Rapid Paper

Effects of Amylase Activity on Changes in Amylogram Characteristics during Storage of Brown Rice

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A series of storage tests was done to find the effects of the amylase activity present in brown rice on the amylogram peak viscosity and total setback. The result indicates that a decrease in amylase activity during the storage causes an increase in the peak viscosity, but the decrease in the amylase activity has nothing to do with the total setback.

The amylogram peak viscosity and setback of rice flour pastes increase during storage.[1] A couple of investigators have cited several factors which are responsible for the changes in amylograms during the storage of rice. Yasumatsu *et al.*[2] reported that methanol extraction by Schoch's method[3] eliminates the difference in peak viscosity caused by storage, and they suggested that the increase in free fatty acids during milled rice storage causes an increase in the peak viscosity of the amylogram. On the other hand, Shibuya *et al.*[4] reported that the increase of free fatty acids hardly influences the amylogram peak viscosity and setback of rice flour pastes. Shibuya *et al.*[5] also reported that the amylogram characteristics of rice starch pastes changes little during storage. Sreenivasan[6] and Desikachar *et al.*[7] studied the effects of amylase activity during storage on the cooking quality of the rice, but the effects of this amylase activity on changes in the amylogram during storage of brown rice has not been studied in detail. Further, Bhattacharya *et al.*[8] reported that the peak viscosity differed appreciably among rice varieties and the variation was quite random. They suggested that further research would be required to explain the random variation among the rice varieties.

The purpose of this paper is therefore to find out whether the amylase activity affects the amylogram peak viscosity and setback of brown rice flour pastes during the storage of brown rice.

MATERIALS AND METHODS

Materials. An Indica/Japonica brown rice (*Sam Kang* variety) was obtained by dehulling with a Satake dehuller at our laboratory, packed in polyethylene bags, and stored at 35°C for 12 months.

The brown rice was milled to flour by passing through a 100-mesh sieve, and the brown rice flour obtained was designated UBR (undefatted brown rice flour). The undefatted brown rice flour was defatted with ether at room temperature for 8 hr to extract free lipids from the flour and the sample was designated EDBR (ether defatted brown rice flour). The ether defatted brown rice flour was again defatted with an 85% methanol solution at 80°C for 4 hr to extract bound lipid from the flour (Schoch's method[3]) and the sample was designated MEDBR (methanol extracted, ether defatted brown rice flour). Brown rice starch was prepared by Dimler's method.[9]

Amylograph. Pasting properties of undefatted and defatted brown rice flours and starch were measured with a Brabender Visco-Amylograph. Forty grams (dry basis) of brown rice flours (100 mesh) and 410 ml of distilled water with and without the addition of 200 mg mercuric chloride were well mixed and heated from 25 to 95°C, held at 95°C for 60 min, and cooled to 50°C to obtain peak viscosity and total setback such that the samples could be characterized viscographycally.[8]

Amylase activity measurements. Amylase activity was measured by Bernfeld's method.[10] One gram of brown rice flour was extracted for 30 min at 37°C with 10 ml of 0.1 M sodium acetate buffer (pH 4.75). The extracts were centrifuged at 27,000 × *g* for 10 min. One ml samples of the supernatant were used to measure amylase activity after 5 min of incubation time with the starch substrate at 37°C. Using a maltose standard curve, amylase activity was calculated and expressed as mg of maltose per min per ml of extract.

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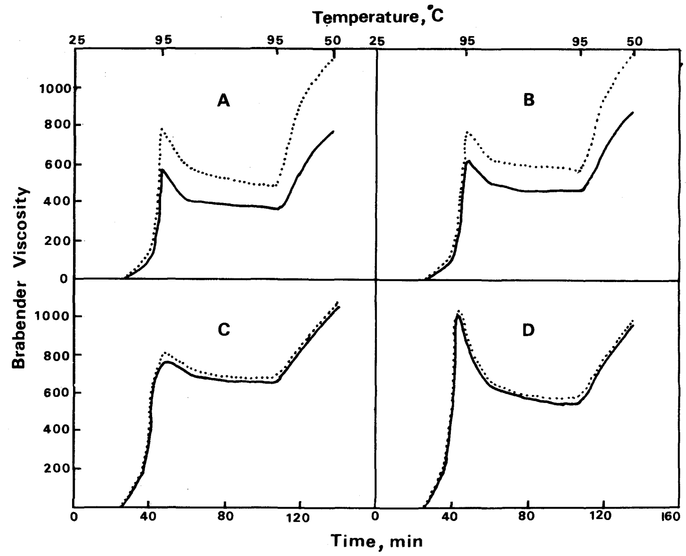

FIG. 1. Amylograms of Undefatted Brown Rice Flour (UBR) (A), Ether Defatted Brown Rice Flour (EDBR) (B), Methanol Extracted Ether-Defatted Brown Rice Flour (MEDBR) (C) and Brown Rice Starch (D) during Storage of Brown Rice at 35°C.

