Effect of Lipids on the Retrogradation of Cooked Rice

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ABSTRACT

Cereal Chem. 67(1):7-10

The effect of lipids on the aging process of cooked rice stored at 5°C was studied by measuring enzyme digestibility and X-ray diffraction patterns. For normal rice, simultaneous digestibility with β-amylase and pullulanase, which is a good measure of degree of gelatinization, increased after 3 hr of storage and then continuously decreased. X-ray diffraction patterns of normal rice immediately after cooking showed a V-type pattern that may be attributed to helical complexes of amylose with lipids in starch. V-type pattern intensity became faint in 5 hr, whereas B-type pattern intensity increased, implying that the starch-lipid complexes were metastable and changed to a more stable structure partly characterized by B-type X-ray pattern via an amorphous state. This can illustrate the way in which lipids affect the retrogradation process of cooked rice. Water-soluble carbohydrates from aged pastes of normal rice starch were also measured and found to increase and then decrease after 5 hr of storage. This may also closely correlate with the behavior of the starch-lipid complex. Gel permeation chromatography of the water-soluble carbohydrates indicated that an increase in solubility by defatting was due not only to the amylose fraction but also to the amylopectin fraction.

Normal starches from cereals contain 0.5~1.3% internal lipids (i.e., lipids extractable only by polar solvents such as water containing methanol, butanol, etc.), which have a remarkable influence on the behavior of starch in gelatinization and retrogradation processes (Lorenz 1976, Ohashi et al 1980, Juliano 1984, Morrison et al 1984, Azudin and Morrison 1986, Morrison and Azudin 1987, Galliard and Bowler 1987). X-ray diffraction study (Zobel et al 1988) and differential scanning calorimetry (Kugimiya et al 1980) have shown helical complexes of amylose with lipids after normal cereal starches gelatinize. The complexes are more resistant to enzymic attack (Hanna and Lelievre 1975, Holm et al 1983) and less soluble in water (Gray and Schoch 1962, Ohashi et al 1980, Hoover and Hadziyev 1981) than amorphous starches. In the present study, enzyme digestibility and X-ray diffraction pattern of cooked rice and water solubility of gelatinized rice starch were measured during storage. Results such as changing enzyme digestibility and water solubility indicated that the helical complexes are metastable and alter their structures during storage.

MATERIALS AND METHODS

Materials

Polished normal rice and polished waxy rice were purchased in Shiga, Japan. Normal rice starch was supplied by Shimada Chemical Industry Co., Niigata, Japan. Palmitic, oleic, and linoleic acids were purchased from Kyoto Wako Pure Chemical Industry Co., Kyoto, Japan, and used without further purification.

Defatting of Rice and Rice Starch

Defatted normal rice and defatted normal rice starch were prepared by Soxhlet extraction with 85% methanol for 72 and 30 hr, respectively. The amounts of the lipids extracted were 1.27 and 0.65% (w/w) of rice and rice starch, respectively.

Reintroduction of Lipids into Defatted Rice

Defatted normal rice (200 g) was suspended in 750 ml of methanol solution containing palmitic (20 g), oleic (33 g), and linoleic acids (43 g), the average composition of fatty acid in rice (Yasumatsu and Moritaka 1964, Yasumatsu et al 1966, Morrison et al 1984). The mixture was refluxed for 5 hr to introduce the lipids into the defatted rice. The rice was separated by filtration and dried after washing with ether. The amount of fatty acids reintroduced was 2.25% (w/w) of the defatted rice.

Methods of Gelatinization

Normal rice (200 g) soaked in water for 30 min was cooked with 1.6-fold (w/w) water by an automatic electric rice cooker (0.7 L). After the cooker was turned off, the rice sat for 15 min, a procedure that conditions the cooked rice for eating. Waxy rice was cooked by the same way with 1.2-fold (w/w) water. One gram of rice starch or defatted rice starch was suspended in 50 ml of water and heated by stirring at 85°C for 30 min to obtain a gelatinized paste. To prepare refatted starch paste, 50 ml of the defatted rice starch suspension was heated with palmitic acid (18 mg dissolved in 2 ml of methanol) as already described so that gelatinization and refatting of starch were achieved simultaneously.

