were always greater than 0.95. From the results in Table III it is apparent that the adsorption of oryzenin on starch, amylose, and amylopectin decreased during storage (equilibrium constants and the n:m ratios decreased). The adsorption of oryzenin was larger on amylose than on amylopectin, but the relative decrease after storage was almost the same on amylose and/or amylopectin.

Stickiness and Oryzenin Binding. During cooking the surface layers, cells, and granules disintegrate and the components leach out from the cells, partially also into the cooking liquid (Rao et al., 1976; El-Said et al., 1980; Deshpande and Bhattacharya, 1982). This destruction enables the interactions between the starch granules and protein bodies. Additionally, some starch granules are mixtures of starch and oryzenin (Juliano, 1985). Thus, during cooking and cooling (stickiness was measured at room temperature) of rice, proteins can easily interact with starch and its components.

Several factors may simultaneously influence stickiness of cooked rice. Molecular weights of oryzenin, amylose, and amylopectin, amylose content, cystine bridges, and oryzenin–starch binding are among these possible factors.

However, from our experiments it became clear that the oryzenin–starch binding seems to be one of the most important factors. As is apparent from Tables I and III and from Figure 2, in the two studied rice varieties stored at different temperatures, there was an almost linear direct relationship between stickiness (measured by n_{max}) and the equilibrium binding constants K_{eq} or the binding ratios n:m of oryzenin on starch, amylose, and/or amylopectin.

Table III. Interaction of Oryzenin with Starch Components from Rice[a]

rice	K_{eq}	n	m	n:m	r
amylose + oryzenin					
medium-grain rice					
control	0.041	0.265	0.107	2.48	0.997
4 °C	0.039	0.258	0.106	2.43	0.998
40 °C	0.029	0.196	0.101	1.94	0.998
long-grain rice					
control	0.025	0.155	0.101	1.53	0.998
4 °C	0.024	0.149	0.100	1.49	0.988
40 °C	0.020	0.116	0.089	1.30	0.988
amylopectin + oryzenin					
medium-grain rice					
control	0.025	0.264	0.115	2.30	0.998
4 °C	0.023	0.259	0.117	2.21	0.999
40 °C	0.016	0.113	0.064	1.76	0.975
long-grain rice					
control	0.013	0.150	0.103	1.45	0.997
4 °C	0.013	0.144	0.102	1.41	0.984
40 °C	0.010	0.090	0.072	1.25	0.987
starch + oryzenin					
medium-grain rice					
control	0.054	0.268	0.099	2.70	0.999
4 °C	0.055	0.261	0.100	2.61	0.999
40 °C	0.040	0.187	0.101	1.85	0.996
long-grain rice					
control	0.035	0.150	0.098	1.53	0.997
4 °C	0.035	0.146	0.101	1.45	0.995
40 °C	0.028	0.117	0.107	1.09	0.990

[a] Medium- and long-grain polished rice was stored at 4 and 40 °C. Control is a postharvest rice. The results are from duplicates, each obtained from 49 concentration combinations. The variation from the mean between duplicates was less than ±2%. The correlation coefficients, r, were always larger than 0.95. Constants K_{eq}, n, m of eq 8 were calculated from absorbance. Constant K_{eq} was calculated from P and S in grams per liter and ΔA in absorbance units.

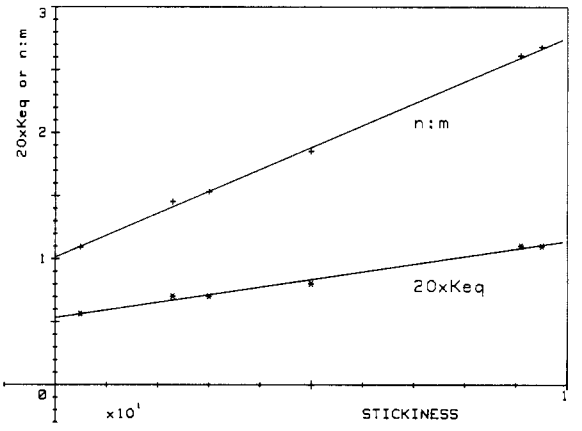

Figure 2. Binding of oryzenin to starch related to stickiness of cooked rice grains. Stickiness is expressed as n_{max}. To fit in the scale K_{eq} is multiplied by 20. Statistical reliabilities are shown in Tables I and III.

This means that the binding of oryzenin to the starch components positively influenced the stickiness of cooked rice.

Generally, the storage of rice grains with the average storage water content 12–14% resulted in a large increase of –SS– intermolecular bridges and molecular weight of oryzenin. The molecular weight of amylopectin and the amylose content in starch also increased. On the other hand, the molecular weight of amylose and the binding of oryzenin on amylopectin and/or amylose decreased. The stickiness of cooked rice grains also decreased during storage. The binding of oryzenin to starch was directly related to the stickiness. These changes were strongly supported by higher storage temperature.

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