CEREAL CHEMISTRY

VOL. 37 JANUARY, 1960 NO. 1

THE RELATIVE EFFECTS OF ENZYMATIC AND PHYSICAL CHANGES DURING STORAGE ON THE CULINARY PROPERTIES OF RICE[1]

H. S. R. DESIKACHAR AND V. SUBRAHMANYAN

ABSTRACT

The amylases were destroyed during the first 5 minutes of cooking milled rice grains, but the solids extracted by the cooking water continued to increase as cooking progressed. The inhibition of amylase action by mercuric salts did not improve the cooking quality of new rice; treatment with formalin or steam-curing of the freshly harvested rice did. The cooking of old rice in amylase solution did not affect its cooking quality.

In aqueous suspensions, fresh-rice flour took more time to settle and left the supernatant liquid more turbid than old-rice flour under similar conditions.

The physical changes that occur during storage have to be considered in explaining the improved cooking quality of old rice.

The improvement in the culinary properties and certain changes in the physicochemical properties of the starch occurring during storage of rice have been reported earlier (4). Two theories have been proposed to explain the better cooking property of the stored rice. The lowering in amylase activity during storage is considered by Sreenivasan (10,11) to be the factor responsible for the improved cooking quality of old rice. A change in the colloidal condition of the rice from the sol to the gel state during storage is believed by Rao (7) to bring about the better cooking quality in the stored grain. The object of the present study was to ascertain the relative importance of these biological and physical changes in determining and explaining the better cooking characteristics of the old grain. The effect of amylases during cooking was studied by determining their rate of inactivation during cooking of new rice. The effect of chemical inactivation of amylases on the culinary quality of the rice and of cooking rice in amylase solutions was also determined. To get some idea as to

[1] Manuscript received June 5, 1958. Communication from the Division of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, India.

the extent to which physical factors could modify the cooking quality of the rice, some changes in the colloidal property of the rice as well as the solubility of starch in perchloric acid were investigated. The effect of soaking rice in certain solvents on its cooking characteristics was also studied.

The differences in cooking quality between old and new rice relate to the lower swelling capacity of the latter and its tendency to yield a thick viscous gruel during cooking. The term *cooking quality* in the present study has therefore been used in a rather restricted sense to denote these two culinary characteristics only.

Materials

Three varieties of rice, Co-11, GEB-24, and S-749, grown locally and representing typical short, medium, and long-grained varieties, respectively, were used in the present study. Rice samples milled from freshly harvested paddy were the source of new rice; that obtained from paddy of the same variety stored for one year served as the source of old rice. Essentially similar differences existed between the new and old samples in the three varieties of rice. The effect of different treatments was also similar in the three varieties tested; hence, results will be reported here for only one variety of milled rice: Co-11 – Bangar Sanna – a fine-grained variety obtained from a paddy breeding station. The old rice sample had the composition: moisture, 12.2%; protein, 5.2%; ether extractives, 2.1%; calcium 9.6 mg.%; and phosphorus, 226 mg.%. The new rice, after drying to the same moisture content as above, had also the same chemical composition.

Methods

Swelling Quality of Rice. Ten-gram rice samples were cooked for 30 minutes in a steam cooker in 50 ml. of water in graduated boiling tubes, the excess gruel was strained off, and the volume and weight of the cooked rice were determined. The effect of cooking the rice in a limited amount of water (two parts) on its swelling quality was also determined in certain cases.

Enzyme Inactivation during Cooking. New rice which is richer in amylases than old stored rice was used for these studies. Ten-gram samples of rice were cooked for different periods in 50 ml. of water on an electric hot plate and then immediately homogenized along with the gruel in a Waring Blendor with 50 ml. of M/15 phosphate buffer of pH 7.0. The homogenate was centrifuged, and the centrifugate was used as a source of alpha- and beta-amylases. For determination of alpha-amylase, 10 ml. of 2% soluble starch solution buffered with an equal volume of phosphate buffer of pH 7.0 were incubated at 37°C. with 2.5 ml. of the enzyme extract, and, at the end of 18 hours, 1 ml. of the mixture was diluted to 150 ml.; 1 ml. of 10*N* sulfuric acid and 1 ml. of N/10 iodine solution were added and the solution made up to 250 ml. The percent fall in blue color of the starch substrate as measured by a Klett-Summerson photoelectric colorimeter (using red filter no. 66) was taken as a measure of alpha-amylase activity. The beta-amylase activity was determined by measuring the release of reducing sugars when 10 ml. of starch solution and 10 ml. of M/5 acetate buffer (pH 4.6) were incubated at 37°C. for 18 hours with 5 ml. of enzyme extract (Giri and Sreenivasan, 6). Reducing sugars were determined by Somogyi's method (9). At each stage of cooking, as employed in the above studies, solids lost by the rice in the gruel were also determined in separate samples of rice.