Preparation of Retrograded Samples

Cooked rices and rice starch pastes were tightly sealed and stored at 5°C to allow retrogradation. For cooked rice, aliquots of the retrograded samples were taken at intervals to be analyzed. These aliquots were precipitated by adding ethanol, and the precipitates obtained were separated by filtration, dried after washing with ether, and analyzed as powder specimens for the measurements of degree of gelatinization and X-ray diffraction.

Degree of Gelatinization

The limiting degree of hydrolysis of the powder was measured by the β-amylase-pullulanase (BAP) method (Kainuma et al 1981, Matsunaga and Kainuma 1986), and the results are referred to as the degree of gelatinization throughout this paper.

X-Ray Diffraction

X-ray diffraction patterns were taken with a Rigakudenki X-ray diffractometer RAD-1A, using the following conditions: target, Cu-Kα radiation (Ni filter); voltage, 35 kV; current, 20 mA; scanning speed, 2° 2 θ/min; time constant, 1 sec. Powder samples were conditioned at 75% rh for one day before taking the X-ray diffraction patterns.

Gel Permeation Chromatography of Starch Paste

Starch pastes stored were centrifuged at 4,000 rpm for 30 min. Total carbohydrates in the supernatants were analyzed by gel permeation chromatography (GPC) under the following conditions: column, 2.5 × 62 cm with Sepharose CL-2B gel, product of Pharmacia Fine Chemicals Co.; eluant, 0.05M NaOH aqueous solution; flow rate, 15 ml/hr; temperature, 15~18°C; elution volume of one fraction, 3 ml. Total carbohydrate in each fraction was measured by the phenol-sulfuric acid method (Hodge and Hofreiter 1962) and a GPC elution pattern was drawn. The area of the GPC elution pattern was normalized as to the amount of the total carbohydrates solubilized in the supernatants. Molecular weight scale was calibrated using enzymatically synthesized amyloses (Kitamura et al 1982).

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RESULTS AND DISCUSSION

Table I shows the degrees of gelatinization of the cooked native rices during storage at 5°C after cooking. The degree for normal rice increased to a maximum value after 3 hr and then decreased. In contrast, for waxy rice the degree of gelatinization was initially high, remained unchanged for 1 hr of storage, and then gradually decreased. The different trends in starch retrogradation reflect differences in lipid and amylose contents of the two kinds of starches.

To examine the role of lipids in these phenomena, defatted and refatted normal rices were prepared and cooked for comparison with native nondefatted rice (Fig. 1). In defatted rice, the degree of gelatinization was higher than that of the native nondefatted rice soon after cooking. The degree remained unchanged for 3 hr of storage and then decreased with time. In refatted rice, like native nondefatted rice, the degree of gelatinization increased to the maximum value after 3 hr of storage and then decreased.

The crystalline nature of these cooked rices was investigated by X-ray diffraction (Fig. 2). In native nondefatted rice soon after cooking, V-type X-ray diffraction patterns characterized by clear diffraction peaks around 2 θ of 12.5 and 20.0° (Mikus et al 1946, Takeo et al 1973) are seen. The V-pattern should result mainly from the formation of helical complexes of starches with lipids. It is said that starch gel apparently shows a V-pattern when the gel is precipitated at high temperature (~70°C) with hot alcohol (Bear 1942). In this study, however, the precipitation was achieved at room temperature with cold ethanol so that the ethanol could make only a minor contribution to the V-pattern observed (Bear 1942). With time, a crystalline structure (the B-type X-ray diffraction pattern, characterized by peaks around 2θ of 5.6, 17.0, 22.0, and 24.0° [Zobel 1964]) became apparent, whereas the diffraction due to the helical complexes weakened. The pattern of the defatted rice soon after cooking is an amorphous one with a trace of V-pattern, which might be due to ethanol; with time, a faint B-pattern is observed. In the case of refatted rice, a more distinct crystalline V-pattern is evident soon after cooking, and development of the B-type crystalline structure is retarded.

