Enzymatic Activities and Rheological Properties of Stored Rice

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ABSTRACT

Amylolytic, proteolytic, lipolytic, and lipoxygenase changes in milled rice obtained from short-, medium-, and long-grain varieties of paddy, one lot stored immediately after harvest at natural moisture and one dried to 12% moisture, were investigated after 1, 6, and 12 months of storage. Paddy samples stored after drying had significantly reduced diastatic, proteolytic, and lipase activities, whereas lipoxygenase activity was not altered with drying. Stored samples had increased activities of proteases and lipases, but amylase activity was greater in freshly harvested samples. Dried samples had reduced contents of free amino acids and free fatty acids. With or without drying, free amino acid and free fatty acid content increased during storage. Peak viscosity of milled rice did not change when paddy was dried before storage but it did increase during storage.

Starch and proteins of milled rice have been studied extensively in relation to the processing, cooking, eating, and nutritional properties of the grains (Juliano et al 1965, Juliano 1968). Storage of milled rice results in high paste viscosity and lower α- and β-amylase activities (Barber 1969). An increase in the free fatty acids and a decrease in amylase during storage of milled rice resulted in an increase in the peak viscosity of the amylograms (Yasumatsu et al 1964, Shin et al 1985).

The relation of amylase activity with the amylographic properties of starch was reported in the literature but the action of proteolytic, lipase, or lipoxygenase enzymes was not reported, particularly when the crop was harvested and stored normally.

Shortages of manual labor, harvesting aids, and drying facilities in the Indian subcontinent are some of the constraints that force farmers to harvest their crops at variable moisture levels. The freshly harvested paddy is commonly stored in gunny bags (65 kg). Although some reports are available in the literature on the effect of storage on enzymatic activities in milled and brown rice, study of the effect of storage of paddy in gunny bags, the normal practice in India, is lacking. In this investigation, we determined the effects of year-long storage on enzymatic activities and rheological properties of paddy stored under ambient conditions at time of harvest or dried to a safe moisture content of 12%.

MATERIALS AND METHODS

Materials

The paddy samples of varieties IR-8, PR-108, and Basmati-370 (short-, medium-, and long-grain varieties, respectively) were obtained from the farms of the Department of Plant Breeding, Punjab Agricultural University, Ludhiana, India. The seeds were sown in the nursery and then transplanted in the main fields. After harvesting, one lot of each variety was stored in triplicate at the original moisture content, and one lot was dried to 12% moisture with a forced-air circulation drier at 35°C and stored. Both lots were stored in gunny bags under ambient conditions for one, six, and 12 months (Fig. 1).

Analytical Methods

Protein, fat, and free fatty acids were determined by AACC approved methods (1976). Protein is expressed as Kjeldahl N × 5.95%. Proteins in water extracts were determined according to the method of Lowry et al (1951), using Folin and Ciocalteu's reagent, with bovine serum albumin as a standard. Free amino acids were determined by the method of Lie (1973) with little modification in the extraction. Free amino acids were extracted from 1 g of finely ground sample with 100 ml of 70% ethanol for 1 hr. Ethanol was evaporated at 40°C and volume adjusted to 50 ml. A 2-ml aliquot was reacted to develop the color. Glycine was used as a standard, and results were expressed as percent glycine.

Enzyme Activity Measurements

Diastatic activity was measured by the Blish and Sandstedt (1933) method (AACC 1976). Results were expressed as milligrams of maltose per 10 g per hour at 30°C.

Proteolytic activity was determined by the Ayres and Anderson (1939) method (AACC 1976), with the modification that protein was determined by the method of Lowry et al (1951) rather than by determination of soluble nitrogen. Results were expressed as milligrams of protein hydrolyzed per 100 g of flour at 45°C for 2 hr.

