Changes of Oryzenin and Starch during Preharvest Maturation of Rice Grains

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Changes in oryzenin (rice storage protein) and starch during the preharvest maturation (14, 30, and 50 days after flowering) of rice grains have been studied. During the synthesis of oryzenin the cysteine content slightly decreased but the cystine bridges slightly increased. The average molecular weight of starch increased only slightly, but the average molecular weight of oryzenin decreased significantly (by 27%) during the studied maturing period. The oryzenin–starch equilibrium binding, which is important for the functional properties of cooked rice, increased during the preharvest period. The changes in oryzenin and its binding to starch in ripening rice were in sharp contrast to the changes in postharvest stored rice, which were always beneficial to the functional properties of the cooked rice grains. This reversed trend before and after harvest means that late harvest could be unfavorable to the functional properties of rice.

Rice quality depends on the time of harvesting. The harvesting time is difficult to determine. The plant produces tillers and panicles in sequence, and thus only a few panicles per plant may have grains with the optimum properties at any time during the harvest season. Moisture content in the grains is the most important characteristic defining the optimum harvest, but there are other factors involved in optimum harvest economy, for example, the energy needed to dry rice, field yield, head yield, and milling yield of the harvested rice grains.

It is up to the producers in different geographical localities to choose the optimum time of harvest. For example, in tropical countries the maximum grain hardness usually occurs at 32 days after flowering (Sajwan et al., 1992). This also results in the highest head yield. Thirty-two days after flowering, grain fissures increased mainly because of the critical grain moisture changes during the day and night.

Optimum harvest time in the United States is, of course, different. For example, the average crop in California is ready for harvest 45–55 days after the first heading (Juliano, 1972, 1985).

After flowering, oryzenin is formed much faster than other protein fractions, globulins, albumins, or prolamines (Palmiano et al., 1968; Cagampang et al., 1976).

Starch remains the main component of the rice grain endosperm from the early developing stages up to maturity. Total protein (except prolamines) in the ripening rice grains increases after flowering (Villareal and Juliano, 1978; Mandac and Juliano, 1978). The ratio of protein fractions in the developing rice grains changes during the preharvest maturing period, but the rice storage protein, called rice glutelin or oryzenin, is always the prevailing protein (75–90% of the total protein).

Thus, because at harvest rice oryzenin constitutes the largest portion of the protein in rice grains (75–90% of total protein), its importance for rice properties is self-evident.

Most of the rice protein studies during the maturing preharvest period of rice grains concentrated on isolation, purification, and analysis of different protein fractions (albumin, globulin, prolamin, and oryzenin).

After harvest, during storage, protein changes are reflected in protein–starch interactions, and these interactions are further reflected in changes in the functional properties of rice (Chrastil, 1990). Thus, it is also important to find the changes in these physicochemical characteristics during the rice maturing period before storage.

In the following paragraphs some of the chemical and physicochemical changes in rice grains during the preharvest (14–50 days after flowering) maturing period have been examined.

EXPERIMENTAL PROCEDURES

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

Preharvest Rice. A typical U.S. long-grain rice variety (Lemont) was obtained from the Crowley Research Station, Crawley, LA. Rice was planted in the greenhouse in Hoagland–Snyder solution as a nutrient (Yoshida et al., 1976). At selected time intervals after flowering, samples were taken in random order. Grains that differed very much from the average grain size, color, or shape were excluded. Rice grains were ground to a flour in a water-cooled micromill (Technilab Instruments, Pequannock, NJ). All samples were ground in the same manner.

Only ripe rice grains (50 days after flowering) were polished (10%). This did not influence the experimental results because oryzenin and starch are present almost exclusively in endosperm.

Defatting. The flour was first extracted for 60 min with 7 volumes of ether plus 7 volumes of MeOH. The extracted flour was filtered through a medium fritted glass filter, and the extraction was repeated twice. Extracts were discarded.

Extraction of Albumin. After the last extraction, the defatted flour was dried in air and extracted twice by 60 min of shaking with 10 volumes of water at 25 °C. Each suspension was immediately centrifuged at 10000*g* in a cold (15 °C) rotor for 20 min, and the supernatants were discarded.

Extraction of Globulin. The flour (still wet) was then extracted twice by 60 min of shaking with 10 volumes of 5% NaCl at 25 °C. Each suspension was immediately centrifuged at 10000*g* in a cold rotor (15 °C) for 20 min, and the supernatants were discarded.

Extraction of Prolamin. The flour (still wet) was then extracted three times by 60 min of shaking with 10 volumes of 55% propanol at 25 °C. Each suspension was immediately centrifuged at 10000g in a cold rotor (15 °C) for 20 min, and the supernatants were discarded.