TABLE I
Changes in the Degree of Gelatinization of Cooked Rices Stored at 5°C

Storage (hr)	Degree of Gelatinization (%)	
	Normal Rice	Waxy Rice
0	88.0 ± 3.9[a]	94.9 ± 1.8
1	91.8 ± 1.6	95.9 ± 1.9
3	97.9 ± 2.0	89.5 ± 2.6
5	90.6 ± 5.8	88.1 ± 2.3
24	83.1 ± 4.2	83.7 ± 3.0
48	59.7 ± 5.5	73.8 ± 6.5
144	44.4 ± 5.1	71.5 ± 2.3

[a] Mean ± SD (three samples).

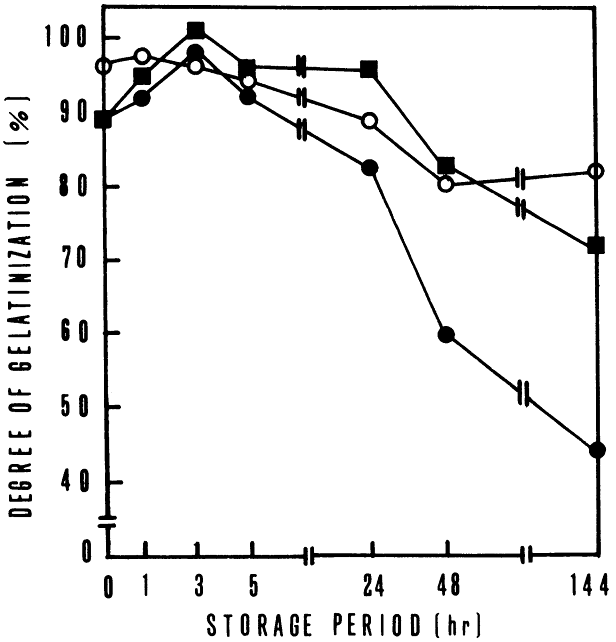

Fig. 1 Effect of lipid on starch retrogradation. Changes in the degree of gelatinization during the storage of cooked normal rices. Nondefatted (native) rice (●); defatted rice (○); refatted rice (■).

It is certain from the results that amorphous starch and helical complexes of starch coexist in cooked normal rice. The helical complex is considered more resistant to enzymic attack than amorphous starch (Hanna and Lelievre 1975, Holm et al 1983) and seems to be less resistant than retrograded starch. Considering this, it may be that the change in degree of gelatinization of cooked rice during storage closely correlates with a structural change of the helical complex of starch with lipid. In our opinion, the helical complex in cooked rice is not stable during storage, and the helix loosens or unwinds to a random or amorphous structure, which changes its susceptibility to amylase. The amorphous starch resulting then changes to a stable, partially

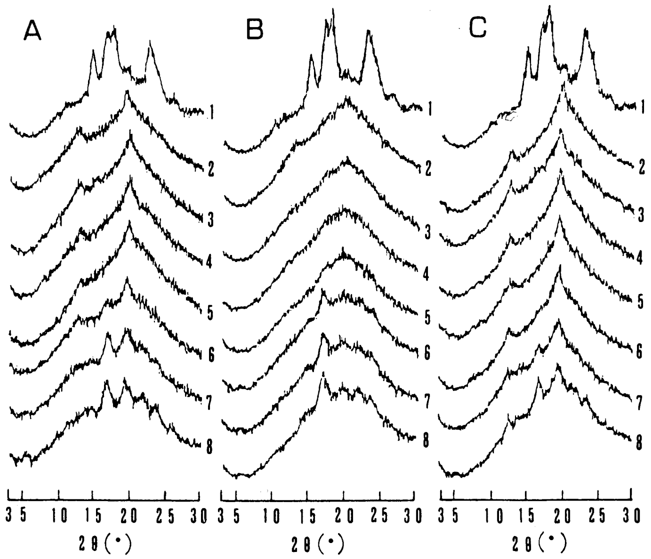

Fig. 2. Effect of lipid on starch retrogradation. Changes in the X-ray diffraction pattern during the storage of cooked normal rices. **A,** Nondefatted (native) rice; **B,** defatted rice; and **C,** refatted rice. Uncooked rice (**1**); Rice cooked and stored for 0, 1, 3, 5, 24, 48, and 144 hr, respectively (**2–8**).

