

Effect of Wheat Flour Aging on Starch-Granule Surface Proteins

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ABSTRACT

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Prime starch was isolated from aged wheat flours by acetic acid extraction (pH 3.5). Starch-granule surface proteins were extracted from these starch samples with 1% sodium dodecyl sulfate (SDS) containing 1% 2-mercaptoethanol at room temperature (15–25°C) and quantified by the Lowry method. The amount of starch-granule surface proteins from aged flours was three to four times greater than the control samples.

The SDS-polyacrylamide gel electrophoresis pattern of these extracted starch-granule surface proteins was different between the control and aged samples. High-performance liquid chromatography clearly showed that the higher molecular mass proteins (22–150 kDa) increased with aging of the wheat flour.

Aging wheat flour causes changes that improve its utilization in breads and cakes. Age-related changes were summarized by Christensen and Kaufmann (1974). Recently, Shelke et al (1992) reported age-related changes in the viscosity of batters and the water-binding ability of wheat flour. Seguchi and Matsuki (1977) and Seguchi (1990a) reported that chlorination and heat treatment of wheat flour improved pancake texture characteristics such as springiness and gumminess and that the effects were caused by a change in the nature of the starch-granule surface character from hydrophilic to hydrophobic. Furthermore, the amount of starch-granule surface proteins increased gradually with chlorination level. The presence of starch-granule surface proteins were reported by Lowy et al (1981), Greenwell and Schofield (1986), and Seguchi and Yamada (1989). Greenwell and Schofield (1986) and Malouf et al (1992) reported that a starch-granule surface protein was negatively correlated to grain hardness. This protein, friabilin (15 kDa), was specifically associated with the starch granule, based on purification of starch granules by cesium chloride (Sulaiman and Morrison 1990) and by immunological detection (Skerritt et al 1990). Eliasson and Tjerneld (1990) reported that wheat proteins were easily adsorbed to wheat starch and the amount of protein adsorbed increased when the starch granule had been heated. In this article, the changes in starch-granule surface proteins from aged-wheat flour are presented.

MATERIALS AND METHODS

The wheat flour used in this study was K Alps (Nitto Flour Milling Co., Tokyo, Japan) made from western white wheat from the 1991 harvest. Protein and ash contents of the wheat flour were 8.2 and 0.38%, respectively, at 13.2% moisture. Freshly milled flour was put into vinyl bags and stored at –20°C until used. Flour (0.5 kg) was placed on an iron plate (26 × 34 × 3 cm) in a 1.0-cm layer and left in a computer-controlled drying oven (DV 41, Yamato Co., or EYELA LTI-600SD, Rikakikai Co., Tokyo, Japan) for the following time and temperature combinations: 10, 20, and 40 hr at 100°C; 12 and 24 days at 60°C; 30 and 189 days at 40°C; and 202 days at 25°C. Other samples were stored for 46, 130, and 233 days at room temperature. After aging, flour samples were stored at –20°C until used. Flour was fractionated by the acetic acid (pH 3.5) method of Sollars (1958) and fractions were freeze-dried. Flour and starch moistures were determined by the method of Tsutsumi and Nagahara (1961). Starch-granule surface proteins were extracted from the prime starch fractions (2.0 g) by shaking overnight with 40 ml of 1% sodium dodecyl sulfate (SDS) solution containing 1% 2-mercaptoethanol at room temperature (Seguchi and Yamada 1989) and quantified using the method of Lowry et al (1951).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described (Seguchi and Yamada 1989). The amount of sample protein loaded per lane on the gel was 3–5 µg. High-performance liquid chromatography (HPLC) was performed using a gel-filtration chromatography (GFC) column (Shodex Protein KW-803, 0.8 × 30 cm, Showa Denko Co., Tokyo, Japan) with 1% SDS containing 2-mercaptoethanol at a flow rate of 1.0 ml/min. Starch-granule surface protein was dissolved in this solution and 30–60 µg protein was injected. The eluate was monitored at 280 nm. Oil-binding capacity of the isolated starches was assessed by vigorously shaking the starch (0.5 g) with rapeseed or corn oil (1.0 ml) and water (5.0 ml) in a test tube (Seguchi 1984a).

