Stickiness of Oryzenin and Starch Mixtures from Preharvest and Postharvest Rice Grains

Joseph Chrastil

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

Stickiness, molecular weight, binding, sulfur bridges, and amylose of isolated oryzenin (rice storage glutelin) and/or starch from preharvest and postharvest rice grains were studied. The molecular weight, amylose content, and stickiness of starch did not change significantly during preharvest ripening or postharvest storage of rice, but the stickiness of oryzenin and its mixtures with starch increased during ripening and decreased during postharvest storage. The stickiness of oryzenin or oryzenin–starch mixtures from preharvest and postharvest rice grains was related to its average molecular weight, cystine bridges, and the oryzenin–starch equilibrium binding constant.

Keywords: *Rice; protein; starch; interactions; stickiness*

Both before and after harvest, starch is the main component of the rice grain endosperm (80–90% of the flour) and the rice storage protein, oryzenin, is the prevailing protein (75–90% of the total protein) (Palmiano et al., 1968; Villareal and Juliano, 1978; Juliano, 1985).

Stickiness is an important functional property of cooked rice grains. By cooking, the protein and starch bodies are partially destroyed (Juliano, 1985; Moore and Carter, 1974; Remsen and Clark, 1978; Lorenz and Kulp, 1991), and this enables the interactions of protein with starch by mutual binding (Chrastil, 1990a,b). These interactions occur mainly in the form of reversible adsorption and are related to the stickiness of cooked rice (Chrastil, 1993a).

After cooking, the rice grain surface is covered by small particles which are evidently responsible for stickiness and which can be carefully washed out from sieved cooked rice grains by cold water in which they suspend. These particles contain mostly oryzenin and starch, but only little albumins or globulins (Chrastil, not shown here).

Greater binding of oryzenin to starch causes greater stickiness and vice versa. The extent of binding is mostly influenced by oryzenin and its composition (Chrastil, 1990b, 1992, 1993a,b; Chrastil and Zarins, 1992; Marshall and Chrastil, 1992).

In our previous works, the relation between protein–starch binding and the stickiness of cooked rice grains was measured indirectly in separate experiments (Chrastil, 1990b, 1992, 1993a). From these works it was apparent that the stickiness of cooked rice grains decreased during storage and was related to the decreased starch–oryzenin binding.

However, it was difficult or impossible to measure the stickiness of cooked rice grains in the early stages of development. Thus, to obtain information about the relative changes in stickiness and interactions of oryzenin, starch, and their mixtures in both developing and stored rice, we have studied these components isolated from both preharvest and postharvest rice grains of the same variety (Lemont). In this manner we could compare these phenomena in different stages of rice development and/or storage.

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).

Preharvest Rice. A typical U.S. long-grain rice variety (Lemont) was obtained from the Crowley Research Station, Louisiana. Rice was planted in the greenhouse in Hoagland-Snyder nutrient solution (Yoshida et al., 1976). At selected time intervals after flowering, samples were taken in random order.

Grains that differed more than 30% 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.

Postharvest Rice. Polished (10% removed) rice grains (less than 1 month after harvest) of the same variety (Lemont) were stored in duplicate in closed jars at 4 and 40 °C for 1 year. After storage, the grains that differed more than 30% from the average grain size, color, or shape were excluded. Rice grain samples were ground to a flour in a water-cooled micromill (Technilab Instruments). All samples were ground in the same manner.

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

Extraction of Globulin. The flour (still wet) was 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 (containing globulins) were discarded.

Extraction of Prolamine. The wet flour (albumin and globulin free) was extracted three times by 60 min of shaking with 10 volumes of 55% 1-propanol at 25 °C. Each suspension was immediately centrifuged at 10000*g* in a cold rotor (15 °C) for 20 min, and the supernatants (containing prolamine) were discarded.