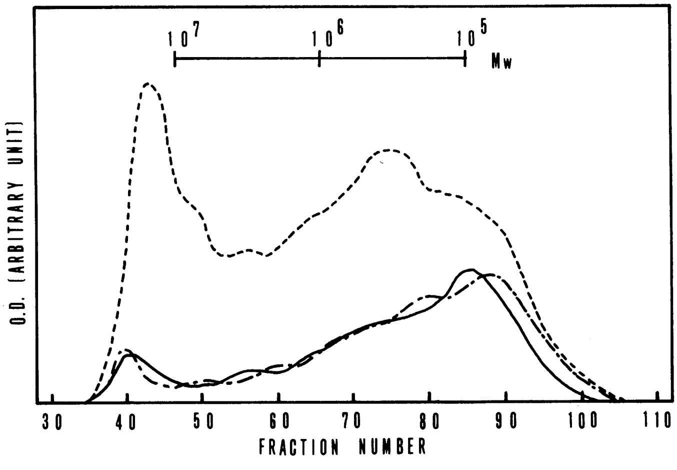

Fig. 3. Effect of lipid on the gel permeation chromatography elution patterns of the water-soluble fractions of normal rice starch pastes. Nondefatted (native) rice starch (—); defatted rice starch (- - -); refatted rice starch (– • –).

TABLE II
Effect of Lipid on Water-Solubility
in the Gelatinization of Normal Rice Starch

Fraction	Water-Soluble Carbohydrate (%) Nondefatted	Defatted
Total[a]	10.4	31.9
Amylose[b]	9.2	20.3
Amylopectin[b]	1.2	11.6

[a] Amount of total carbohydrate in the supernatant of starch paste was measured by the phenol-sulfuric acid method.

[b] Calculated from the area of gel permeation chromatography elution pattern.

crystalline structure that gives a B-type X-ray diffraction pattern. This may reasonably explain the changes in cooked rice digestibility observed in this study.

To further investigate an interaction between starch components (amylose, amylopectin) and fatty acid, GPC analyses were carried out. The gelatinized normal starch paste was centrifuged, and the supernatant and the residue were separated. Figure 3 shows GPC elution patterns of the water-soluble carbohydrates in the supernatants from the nondefatted, defatted, and refatted normal rice starch pastes before storage. The elution patterns show that the carbohydrates can be divided into two major fractions, namely, higher molecular weight and lower molecular weight fractions, respectively. The higher molecular weight fraction combines fractions no. 52 and below and shows iodine staining color with λ_{max} of 580 nm (in $10^{-3} N$ I_2, $10^{-2} N$ KI solution). The lower molecular weight fraction, combining fractions above no. 52, had a color with λ_{max} of 620 nm. Hence, the higher molecular weight fraction consists essentially of amylopectin and the lower molecular weight fraction of amylose (Richter et al 1968, Yamada and Taki 1976).

Amounts of the water-soluble carbohydrate were evaluated from the area of the elution pattern (Table II). For nondefatted rice starch, 10.4% of total carbohydrate in the starch paste was soublilized, 9.2% amylose and 1.2% amylopectin. By removing lipids, the water-soluble carbohydrate is much increased in all molecular weight fractions even amylopectin; that is, 20.3% in amylose and 11.6% in the amylopectin fraction. In the refatted starch paste, although it is not obvious whether lipids were reintroduced as they were before defatting, the elution pattern returns to the one quite similar to that of native nondefatted starch. These can be interpreted in terms of the interaction between lipids and starch, since the solubility of starch in water decreases when starch-lipid complexes are formed (Gray and Schoch 1962, Ohashi et al 1980, Hoover and Hadziyev 1981).