Preparation of Oryzenin. The flour after prolamin extraction (still wet) was washed with 10 volumes of water (to wash the

remaining salt and alcohol) and then extracted three times by 60 min of shaking with 10 volumes of 0.05 M NaOH at 25 °C. Each suspension was immediately centrifuged at 10000*g* in a cold rotor (15 °C) for 20 min. The combined supernatants were filtered as above, and the oryzenin was precipitated by saturation with $(NH_4)_2SO_4$. The precipitate was then extensively dialyzed against cold water and freeze-dried.

Preparation of Starch. After the extraction of oryzenin, the residue (still wet) was extensively dialyzed against cold water and freeze-dried. It was then extracted twice by 60 min of shaking with 10 volumes of DMSO. Each suspension was immediately centrifuged at 10000*g* in a cold rotor (15 °C) for 20 min. The combined supernatants were extensively dialyzed against cold water and freeze-dried.

Protein Content in Oryzenin or Starch. Oryzenin or starch was dissolved in 0.1 M NaOH (10 mg/mL). Oryzenin solution was diluted 1:50, and starch solution was used directly for protein determination by Lowry's method (Lowry et al., 1951).

Starch Content in Oryzenin or Starch. Starch content in the oryzenin or starch fractions prepared from rice flour was determined according to a modified colorimetric method of Montgomery (1961).

Viscosity. Viscosities of oryzenin and starch were measured at 25 °C with a Brookfield con/plate digital viscosimeter Model DV-II using a 0.5-mL cone. Intrinsic viscosities (Billmeyer, 1984) were estimated from 10 concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0%) of oryzenin or starch in 0.1 M NaOH by the statistical regression analysis of the duplicates.

Molecular Weight of Oryzenin. The molecular weight of oryzenin was determined from the intrinsic viscosity by the equation (Chrastil, 1990)

$$M_\eta = (2.685 \times 10^{13})[\eta]^{8.197} \quad (1)$$

Cysteine and Cystine in Oryzenin. Oryzenin was dissolved in 88% formic acid (10 mg/mL), and the free -SH and -SS- bonds in oryzenin were determined by the direct method (Chrastil, 1989) without the hydrolysis of oryzenin. The results were expressed as an average from triplicate samples. The standard deviation of the mean of the triplicates was <0.003% S.

Molecular Weight of Starch. The molecular weight of starch was determined from the intrinsic viscosity by the equation (Chrastil, 1990)

$$M_\eta = (1.023 \times 10^{7})[\eta]^{4.271} \quad (2)$$

Amylose Content in Starch. Starch was dissolved in 0.1 M NaOH (2.5 mg/mL), and the amylose content was determined by a colorimetric method (Chrastil, 1987). Two hundred microliters of starch solution was pipetted into 5 mL of 0.5% TCA in 10-mL test tubes. The solution was 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 620 nm vs H_2O in a Shimadzu 260 double-beam spectrophotometer. The standard was pure potato amylose, and the blank was H_2O.

Binding of Oryzenin to Starch. The equilibrium binding constants of oryzenin to starch were determined in 0.05 M NaOH from the equation (Chrastil, 1990)

$$\Delta A = K_{eq} P^n S^m \quad (3)$$

where ΔA is the difference in absorbance at 285 nm, P (g/L) is the concentration of protein and S (g/L) is the concentration of starch in the mixture. The constants of eq 3 were calculated by regression analysis of the differential absorption spectra of 20 selected mixtures of oryzenin and starch measured at 285 nm.

RESULTS AND DISCUSSION

Starch. The average molecular weight of starch from the ripening rice grains (14, 30, and 50 days after flowering) increased slightly. A similar slight increase was observed also in the postharvest stored rice grains (Chrastil, 1990, 1992). Thus, there was no discontinuity of that property before and after harvest. The same was true about the amylose content in preharvest and postharvest starch.

Table I. Preharvest Changes of Starch in Maturing Rice Grains[a]

	days after flowering		
starch	14	30	50
intrinsic viscosity [η] (L g^{-1})	0.0530	0.0624	0.0760
av molecular weight ($\times 10^{-6}$)	3.38	3.59	3.87
amylose content in starch (%)	24.7	25.1	24.4

[a] The values are averages from triplicates. In all cases deviations between triplicates were smaller than ±5% of the mean.