——, flours or starch at storage time of 0 month; ------, flours or starch at storage time of 12 months.

TABLE I. AMYLOGRAM PEAK VISCOSITY AND TOTAL SETBACK OF BROWN RICE FLOUR PASTES WITH AND WITHOUT ADDITION OF MERCURIC CHLORIDE DURING THE STORAGE OF BROWN RICE AT 35°C

Type of flour[b]	Peak viscosity				Total setback			
	Before storage		After 12 months		Before storage		After 12 months	
	Control	$HgCl_2$[a] added	Control	$HgCl_2$ added	Control	$HgCl_2$ added	Control	$HgCl_2$ added
Undefatted brown rice flour (UBR)	580±10	860±10	780±10	940±10	420±10	450±10	670±10	620±10
Ether defatted brown rice flour (EDBR)	620±10	840±10	770±10	890±10	410±10	400±10	650±10	630±10
Methanol extracted ether defatted brown rice flour (MEDBR)	760±20	800±20	820±20	840±20	400±20	410±20	380±20	390±20
Brown rice starch	1020±20	1010±30	1030±30	1040±30	410±20	310±30	400±20	310±30

[a] 200 mg mercuric chloride added to a mixture of 40 g brown rice flours and 410 ml distilled water.
[b] Mean ± S.D. based on 3 samples.

RESULTS AND DISCUSSION

Amylogram characteristics of undefatted and defatted brown rice flours and starch stored for 0 and 12 months is shown in Fig. 1. Peak viscosity and total setback of UBR increased as shown in Fig. 1A, and free fatty acids in this flour increased from 9 mg/40 g brown rice to 62 mg/40 g brown rice during the 12 month storage period at 35°C. In the meantime, EDBR, which does not contain free fatty acids, also showed an increase in both peak viscosity and total setback as shown in Fig. 1B. From these results, we can confirm the finding of Shibuya *et al.*,[4] who reported that an increase of free fatty acids hardly affects the increase of peak viscosity of rice flour pastes.

Table I shows that the peak viscosities of both UBR and EDBR increased substantially during the 12 month storage period at 35°C, and Table II shows that amylase activity present in the UBR and EDBR decreased substantially during the same storage period. To find out whether the increase in the peak viscosity is due to the decrease of amylase activity during the storage period, each sample was tested with and without addition of mercuric chloride which is a potent inhibitor of the amylase.[7] Table I shows that the peak viscosities of UBR and EDBR are indeed increased when treated with mercuric chloride. This means that the peak viscosity is affected by the amylase activity. In the meantime, the peak viscosity of MEDBR did not increase significantly during the storage, regardless of the presence of mercuric chloride. This result can be explained partly by the fact that the amylase activity was seriously inactivated during methanol extraction for 4 hr at 80°C and the difference in the amylase activity present in MEDBR before and after the storage period was negligible (Table II). The peak viscosity of brown rice starch also did not change during storage, regardless of the treatment with mercuric chloride. Therefore, we suggest that the decrease in the amylase activity present in the UBR during the storage causes the increase in the amylogram peak viscosity of UBR during the same storage period. Furthermore, the difference in the amylase activity among the rice varieties appears to be partly responsible for the random variation in the peak viscosity for the rice varieties which was reported by Bhattacharya *et al.*[8]

Total setback is used as an index of the stickiness of cooked rice.[8,11] Table I shows that the total setback of UBR and EDBR increased substantially during the 12 month storage period at 35°C, but their total setback did not show a significant increase when treated with mercuric chloride (Table I). From this result, we can say that increases in the total setback of UBR and EDBR during the storage are independent of amylase activity during the same storage period. Therefore, we suggest that the decrease in amylase activity during the storage hardly affects the total setback of UBR. This result indirectly supports the findings of Desikachar *et al.*,[7] who reported that amylase activity is not significant in the cooking quality of rice. In the meantime, removal of bound lipids by methanol extraction eliminates the increase in the total setback caused by the storage as shown in Table I and from this result, we can suggest that an increase in the total setback of UBR during the storage appears to be due to the changes in some structural components such as bound lipid.

TABLE II. AMYLASE ACTIVITY IN VARIOUS BROWN RICE FLOURS DURING STORAGE OF BROWN RICE AT 35°C

Type of flour[a]	Amylase activity (mg maltose/min/ml extracts)	
	Before storage	After 12 months
Undefatted brown rice flour (UBR)	0.206±0.012	0.114±0.009
Ether defatted brown rice flour (EDBR)	0.170±0.015	0.090±0.009
Methanol extracted ether defatted brown rice flour (MEDBR)	Nil	Nil

[a] Mean±S.D. based on 3 samples.

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