Chemical Inactivation by Mercuric Salts. Ten grams of fresh rice were cooked in 50 ml. of 0.2% mercuric chloride and mercuric acetate solutions, to inactivate the amylases (1), and the swelling number of the rice was determined according to Rao (8).

Cooking of Rice in Amylase Solutions. Centrifuged human saliva diluted with 20 volumes of water and also an extract obtained by shaking new-rice flour with five parts of water overnight were used as a source of the amylases. Ten grams of rice were soaked in 50 ml. of the above enzyme extracts for 30 minutes and then cooked in the same solution by steaming for 30 minutes. The swelling number and the loss of gruel solids were determined after cooking.

Treatment with Certain Solvents. New rice was soaked overnight (16 hours) at laboratory temperature (25°–26°C.) in chloroform, 95% ethyl alcohol, and 10% formalin, strained and freed of adhering residual solvent by thorough washing with water. Rice thus treated was then cooked in five parts of water as usual and the swelling quality determined.

Solubility of Rice Starch in Perchloric Acid. Two grams of rice flour passed through 100-mesh sieve were extracted overnight with 50 ml. perchloric acid solution of different concentrations at laboratory temperature, and the starch in the filtrate was determined by precipitation with alcohol (90% V/V), weighing the precipitate after repeated washing with alcohol, and drying to constant weight at 105°C.

Settling Characteristics of Raw and Boiled Rice Flour Suspensions. One percent suspensions of fresh- and old-rice flour obtained by powdering and then passing through a 100-mesh sieve were allowed to settle at 25°–26°C. in 100-ml. graduated cylinders of the same diameter, and the time needed for settling of the suspended particles was noted. The time at which clear demarcation into two separate layers (of settled flour and top supernatant) became visible was noted. The mixture was allowed to settle further without disturbance for 2 hours, and the turbidity in the top layers was measured in a Klett-Summerson photoelectric colorimeter using blue filter no. 42. Similar observations were made on the suspensions, which had been boiled for 10 minutes and then cooled to laboratory temperature (25°–26°C.).

Results and Discussion

The fact that old stored rice absorbs more water during cooking and hence increases in both weight and volume has been observed by earlier workers. Further interesting observations made in the present study (Table I) show that the improved swelling characteristics of old

TABLE I
BULK DENSITY OF COOKED RICE
(Ten grams rice cooked in steam for 30 minutes)

	Cooked in Excess Water and Gruel Discarded			Cooked in Two Volumes Water		
	Weight	Vol.	Bulk Density	Weight	Vol.	Bulk Density
	g	*ml*		*g*	*ml*	
New	35.3	42	0.84	30.5	29	1.05
Old	42.9	55	0.78	30.5	37	0.83

rice can be considered to be due to two distinct factors: It has the capacity to imbibe more water than the new rice, contributing to an increase in both weight and volume. It also has the ability to swell more in volume than new rice at constant weight, and this factor is responsible for the fluffiness and lower bulk density of cooked old rice as compared with new rice.

Data presented in Table II show that within the first 5 minutes of cooking, practically all the alpha- and beta-amylases present in the rice grain were inactivated. The loss of solids into the cooking water, which is an indication of its tendency to become pasty during cooking, has also been determined at different stages of cooking. It is clear from the data (Table II) that even after the enzymes were inactivated, the grain continued to lose solids into the cooking water. The rate of loss of solids in the gruel is, in fact, greater in the later stages of cooking than in the first few minutes of boiling when the amylases may be expected to exert their action. When mercuric salts were added to the cooking water, the cooking quality of new rice was not improved. (The swelling number of control untreated sample was 2.6;

TABLE II

DESTRUCTION OF α- AND β-AMYLASES IN RELATION TO PASTINESS DURING COOKING OF FRESH RICE

	Amylase Activity		Pastiness as Measured by	
Time of Cooking	Alpha (as Percent Fall in Blue Value)	Beta (as mg. Maltose Released)	Solids Leached out in Gruel	Solids Leached out between Successive Heating Periods
minutes			*g*	*g*
0	83.7	7.9	0.17	...
3	25.0	4.5	0.42	0.25
5	Nil	0.7	0.51	0.09
10	Nil [a]	Nil	0.74	0.23
15	Nil [a]	Nil	1.17	0.37
20	Nil [a]	Nil	1.51	0.34