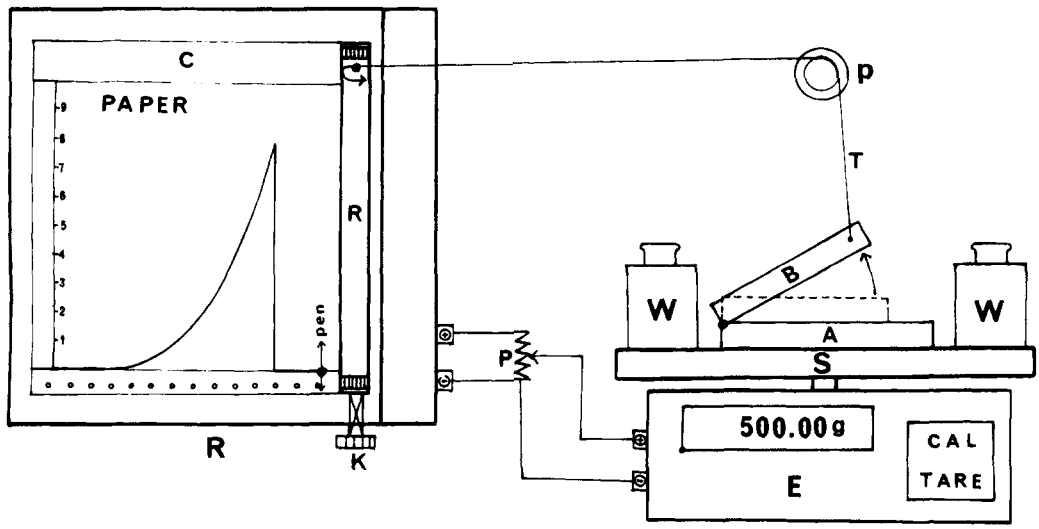

Figure 1. Schematic arrangement for measuring stickiness of rice oryzenin, starch, and their mixtures. For clarification the recorder in the picture is in vertical position. In real arrangement it was horizontal. E, electronic balance; R, recorder; W, weights; S, scale; A, lower plexiglass plate fixed to the scale; B, movable upper plexiglass plate; T, thread fixed to the roller and upper plate; p, pulley; P, potentiometer; R, roller; K, roller adjusting knob; C, cutoff part of the recording paper.

Preparation of Oryzenin. The wet flour after prolamine extraction was washed with 10 volumes of water (to wash out 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. The dry oryzenin powder was ground in a water-cooled micromill (Technilab Instruments) and sieved to particle size <100 μm.

Preparation of Starch. After oryzenin extraction, the wet residue 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. The dry starch powder was ground in a water-cooled micromill (Technilab Instruments) and sieved to particle size <100 μm.

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 according to the method of 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/or 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 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, 1990b)

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

Cysteine and Cystine in Oryzenin. Oryzenin was dissolved in 88% formic acid (10 mg/mL), and the free −SH and −S−S− bonds in oryzenin were determined by the direct method (Chrastil, 1989) without the hydrolysis of oryzenin. The results were expressed as an average from duplicate samples.

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

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

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 0.1 M NaOH from the equation (Chrastil, 1990b)

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

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.

Measurement of Stickiness. Stickiness of starch, oryzenin, or their mixtures was measured with the equipment shown in Figure 1. A 1 cm thick plexiglass plate (5 cm wide and 8 cm long) was fixed to the scale of the digital electronic balance (Denver Instrument Co.). This lower plate was connected at the end by a tiny door post with another upper 1 cm thick plexiglass plate (1 cm wide and 5 cm long). The touching surface with the lower plate was 1 cm × 4 cm. The upper plate was connected by means of a small pulley with the moving roller of a recorder (Houston Instrument Division of Bausch & Lomb).

The recording paper on the side where the thread from the pulley was fixed to the roller was cut off (2 cm). To adjust the correct voltage, the recorder (printer) outlet of the balance was connected to the recorder inlet through a potentiometer (10 kΩ).