RESULTS AND DISCUSSION

Increasing Starch-Granule Surface Proteins by Aging

Up to three to four times more starch-granule surface protein was extracted from prime starch after various aging treatments compared to control flours (Table I). Little or no increase in the amount of starch-granule surface protein could be observed at the shortest time periods at each aging temperature. The results in Table I indicate that a longer aging time is necessary to obtain the same increase in the amount of starch-granule surface proteins from flours aged at lower temperatures. The significantly different amount of surface protein between the flours aged at 25°C for 202 days and at room temperature for 233 days would result from the lower average temperature (15–25°C) and possibly from the variance in relative humidity under ambient conditions.

TABLE I
Starch-Granule Surface Protein (%)

Treatment ^a	Percentage
Control	0.06 ± 0.01 ^b
100°C	
10 hr	0.09 ± 0.01
20 hr	0.10 ± 0.01
40 hr	0.19 ± 0.03
60°C	
12 days	0.07 ± 0.01
24 days	0.17 ± 0.01
40°C	
30 days	0.07 ± 0.02
189 days	0.20 ± 0.02
25°C	
202 days	0.24 ± 0.01
Room temperature	
46 days	0.06 ± 0.01
130 days	0.19 ± 0.02
233 days	0.15 ± 0.02

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^aThree replicates.

^bStandard deviation.

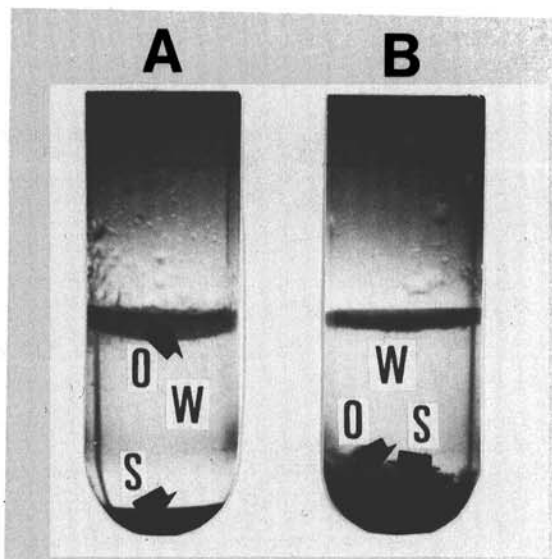


Fig. 1. Starch extracted from control wheat flour (A) or flour aged at room temperature for 233 days (B) and mixed with oil and water. O = oil, S = starch, W = water.

Hydrophobicity (Lipophilization) of the Aged Starch-Granule

It was observed by Seguchi (1984a and 1984b) that wheat starch granules bound oil after chlorination and heat treatment (120°C for 2 hr). Seguchi (1990b) indicated an increase in the amount of starch-granule surface protein separated from chlorinated wheat flour by the Sollars (1958) fractionation method. Seguchi (1990c) suggested that the hydrophobicity (lipophilization) resulted from changes in the relationship between flour components such as gluten and tailings fractions and influenced the bubbling stability in cake batter (Seguchi 1987, Seguchi and Yamada 1988). The oil-binding capacity of aged wheat starch (room temperature for 233 days) indicated hydrophobicity (lipophilization) (Fig. 1) as did the effects of chlorination and heat treatment. Other aging treatments that produced an increase in starch-granule surface protein produced similar results. The increase in hydrophobicity (lipophilization) due to aging should increase the amount of starch-granule surface proteins based on previous observations (Seguchi 1990b).

