Effects of Secondary Structures of Heated Egg White Protein on the Binding Between Prime Starch and Tailings Fractions in Fresh Wheat Flour

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ABSTRACT

Dried egg white protein was heated at 120°C for 1 hr, added to a fresh wheat flour (protein 8.6%), and the protein and wheat flour were subjected to acetic acid (pH 3.5) fractionation. The results showed that egg white protein increased the binding between prime starch (PS) and tailings (T) fractions in wheat flour. Several conditions for heating of egg white protein were examined to determine 1) the effect of the amount of water added to the protein before heating; 2) the effect of heating time (hr) on protein at 120°C; and 3) the effect of heating temperature on the binding between PS and T fractions. The amount of protein per 50.0 g of wheat flour was further examined for the maximum binding between PS and T fractions. The heated egg white protein was analyzed by Fourier transform infrared (FT-IR) spectroscopy, and the changes in the secondary structures (α-helix, β-sheets, and others) of the protein caused by heating were studied. When egg white protein was heated at 120°C for 8 hr, 9.0% of the α-helix structures of egg white protein decreased to 3.0%, and 37.0% of the β-sheet structures increased to 41.0%. The decrease of α-helix and increase of β-sheet structures of heated egg white protein were related to the increase in the binding between PS and T fractions in the same heated egg white protein and wheat flour sample. A relationship between the structural changes in heated egg white protein (180°C, 1 hr) and the binding between PS and T fractions in the heated egg white protein and wheat flour was also observed.

Although a number of studies on the aging of wheat flour to improve the textures of wheat products have been reported (Pomeranz 1992), the mechanism responsible is not well understood. Seguchi et al (1998) observed the improving effects of aging soft wheat flour (protein content 8.2%) on the springiness and gumminess textures of pancakes. The acetic acid (pH 3.5) fractionation of wheat flour indicated that a separation of the prime starch (PS) and tailings (T) fractions gradually decreased with the increased storage time of wheat flour, which was caused by the interaction between PS and T fractions, and the protein content in the mixture of PS and T fractions increased with storage time. Seguchi et al (1998) indicated that the increase in the interaction between PS and T fractions caused by the aging of wheat flour was highly related to the improvement of pancake springiness (r = 0.9821). Seguchi (1993) reported that starch granules isolated from stored wheat flour were hydrophobic and that the amount of starch granule surface protein was 3–4× higher than in the control. Then Seguchi et al (1998) suggested that the increase in the interaction between PS and T fractions by aging was caused by the hydrophobicity of starch granule surface protein. These phenomena were similar to those of chlorinated (Seguchi and Matsuki 1977; Seguchi 1990a) and heat-treated wheat flour (Seguchi 1990b). The phenomena also suggested that the improvements in pancake were due to a change in the interaction between PS and T fractions, which may be caused by the change in wheat protein.

The main objective of this study was to make clear the change of secondary structure of wheat protein by aging, and how it relates to the binding between PS and T fractions and the improvement of pancake springiness. However, because wheat protein is composed of many kinds of proteins, it is difficult to determine a more precise role of the protein in the binding between PS and T fractions in aged wheat flour. So, various pure proteins (unheated and heated) were blended with fresh wheat flour and the effects of the pure proteins on the binding of PS and T fractions were examined. Among pure proteins, we observed the remarkable effects of heated egg white protein on the increase of the binding between PS and T fractions. So, the effects of various heating conditions (heating temperature, time, and amount of added water to protein) of the egg white protein on the binding between PS and T fractions were studied. In addition, the relationship between the structural changes (α-helix, β-sheet, and others) of heated egg white protein and the binding between PS and T fractions in the heated protein and wheat flour were studied.

MATERIALS AND METHODS

Wheat Flour and Protein

Wheat flour (cv. Alps) was donated by the Nitto Flour Milling Co., Japan. Protein conversion was N × 5.7 (Approved Method 46-10, AACC 2000), and ash was determined according to Approved Method 08-01 on a 14.2% moisture basis. The protein and ash contents of the wheat flour were 8.6 and 0.36%, respectively. Commercially purchased protein, egg white protein, low gel (Kewpie Co. Ltd., Japan), gliadin, and glutenin (Asama Chemical Co. Ltd., Japan), bovine serum albumin (Sigma), and casein (Merck) were used.

Dried protein (0.5 g) was poured into a petri dish (15.5 cm diameter, 2.0 cm depth), mixed with water (2.0–10.0 mL), and left in a computer-controlled drying oven (DV 41, Yamato Co.) at 120–180°C for various times. Dried protein (5 g) and wheat flour (50 g) were blended and sifted three times through a 30-mesh sieve.