The balance was adjusted by weights to 500 g, and the pen on the recorder was adjusted to zero. Fifty milligrams of powdered oryzenin or starch or 25 mg plus 25 mg of oryzenin–starch mixture was mixed quickly to a homogeneous paste with 0.1 mL of distilled water and applied to the small plexiglass

Table 1. Changes of Starch in Rice Grains[a]

	Preharvest Rice		
	days after flowering		
	14	50	r
av molecular wt ($\times 10^{-6}$)	3.38 ± 0.1	3.87 ± 0.15	0.911
amylose content in starch (%)	24.7 ± 1	24.4 ± 1	0.152
	Postharvest Rice		
	storage temp		
	4 °C	40 °C	r
av molecular wt ($\times 10^{-6}$)	3.49 ± 0.14	3.75 ± 0.11	0.737
amylose content in starch (%)	25.1 ± 1	26.0 ± 1.1	0.445

[a] The values are averages from duplicates with standard deviations of the mean; r is the correlation coefficient ($r = 1$ when groups are absolutely different and $r = 0$ when they are equal).

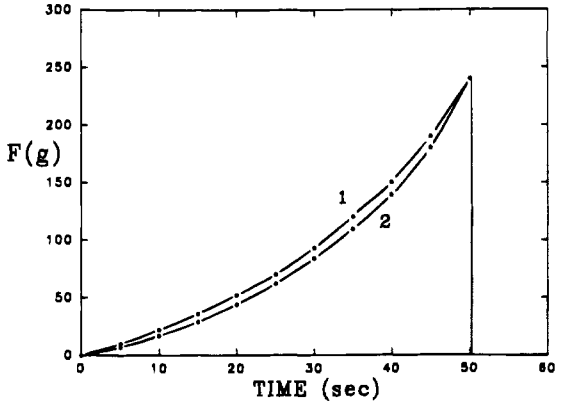

Figure 2. Recorded stickiness of preharvest rice starch. Starch was not preheated. 1, 14 days after flowering; 2, 50 days after flowering.

surface. Some samples were preheated in sealed tubes for 10 min to 100 °C (to gelatinize starch) and cooled to room temperature before use.

The two plexiglass surfaces were compressed to obtain a homogeneous layer of the paste between them and the edges were cleaned of paste. After 1 min, the recorder was started (2 cm/min, 200 mV). When the upper plexiglass was lifted, the stickiness of the sample decreased the weight on the scale and moved the pen on the recorder. The curve was recorded until the two plexiglass surfaces split apart and the recorded resistance curve returned to zero (starting value). The maximum on the curve was chosen as the measure of the sticking resistance of the paste.

RESULTS AND DISCUSSION

Starch. Isolated starch does not necessarily reflect the aggregated native state, but the relative values should more or less represent integrated changes occurring in native form. Purified starch contained more than 99% starch. In agreement with previous results (Chrastil, 1990b, 1992), during ripening (14 and 50 days after flowering) and/or storage (at 4 and 40 °C) of rice grains, the average molecular weight of starch increased only slightly. The same was true about the amylose content in preharvest and postharvest starch. There was no significant change in amylose content in starch during the preharvest ripening or postharvest storage of rice grains (Table 1).

Stickiness of starch also did not change during ripening (Figure 2) or storage (Figure 3). As is apparent from these figures, the stickiness of starch was slightly higher in stored rice than in ripening rice (240 and 293 g of stress for complete separation of the plates).

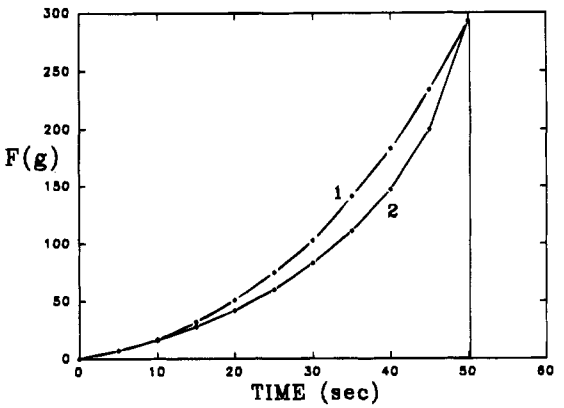

Figure 3. Recorded stickiness of postharvest rice starch. Starch was not preheated. 1, stored at 4 °C; 2, stored at 40 °C.