SDS-PAGE and HPLC Patterns of Starch-Granule Surface Proteins from Aged Flour

The differences between aged and control starch-granule surface proteins were examined with SDS-PAGE and HPLC. Figure 2 shows the SDS-PAGE patterns of surface proteins of starch granules collected from control and aged (202 days, 25°C) flours. The other aging treatments showed equivalent results. The differences in the patterns between the control and aged samples indicate that aging resulted in new proteins (31–66.2 kDa) being bound to starch granules. Changes in the relative amount of each protein band due to aging were not analyzed. HPLC patterns also show differences between the two treatments (Fig. 3). Figure 3A shows the HPLC-elution profile of starch-granule surface proteins from control flour, and Figure 3B shows the changes in the elution profile from aged flour (at room temperature for 130 days), indicating an increase in larger molecular mass (22–150 kDa) proteins. Other samples of starch-granule surface protein from aged flours indicated the same result.

CONCLUSIONS

Wheat starch granules fractionated from wheat flour aged at 25°C for 202 days, or at higher temperatures for a shorter time, exhibited increased hydrophobicity (lipophilization) and contained more starch-granule surface protein. The increased starch-granule surface protein was fractionated by SDS-PAGE and HPLC. Seguchi (1990b) observed the same increase in hydro-

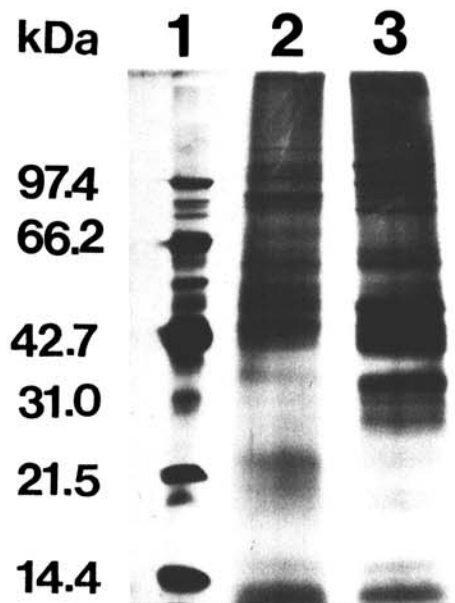


Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns showing molecular weight markers (lane 1), and starch-granule surface proteins extracted from control wheat flours (protein = 8.9 µg) (lane 2), and flour aged at 25°C for 202 days (protein = 7.8 µg) (lane 3).

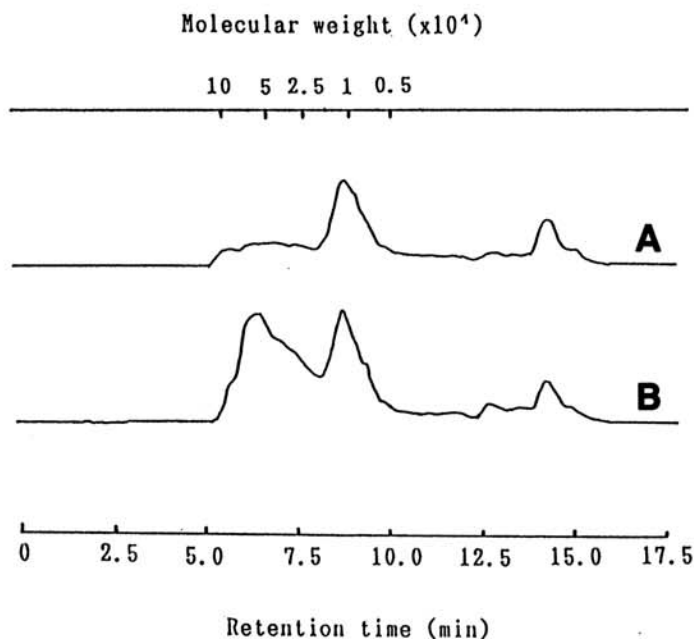


Fig. 3. High-performance liquid chromatography (gel-filtration chromatography) of surface proteins of starch granules collected from control wheat flour (protein = 30 µg) (A) and flour aged at room temperature for 130 days (protein = 57 µg) (B).

phobicity (lipophilization) and starch-granule surface protein after chlorination of wheat flour. This suggested a relationship between these obtained characteristics and improvements in pancake texture. Therefore, aging treatment of wheat flour should also have an improving effect on the pancake texture.

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