[a] A slight increase over original blue color was observed in these cases. This might be due to some physical changes in the gelatinized starch present in the enzyme extract added to the reaction mixture.

that of samples cooked in mercuric chloride and acetate solutions was 2.5 and 2.6, respectively.) When new and old rice were cooked in amylase solutions, it was found that the cooking quality of the rice samples was not materially affected by the enzymes (Table III). This

TABLE III

EFFECT OF ADDITION OF AMYLASES ON THE COOKING QUALITY OF RICE

Treatment	Old Rice		New Rice	
	Swelling[a] No.	Relative Loss of Gruel Solids	Swelling[a] No.	Relative Loss of Gruel Solids
		g		*g*
Control	3.3	0.26	2.5	0.35
Control + salivary amylase	3.3	0.26	2.7	0.37
Control + mixed amylases from new rice flour	3.3	0.28	2.7	0.38

[a] Ratio of weight of water absorbed during cooking to weight of raw rice.

was so although the rice samples were soaked previously for 30 minutes in the enzyme extract and slowly cooked in the same solution to give maximum scope for enzymes to react. These facts, taken together, indicate that factors apart from the amylase systems present in rice are important in determining the cooking quality of the rice.

Among solvents which were tried to alter the native physical condition of the starch, chloroform and alcohol did not improve the cooking quality to a noticeable extent. Prior soaking of the new rice in 10% formalin overnight (16 hours), however, enhanced the swelling quality of new rice to the same level as that of old rice, the swelling numbers of new, old, and formalin-treated new rice being 2.5, 3.3, and

3.3, respectively. Also, the cooked grains did not stick to one another. Formalin is known to bring about cross-linking across starch molecules and also to combine with proteins and is used for this reason as a structural fixative agent. The fact that formalin improved the cooking quality of the new rice therefore indicates that the better cooking quality of the old rice could be due to its hardening during storage. In further support of this, 100 g. of new and old rice grains were extracted with water by gentle shaking in a mechanical shaker for 2 hours, and more of solids were found to be extracted from the new rice grains (2.16 g. and 1.76 g. from new and old rice, respectively). Testing the hardness of new and old rice (in the same variety and dried to the same moisture content) in a stiffness or flat-crush testing apparatus (supplied by Gaydon & Co., England, and used in testing of packaging materials) gave indications that the old stored grain was harder than the new grain.

Extraction of new- and old-rice flour with 3% sodium chloride solution showed that the protein in the old rice was less soluble and had suffered denaturation to some extent. Possibility of the existence of similar differences in the solubility of starch in perchloric acid of different concentrations was therefore studied. Data presented in Table IV have not, however, shown any clear-cut trends, although

TABLE IV

SOLUBILITY OF STARCH IN NEW AND OLD RICE IN PERCHLORIC ACID

PERCHLORIC ACID USED FOR EXTRACTION (WT/VOL.)	WEIGHT OF STARCH EXTRACTED	
	New	Old
%	*mg*	*mg*
9.2	6.2	5.7
11.5	45.0	32.5
13.8	620.0	622.5
16.1	687.3	671.2

there are indications that the starch in the old grain is less soluble than that in the new grain. Further experiments under more controlled conditions and with selective solvents for the amylose and amylopectin constituents separately are necessary to confirm the small differences in solubility observed in the present experiments.

Studies on the settling characteristics of rice flour showed that the particles in the new-rice flour tended to settle more slowly than those in the old-rice flour (Table V). The supernatant in the case of new-rice flour was also more turbid. The same was true for the cooked suspensions, too. The flocculent nature of the particles in the new rice was also indicated by the fact that, even after standing for 24 hours, the

TABLE V
TURBIDITY AND SETTLING CHARACTERISTICS OF SUSPENSIONS OF NEW AND OLD RICE FLOUR

	Turbidity Reading of Supernatant after Settling for 2 Hours		Time Needed for Separation into Two Layers when Allowed to Settle under Gravity	
	Uncooked	Cooked	Uncooked	Cooked
			minutes	*minutes*
New	31	192	10	7
Old	13	157	2	1½

volume occupied by the particles that had settled to the bottom was more in the case of new rice. The faster settling rate of particles in the case of old rice could be due either to a change in the property of the rice or to the lower viscosity of the supernatant fluid (4).