Table 2. Changes of Oryzenin in Rice Grains[a]

	Preharvest Rice		
	days after flowering		
	14	50	r
av molecular wt ($\times 10^{-3}$)	161 ± 6	125 ± 5	0.981
cysteine (% S)	0.16 ± 0.008	0.14 ± 0.007	0.821
cystine (% S)	0.18 ± 0.009	0.16 ± 0.008	0.782
	Postharvest Rice		
	storage temp		
	4 °C	40 °C	r
av molecular wt ($\times 10^{-3}$)	132 ± 5	214 ± 9	1.000
cysteine (% S)	0.17 ± 0.008	0.14 ± 0.007	0.918
cystine (% S)	0.16 ± 0.008	0.18 ± 0.009	0.782

[a] The values are averages from duplicates with standard deviations of the mean; r is the correlation coefficient ($r = 1$ when groups are absolutely different and $r = 0$ when they are equal).

Oryzenin. Purified oryzenin contained 2–3% bound carbohydrate in agreement with previous findings (Chrastil, 1990b, 1993a; Chrastil and Zarins, 1992). Total cysteine plus cystine content decreased slightly during ripening, but it did not change during storage (Table 2.) Cystine bridges decreased slightly during ripening. This was different from postharvest stored rice grains for which a relative increase of –S–S– bridges was found during storage, especially at higher storage temperatures.

Another difference between the preharvest and postharvest rice grains was the average molecular weight of purified oryzenin. In the ripening preharvest rice grains the average molecular weight of oryzenin decreased slightly, but in stored rice the average molecular weight of purified oryzenin increased greatly during storage.

As with starch, the average molecular weight of isolated oryzenin represents just the relative changes in the oryzenin which should reflect in some more complicated way the changes in native aggregated form of this protein.

Stickiness of oryzenin showed a reverse trend to its molecular weight. The stickiness of oryzenin from the ripening rice grains increased, but the stickiness of oryzenin from postharvest stored rice grains decreased (Figures 4 and 5).

Mixtures of Oryzenin with Starch. The changes in oryzenin and starch were reflected in the starch–oryzenin binding (Table 3). The equilibrium binding constant, K_{eq}, and the equilibrium binding ratio oryzen-

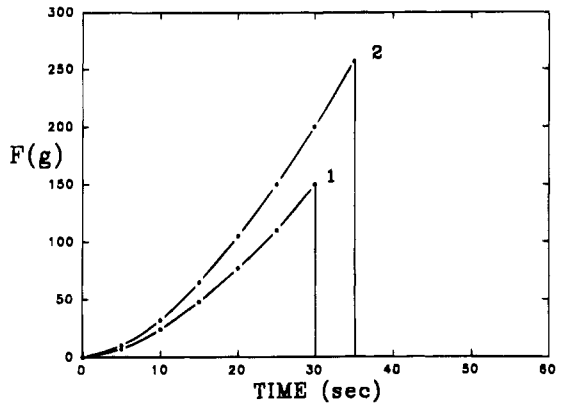

Figure 4. Recorded stickiness of preharvest rice oryzenin. Oryzenin was not preheated. 1, 14 days after flowering; 2, 50 days after flowering.

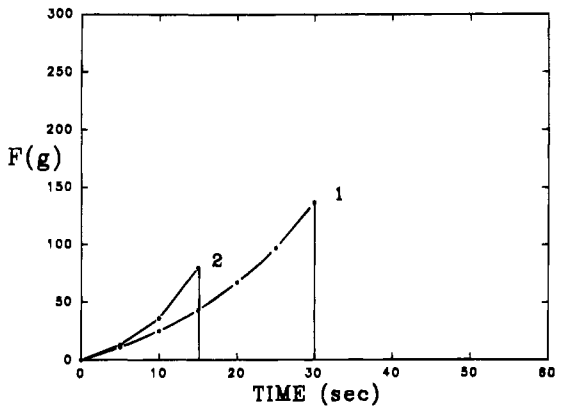

Figure 5. Recorded stickiness of postharvest rice oryzenin. Oryzenin was not preheated. 1, stored at 4 °C; 2, stored at 40 °C.