Lipase activity was expressed as the percentage of oleic acid on a fat basis using the AACC (1976) quick method for free fatty acids. Lipoxygenase activity was determined using a modification of the method of Summer (1943). The enzyme extract was obtained by macerating a 5-g sample with 25 ml of distilled water in a mortar and pestle. The sample was filtered through Whatman No.1 filter paper and the filtrate used as enzyme extract. Two milliliters of enzyme extract was used to develop the color. Absorbance was measured at 520 nm on a Spectronic-20 spectrophotometer. Results were expressed as:

$$\underset{(\mu\text{l of } O_2\cdot g^{-1}\cdot \text{min}^{-1})}{\text{Lipoxygenase activity}} = \frac{\text{Total } Fe^{+++}}{(\text{Time of reaction} \times \text{Weight of sample in enzyme extract})} \times 0.2866$$

Rheological Characteristics

Rheological characteristics were studied using the amylograph method of Hallick and Kelly (1959). A 50-g sample of powdered

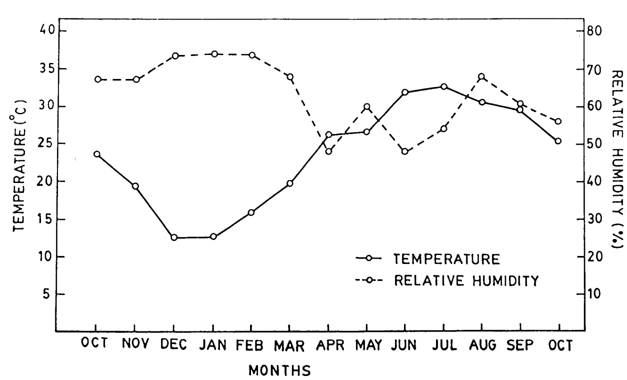

Fig. 1. Mean temperature and relative humidity during storage (1986–1987) of paddy in gunny bags under normal atmospheric conditions.

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TABLE I
Moisture Content of Paddy at Time of Harvesting and During Storage

Variety	Harvest	Storage Time					
		1 Month		6 Months		12 Months	
		Not Dried[a]	Dried[b]	Not Dried	Dried	Not Dried	Dried
IR-8	20.6	15.4	12.9	9.3	8.8	11.3	11.0
PR-108	19.7	14.9	13.1	8.8	8.7	10.6	10.3
Basmati-370	18.6	14.1	12.4	8.4	8.4	10.2	10.0

[a] Sample stored without drying.
[b] Sample dried to 12% moisture prior to storage.

rice (40 mesh) was suspended in 450 ml of distilled water and transferred to the revolving stainless steel bowl of the Brabender Amylograph. Temperature rise was set at 1.5°C per min. Amylograms were interpreted for peak viscosity (Brabender units), peak temperature (°C), and gelatinization temperature (°C).

Statistical Analysis of Data

Analysis of variance and least significant difference were computed at the 5% level by the method of Pence and Sukhatme (1967). The design used was a three factor factorial.

RESULTS AND DISCUSSION

Moisture Content, Temperature, and Relative Humidity

The moisture content of paddy at the time of harvesting and the changes that occurred during storage at ambient temperature are presented in Table I. In all the samples, the moisture content of paddy decreased sharply within a month, probably due to low temperatures and dry weather (Fig. 1). The moisture decreased further up to six months of storage. With the onset of the monsoon season, moisture again increased but remained below the level found after one month. This study clearly pointed out that the moisture content equilibrated according to the temperature and relative humidity regardless of initial moisture content. Drying prior to storage was of little use in monitoring moisture in the stored paddy. Among the different varieties, variation of moisture content was significant; a higher value was recorded for IR-8 than for PR-108 or Basmati-370.

Diastatic Activity and Rheological Properties of Milled Rice

The influence of drying and of storage time of paddy on diastatic activity of milled rice prepared from that paddy is shown in Table II. Samples stored without drying had significantly higher diastatic activity, indicating that moisture plays an important role in determining diastatic activity. Duration of storage also had a significant effect on diastatic activity. In general, activity decreased with increased storage time. Overall, the mean enzymatic activity in samples stored for one month was 86.8 mg maltose/10 g; this decreased to 50.7 mg after six months and then increased to 57.1 mg after 12 months. This fluctuation appears to be due to the changes in moisture and temperature during the year. Other researchers have found comparably low values of diastatic activity in stored rice (Shin et al 1985, Narayana et al 1954, Srinivasan 1939). Diastatic activity of the three varieties differed significantly; highest activity was found in the long-grain variety.