Table II. Preharvest Changes of Oryzenin in Maturing Rice Grains[a]

	days after flowering		
oryzenin	14	30	50
intrinsic viscosity [η] (L g^{-1})	0.00997	0.00975	0.00925
av molecular weight ($\times 10^{-3}$)	121	110	88
cysteine (% S)	0.16	0.14	0.14
cystine (% S)	0.18	0.19	0.19

[a] The values are averages from triplicates. In all cases deviations between triplicates were smaller than ±5% of the mean.

There was no significant change in amylose content in starch during the preharvest ripening of rice grains (Table I).

Oryzenin. The purified oryzenin fraction contained 2–3% bound carbohydrate. From this point of view its composition was similar to that of postharvest oryzenin (Chrastil, 1990).

The total cysteine plus cystine content decreased slightly after flowering (Table II). Because oryzenin and its ratio relative to other protein fractions increase greatly after flowering (Palmiano et al., 1968; Cagampang et al., 1976), from the relative decrease of the cysteine plus cystine content it was apparent that the subunits with lower cysteine content were added to the oryzenin molecule more in the last ripening stages than at the early stages after flowering.

The cystine bridges increased slightly after flowering. This was very different from postharvest stored rice grains, where a much greater increase of -SS- bridges was found during storage (Chrastil, 1990).

Another difference from the postharvest rice grain profile was the average molecular weight of purified oryzenin. In stored rice the average molecular weight of purified oryzenin increased greatly during storage, but in the ripening preharvest rice grains it decreased slightly (Table II).

All of these changes were reflected in the starch–oryzenin binding (Table III). The equilibrium binding constant, K_{eq}, increased, and the equilibrium binding ratio oryzenin/starch (n/m) increased. Under the same conditions, the binding of purified albumin or globulin to the starch was very low.

This trend was reversed after harvest. The binding of oryzenin to starch decreased during storage of rice grains, and this was related to changes in the functional properties of rice (cooking properties, stickiness, dough leavening, etc.). For example, the average molecular weight of oryzenin in the stored rice grains was related to the stickiness of cooked rice grains and to the dough leavening of rice flour (Chrastil, 1990, 1992). The changes during storage generally improved these functional properties of rice grains mainly because of oryzenin molecular weight increase and simultaneous oryzenin–starch binding decrease. Before harvest, most physicochemical changes showed a reverse trend when compared to storage changes. Thus, we may conclude that although late harvest might

Table III. Binding of Oryzenin and Starch from Maturing Rice Grains[a]

days after flowering								
14			30			50		
O	S	ΔA	O	S	ΔA	O	S	ΔA
0.25	0.25	0.040	0.25	0.25	0.039	0.25	0.25	0.032
0.25	0.50	0.062	0.25	0.50	0.062	0.25	0.50	0.054
0.25	0.75	0.080	0.25	0.75	0.082	0.25	0.75	0.073
0.25	1.00	0.096	0.25	1.00	0.099	0.25	1.00	0.090
0.50	0.25	0.051	0.50	0.25	0.055	0.50	0.25	0.055
0.50	0.50	0.079	0.50	0.50	0.088	0.50	0.50	0.093
0.50	0.75	0.103	0.50	0.75	0.116	0.50	0.75	0.126
0.50	1.00	0.123	0.50	1.00	0.141	0.50	1.00	0.151
0.75	0.25	0.059	0.75	0.25	0.067	0.75	0.25	0.076
0.75	0.50	0.092	0.75	0.50	0.108	0.75	0.50	0.128
0.75	0.75	0.119	0.75	0.75	0.142	0.75	0.75	0.174
0.75	1.00	0.142	0.75	1.00	0.173	0.75	1.00	0.216
1.00	0.25	0.066	1.00	0.25	0.077	1.00	0.25	0.095
1.00	0.50	0.101	1.00	0.50	0.124	1.00	0.50	0.161
1.00	0.75	0.132	1.00	0.75	0.164	1.00	0.75	0.218
1.00	1.00	0.158	1.00	1.00	0.200	1.00	1.00	0.271
K_{eq} = 0.158			K_{eq} = 0.199			K_{eq} = 0.271		
n = 0.358			n = 0.500			n = 0.790		
m = 0.633			m = 0.681			m = 0.753		
$n:m$ = 0.566			$n:m$ = 0.734			$n:m$ = 1.048		
r = 1.000			r = 1.000			r = 1.000		

[a] Averages from duplicates. O, oryzenin (g/L); S, starch (g/L); ΔA, difference in absorbance at 285 nm; r = nonlinear regression correlation coefficient; oryzenin and starch in 0.05 M NaOH; other constants explained in text.

be beneficial to the starch and protein contents, it would be unfavorable to the functional properties of rice grains.

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