Conclusion

Alpha-amylase in cereals, as in rice (unpublished work), is generally known to be relatively stable to high temperatures. The possibility, therefore, existed that during the cooking of intact rice grains, before the inside of the grain could attain the inactivation temperature, the enzyme may act on the starch to influence adversely the cooking quality of the rice. The present study has, however, shown that both the alpha- and beta-amylases are destroyed within the first 5 minutes of cooking of rice grains. This fact, together with the failure to improve the cooking quality of new rice by chemical inactivation of the amylases or to induce pastiness in old rice by the addition of amylase to the cooking water, shows that the amylases do not play a significant role in determining the cooking quality of the rice. The relative differences in culinary quality between new and old rice cannot therefore be explained on the basis of the difference in their amylase activities.

Changes in the solubility and settling characteristics of rice observed in the present study lend further support to the physical theory of sol-gel transformation earlier proposed by Rao (7). A mild form of "wet-heat treatment" has been proposed and successfully applied as a curing method to improve the cooking quality of new rice (5). Parboiling of paddy, which uses a similar form of heat-treatment, is also known to modify the colloidal property of the rice to a gel state (8). It is, therefore, likely that the physical changes during storage bring about a hardening in structure and thus improve the quality of the grain. The fact that formaldehyde, which hardens the structure of tissues by formation of cross links, can also bring about improvement in the cooking quality of the new grain gives further evidence to this possibility.

Reference can be made here to the changes responsible for the staling of bread (2). Whereas the changes that occur during holding of the bread are undesirable, storage changes in the case of the rice grain are desired by consumers. The changes in the physical properties of rice observed here are analogous, at least partially, to the changes observed during the staling of bread. The lower volume of sediment when aqueous suspensions of stale bread crumbs or stored rice particles are allowed to settle as compared with fresh bread or rice particles, respectively, is an instance of comparison (3). Another common feature is the tendency to form lumps during hydration (munching of bread or cooking of rice). This may be related to the difficulty in the digestion of fresh bread or rice. A decrease in the amount of soluble amylose is also observed during the process of bread staling and storage of rice (3,4). A difference between the two processes, however, relates to the capacity for water uptake. Stale bread crumbs have a lower capacity to absorb water and swell (3); stored rice absorbs more water during cooking than fresh rice although, when soaked in cold water or exposed to a humid atmposphere, fresh rice imbibes a slightly larger amount of water than old rice (4). It is proposed to investigate further similarities or differences that may exist between the changes during the two processes.

Literature Cited

1. Bailey, J. M., Thomas, Gwen J., and Whelan, W. J. Selective inhibition of enzymic impurities associated with potato phosphorylase. Biochem. J. **49:** lvi-lvii (1951).
2. Bice, C. W., and Geddes, W. F. The role of starch in bread staling. *In* Starch and its derivatives, ed. by J. A. Radley; vol. II. Chapman & Hall: London (1953).
3. Cathcart, W. H. Review of progress in research on bread staling. Cereal Chem. **17:** 100–121 (1940).
4. Desikachar, H. S. R. Changes leading to improved culinary properties of rice on storage. Cereal Chem. **33:** 324–328 (1956).
5. Desikachar, H. S. R., and Subrahmanyan, V. The curing of freshly harvested paddy. Parts I and II. J. Sci. Industrial Research (India) **16A:** 365–370 (1957).
6. Giri, K. V., and Sreenivasan, A. Das Amylasesystem des Reiskornes während des Reifens und Keimens. Biochem. Z. **289:** 155–166 (1937).
7. Sanjiva Rao, B. Investigations on rice. Current Sci. **6:** 446–447 (1938).
8. Sanjiva Rao, B., Vasudeva Murthy, A. R., and Subrahmanyan, R. S. The amylose and the amylopectin contents of rice and their influence on the cooking quality of the cereal. Proc. Indian Acad. Sci. **36:** 70–80 (1952).
9. Somogyi, M. Notes on sugar determination. J. Biol. Chem. **195:** 19–23 (1952).
10. Sreenivasan, A. Studies on quality in rice. IV. Storage changes in rice after harvest. Indian J. Agr. Sci. **9:** 208–222 (1939).
11. Sreenivasan, A. Veränderungen der enzymatischen Hydrolyse von Stärke im Reis während der Lagerung. Biochem. Z. **301:** 210–218 (1939).