Amylograph measurements showed that drying of paddy before storage did not significantly affect peak viscosity of milled rice (Table III). However, there was a slight increase in peak viscosity with increased storage time. The increased paste viscosities were the result of concomitant changes in diastase enzymes and free fatty acids in the stored rice. This increase in peak viscosity of stored rice was reported previously; Barber (1969) reported that storage of milled rice results in higher paste viscosity and lower activity of both α- and β-amylases. Yasumatsu et al (1964) showed that the increase in free fatty acids in stored rice corresponds to the increase in peak viscosity. Short-, medium-, and long-grain varieties also had significantly different values for peak viscosity; short-grain varieties had higher peak viscosity than did long and medium grain varieties.

TABLE II
Effect of Drying and Storage Time on Diastatic Activity of Milled Rice[a]

Variety	Storage Time					
	1 Month		6 Months		12 Months	
	Not Dried	Dried	Not Dried	Dried	Not Dried	Dried
IR-8	86.2	58.7	63.4	33.7	84.7	42.1
PR-108	70.5	60.7	53.1	37.7	40.5	37.4
Basmati-370	124.9	119.7	57.5	58.6	77.5	60.4

[a] Values given are diastatic activity expressed in milligrams of maltose per 10 g of rice.

TABLE III
Amylograph Data for Milled Rice

Variety	Storage Time					
	1 Month		6 Months		12 Months	
	Not Dried	Dried	Not Dried	Dried	Not Dried	Dried
Peak consistency (BU)						
IR-8	1,025	1,037	1,110	1,080	1,100	1,093
PR-108	470	442	593	513	595	522
Basmati-370	675	660	697	700	743	750
Peak temperature (°C)						
IR-8	93.3	93.4	94.5	91.0	93.3	92.8
PR-108	91.5	91.9	91.9	92.4	92.1	92.4
Basmati-370	92.1	91.8	91.8	92.0	93.4	91.3
Gelatinization temperature						
IR-8	77.5	72.8	74.3	71.5	77.0	77.5
PR-108	75.8	74.5	77.4	75.0	79.3	79.5
Basmati-370	83.0	80.5	80.5	80.3	79.8	77.1

The effect of drying on the gelatinization temperature and peak temperature was significant (Table III). Higher values were recorded in the samples stored without drying. Duration of storage did not significantly affect either gelatinization temperature or peak temperature.

Proteins, Proteolytic Activity and Free Amino Acids of Milled Rice

Effects of drying prior to storage and of storage time on protein content, water-soluble proteins, proteolytic activity, and free amino acid content are shown in Table IV.

Protein content was not significantly affected by drying, but protein content of milled rice decreased significantly during storage of paddy. Beloglazona et al (1976) also reported a decrease in protein content of rice during storage.

Drying before storage caused a significant decrease in water-soluble proteins. The varieties differed significantly in water-soluble protein content; long-grain varieties had more water-soluble protein than either the short- or medium-grain varieties.

Proteolytic activity decreased significantly as a result of drying, thereby showing the effect of moisture on proteolytic activity. During storage, proteolytic activity significantly increased in both samples. The mean value for proteolytic activity for samples stored one month was 97.5 mg/100 g. This value increased to 189 mg/100 g after 12 months. Proteolytic activity was higher at the higher temperatures prevailing during the summer months (Mazzini et al 1980).

Proteolytic activity was significantly higher in long-grain varieties, and lowest in medium-grain varieties, contrary to the findings of Lorenz and Saunders (1978), who reported that long-grain varieties have the lowest proteolytic activity.

Free amino acid content was significantly higher in samples stored without drying than in dried samples. This difference appears to be due to lower proteolysis at the low moisture levels of the dried grain. As a result of increased proteolytic activity during storage, the free amino acid content of milled rice increased proportionately to storage time of the paddy. Varieties differed significantly in free amino acid content.