Figure 4 shows the GPC elution patterns of water-soluble carbohydrates in nondefatted (native) and defatted normal starch pastes after 0, 5, or 144 hr of storage. In nondefatted (native) rice starch paste (Fig. 4A) the amount of water-soluble carbohydrate increased after 5 hr of storage in all molecular weight fractions, even amylopectin, and decreased with the retrogradation process after 144 hr of storage, molecules of molecular weight $<10^6$ being particularly water-insoluble. (The molecular weight scale was calibrated using emzymatically synthesized amyloses. Although the scales are conveniently used for showing the molecular sizes of the samples, they do not show the true molecular weights.) In the defatted rice starch paste (Fig. 4B), however the amount of water-soluble carbohydrate continuously decreased with time; molecules of molecular weight $<10^6$ in particular showed a noticeable decrease in water solubility. Hence, it can be considered that lipids suppress the rate of amylose retrogradation. This corresponds with the result shown in Figure 2, in which lipids retard the development of a retrograded crystalline structure.

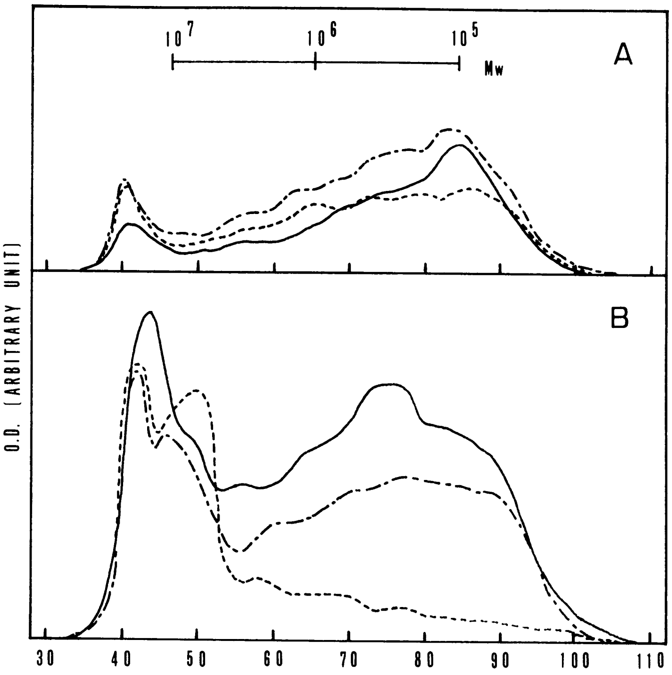

Fig. 4 Effect of storage on the gel permeation chromatography elution patterns of the water-soluble fractions of normal rice starch pastes. **A,** Nondefatted (native) rice starch; **B,** defatted rice starch. Storage periods: 0 hr (—), 5 hr (- • -), and 144 hr (- - -).

It is well known that amylose forms complexes with lipids, whereas amylopectin shows little evidence of interaction with lipids, although some kinds of hydrophilic surfactants are postulated to form weak complexes with amylopectin (Evans 1986, Eliasson et al 1988). From this point of view, it is interesting that the water-soluble amount of amylopectin in normal starch paste is much enhanced by defatting (Fig. 3). The amount of water-soluble amylopectin in nondefatted starch paste increases as the amount of soluble amylose increases, which is seen early in the aging process (Fig. 4A). These facts indicate that lipids can indirectly affect the behavior of amylopectin toward water through complex formation with amylose, which may be associated with amylopectin within a starch granule as postulated by Blanshard (1987). Finally, it seems worthy to mention that the presence or absence of lipid in the paste of waxy rice starch (data not shown) did not affect the amount of the water-soluble carbohydrate on gelatinization.

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[Received December 27, 1988. Revision received June 30, 1989. Accepted July 6, 1989.]