Table 3. Changes of Starch–Oryzenin Binding in Rice Grains[a]

	Preharvest Rice		
	days after flowering		
	14	50	r
equilibrium constant (K_{eq})	0.030 ± 0.001	0.038 ± 0.002	0.939
oryzenin:starch (n:m)	0.62 ± 0.03	1.24 ± 0.06	1.000
	Postharvest Rice		
	storage temp		
	4 °C	40 °C	r
equilibrium constant (K_{eq})	0.037 ± 0.0005	0.029 ± 0.0001	0.996
oryzenin:starch (n:m)	1.21 ± 0.06	0.98 ± 0.04	0.929

[a] The values are averages from duplicates with standard deviations of the mean; r is the correlation coefficient ($r = 1$ when groups are absolutely different and $r = 0$ when they are equal).

in:starch (n:m) in ripening rice grains increased but in postharvest stored rice grains, decreased.

The binding experiments were effected in alkaline medium (to dissolve both components). Nevertheless, it was shown by us in a series of adsorption experiments (not shown here) that the binding of the same rice components in neutral or slightly acidic medium is stronger than in alkaline medium, but the relative qualitative differences between the samples remained similar.

Stickiness of oryzenin–starch mixtures also showed a reverse trend to the molecular weight of oryzenin. The stickiness of oryzenin–starch mixtures from preharvest ripening rice grains increased greatly (Figure 6) but in

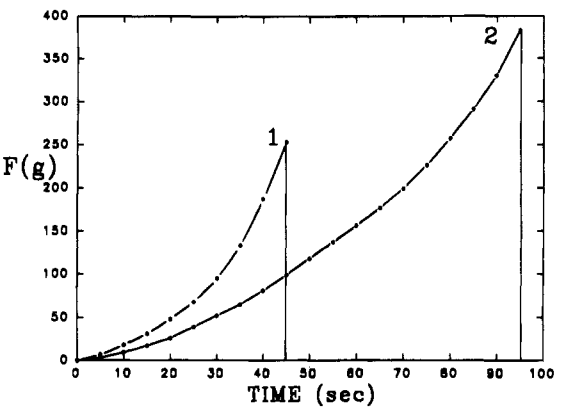

Figure 6. Recorded stickiness of preharvest rice starch–oryzenin mixture. The mixture was not preheated. 1, 14 days after flowering; 2, 50 days after flowering.

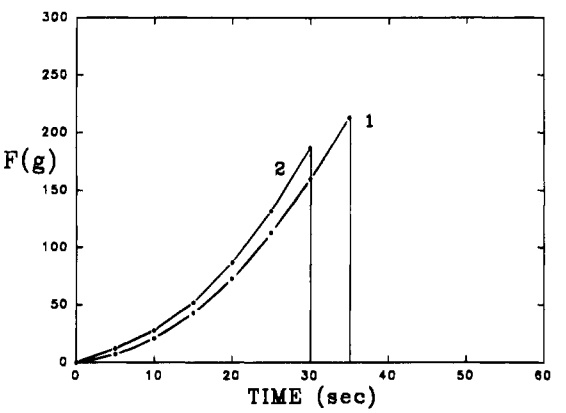

Figure 7. Recorded stickiness of postharvest rice starch–oryzenin mixture. The mixture was not preheated. 1, stored at 4 °C; 2, stored at 40 °C.

stored rice grains, decreased after storage, especially at high storage temperatures (Figure 7).

Stickiness of preheated (gelatinized) starch or its mixtures was higher and of preheated oryzenin slightly lower, but the relative trend was the same as above. The greatest stickiness was again found with the harvested rice grains 50 days after flowering (not shown here).