Lipase, Lipoxygenase, Fat, and Free Fatty Acid Content of Milled Rice

No significant effect on crude fat content was found to be caused either by drying or by storage. Short- and medium-grain varieties had a mean value of 1.1% and long-grain varieties 1.2% throughout. This finding agrees with those of Yasumatsu and Moritaka (1964) and Ramarathnam and Kulkarni (1983).

The effect of drying and the duration of storage on the lipase activity, free fatty acid content, and lipoxygenase activity of milled rice are shown in Table V. Milled rice from paddy dried before storage showed a significant decrease in lipase activity. This is a favorable effect with respect to rice quality. Storage of paddy resulted in a significant increase in lipase activity of milled rice. Noda and Kobayashi (1968) reported that lipase activity of bran from paddy stored for one year was higher than that from the freshly harvested crop.

Drying of paddy before storage lowered the free fatty acid content of the milled rice, apparently due to decreased lipolytic activity at low moisture levels. Free fatty acid content then increased significantly during storage, due to lipolytic activity over a prolonged period. The long-grain variety had significantly higher free fatty acid content than either short or medium varieties.

Drying of paddy before storage did not affect lipoxygenase activity, but activity increased significantly during storage. The short-grain variety had a higher lipoxygenase value than long- or medium-grain varieties.

TABLE IV
Effect of Drying and Storage Time on Protein Content, Water-Soluble Proteins, Proteolytic Activity, and Free Amino Acids of Milled Rice

	Storage Time					
	1 Month		6 Months		12 Months	
Variety	Not Dried	Dried	Not Dried	Dried	Not Dried	Dried
Total protein (%)						
IR-8	7.4	7.5	7.3	7.3	7.2	6.5
PR-108	7.2	7.3	7.3	7.5	7.0	7.1
Basmati-370	9.1	9.7	8.6	9.1	8.8	9.0
Water-soluble proteins (%)						
IR-8	0.38	0.42	0.72	0.56	0.53	0.46
PR-108	0.36	0.40	0.57	0.56	0.48	0.48
Basmati-370	0.48	0.51	0.72	0.69	0.65	0.61
Proteolytic activity (mg of protein hydrolyzed$\cdot$100 $g^{-1}\cdot$2 hr^{-1})						
IR-8	112.8	84.3	158.2	123.3	226.7	157.5
PR-108	86.1	39.8	134.1	86.7	209.4	125.0
Basmati-370	157.3	104.4	210.0	123.7	255.8	158.2
Free amino acids (% as glycine)						
IR-8	0.39	0.34	0.42	0.40	0.46	0.42
PR-108	0.34	0.31	0.46	0.39	0.48	0.42
Basmati-370	0.34	0.40	0.40	0.33	0.45	0.34

TABLE V
Effect of Drying and Storage Time on Lipase Activity, Free Fatty Acid Content, and Lipoxygenase Activity of Milled Rice

	Storage Time					
	1 Month		6 Months		12 Months	
Variety	Not Dried	Dried	Not Dried	Dried	Not Dried	Dried
Lipase activity[a]						
IR-8	14.8	10.7	42.3	30.5	64.0	53.9
PR-108	19.4	11.9	44.5	38.2	75.6	49.0
Basmati-370	25.6	12.3	65.2	56.7	88.2	72.1
Free fatty acids (% as oleic acid)						
IR-8	0.16	0.12	0.45	0.32	0.63	0.55
PR-108	0.21	0.14	0.47	0.41	0.67	0.50
Basmati-370	0.31	0.14	0.79	0.69	1.05	0.83
Lipoxygenase activity (μl of $O_2\cdot g^{-1}\cdot min^{-1}$)						
IR-8	1.6	0.9	2.0	1.9	3.2	3.2
PR-108	0.8	0.7	1.4	1.6	3.2	3.4
Basmati-370	0.8	0.6	1.9	1.9	3.2	4.1

[a] % Fat activity on % fat basis.

CONCLUSIONS

Peak viscosity of milled rice increased with time of storage of paddy due to an increase in free fatty acid content and a decrease in diastatic activity during storage. Drying of freshly harvested paddy was beneficial in decreasing the activity of amylases, proteases, and lipases, the enzymes responsible for deterioration of rice during storage.

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