Conclusions. From these results it is apparent that the stickiness and the binding interactions of starch, oryzenin, or their mixtures are related to their relative average molecular weights. This is not surprising because stickiness is related to surface tension and the surface tension is indirectly related to the molecular weight by Eötvös or Einstein equations (Freundlich, 1930; Eisberg and Resnick, 1974; Adamson, 1979).

It is also apparent that the changes in stickiness are caused mainly by the physicochemical and chemical changes of oryzenin (molecular weight, composition of peptide subunits, and cystine bridges) and to a smaller extent by starch. This agrees with our previous work (Chrastil, 1990b, 1992; Chrastil and Zarins, 1992) in which it was shown that the average molecular weight of oryzenin from stored rice grains was related to the stickiness of cooked rice grains and to the dough leavening of rice flour.

These results also agree with the well-known fact that freshly harvested rice often becomes a sticky paste upon cooking but after several weeks of storage, the stickiness is much reduced (Juliano, 1985; Tsugita et al., 1983).

LITERATURE CITED

Adamson, A. W. *Physical Chemistry;* Academic Press: New York, 1979.

Billmeyer, F. W., Jr. *Textbook of Polymer Science*; Wiley: New York, 1984.

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

Chrastil, J. Spectrophotometric Determination of Cysteine and Cystine in Peptides and Proteins Based on New Color Reaction. *Analyst* **1989**, *114,* 1133–1136.

Chrastil, J. Chemical and Physicochemical Changes of Rice During Storage at Different Temperatures. *J. Cereal Sci.* **1990a**, *11,* 1–85.

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. Correlations between the Physicochemical and Functional Properties of Rice. *J. Agric. Food Chem.* **1992**, *40,* 1683–1686.

Chrastil, J. *Effect of Storage on the Physicochemical Properties and Quality Factors of Rice;* Marshall, W. E., Wadsworth, J. I., Eds.; Rice Science and Technology, Dekker: New York, 1993a; pp 52–53, 58–59, 69.

Chrastil, J. Changes of Oryzenin and Starch During Preharvest Maturing of Rice Grains. *J. Agric. Food Chem.* **1993b**, *41,* 2242–2244.

Chrastil, J.; Zarins, Z. Influence of Storage on Peptide Subunit Composition of Rice Oryzenin. *J. Agric. Food Chem.* **1992**, *40,* 927–930.

Eisberg, R.; Resnick, R. *Quantum Physics of Atoms, Molecules, Solids and Particles;* Wiley: New York, 1974.

Freundlich, H. *Colloid and Capillary Chemistry;* Methuen: London, 1930.

Juliano, B.O. *Rice: Chemistry and Technology;* AACC: St. Paul, MN, 1985; pp 175-205, 443–524.

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

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

Marshall, E. W; Chrastil, J. *Interactions of Food Proteins With Starch; Biochemistry of Food Proteins*; Hudson, B. J. F., Ed.; Elsevier: London, 1992.

Montgomery, R. Further Studies of the Phenol-Sulfuric Acid Reagent for Carbohydrates. *Biochim. Biophys. Acta* **1961**, *48,* 591–593.

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

Palmiano, E. P.; Almazan, A. M.; Juliano, B. O. Physicochemical Properties of Protein of Developing and Mature Rice Grain. *Cereal Chem.* **1968**, *45,* 1–12.

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

Tsugita, T.; Ohta, T.; Kato, H. Cooking Flavor and Texture of Rice Stored Under Different Conditions. *Agric. Biol. Chem.* **1983**, *47,* 543–549.

Villareal, R. M.; Juliano, B. O. Properties of Glutelin from Mature and Developing Rice Grain. *Phytochemistry* **1978**, *17,* 177–182.

Yoshida, S.; Forno, D. A.; Cock, J. H.; Gomez, K. A. *Laboratory Manual for Physiological Studies of Rice;* International Rice Research Institute: Manila, Philippines, 1976; pp 64–66.

Received for review March 15, 1994. Accepted July 25, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1994.