Fractionation with Acetic Acid (pH 3.5)

Wheat flour was fractionated by the method of Seguchi et al (1998). The homogenization step was performed with a mortar and pestle (120 and 70 rpm, respectively) (Nittokagaku Co., ANM-150 Type Japan) (Seguchi et al 1998). Wheat flour (50 g) was mixed with 150 mL of water and homogenized for 20 min at room temperature. After centrifugation at 1,700 × g for 20 min at room temperature, the supernatant was freeze-dried and weighed to recover the water-soluble (WS) fraction. The pellet was homogenized in 125 mL of 0.136N acetic acid solution (pH 3.5) for 20 min and centrifuged. The resulting pellet was further homogenized in 75 mL of 0.0283N acetic acid solution (pH 3.5) and centrifuged. The two supernatants after centrifugation were combined, freeze-dried, and weighed to recover the gluten (G) fraction. The pellet was homogenized in 150 mL of water and

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adjusted to pH 5.0 with 5*N* NaOH solution. After centrifugation, two layers of the pellet appeared. The upper yellowish and viscous layer was the tailings (T) fraction, while the white and nonviscous layer on the bottom was the prime starch (PS) fraction. Those textures of PS and T fractions were so different that we could easily separate them by handling with a spatula. Both fractions were air-dried without washing and weighed. Microscopic observation indicated that large and small starch granules were present in the PS and T fractions, respectively. Protein and wheat flour samples were also fractionated as above. Insoluble proteins were mainly present in the tailings (T) fraction.

FT-IR Measurement

The FT-IR transmission spectra were recorded on a FT-IR-480C Plus spectrophotometer (Jasco Co., Tokyo, Japan). Egg white protein powder was measured with KBr. After the powder and KBr were homogenized with an agate mortar and pestle, the mixed powder was pressed into pellets using an MT-1 Micro KBr die kit and MP-1 minipress (Jasco Co.). For each spectrum, a total of 50 scans were collected at 4 cm^{-1} resolution. For the secondary structure analysis of the protein, deconvolution of the each spectrum was performed using Jasco FT-IR software according to the methods of Fourier self-deconvolution (FSD) (Kauppinen et al 1981) and the finite impulse response operator (FIRO) (Jones and Shimokoshi 1983). The spectra were analyzed by second derivatization (Susi and Byler 1983, 1986) and Gaussian curve fitting (Abbott et al 1991; Fu et al 1994) in the amide I region (1,600 to 1,700 cm^{-1}) using IR-SSE software (Jasco Co.). The principle of this software is based on the method of Sarver and Krueger (1991).

Statistical Analysis

Experiments were performed three times, and the average and standard deviation were calculated.

RESULTS AND DISCUSSION

Effect of Various Proteins on the Binding Between PS and T Fractions in the Protein and Wheat Flour Samples

When there was no addition of protein to the wheat flour, the PS and T fractions were separated almost equally in the soft wheat flour by acetic acid (pH 3.5) fractionation (Table I). The PS fraction consisted of large starch granules (14.1 ± 0.6 µm), and the T fraction consisted of small starch granules (4.12 ± 0.28 µm), lipids, water-insoluble polysaccharides, and proteins. The effects on the recovery of the prime starch (PS) fraction by various proteins (unheated gliadin and egg white protein, heated gliadin, glutenin, egg white protein, bovine serum albumin, and casein) that were added to fresh wheat flour were tested. Unheated gliadin did not decrease the PS fraction (no protein 100%, unheated gliadin 107%); however, unheated egg white protein (64.3%) and every heated protein decreased the PS fraction (heated gliadin 67.4%, egg white protein 45.7%, glutenin 88.6%, bovine serum albumin 57.1%, and casein 80.0%), which indicates binding between PS and T. The decrease of the PS fraction with heated egg white protein was particularly remarkable, which may be due to the increased hydrophobicity of the protein by heating. Seguchi et al (1998) indicated that aging of soft wheat flour for 150 days at room temperature could bind PS and T fractions completely, and Kusunose et al (2002) suggested that the binding could be caused by the wheat protein. However, because the wheat protein was composed of various proteins, the relationship between the increase of binding between PS and T fractions and the precise change of protein structure by aging was not clear. Because the characteristics of the egg white protein molecule is generally well known, we selected the egg white protein as a model protein for altering wheat protein. The reason for this methodology to study the effect of aging flour on pancake texture involved the close relationship between improvement of pancake springiness and increased interaction between PS and T fraction (Seguchi et al 1998).

When 5.0 g of unheated egg white protein was added to the wheat flour (50 g), the protein portion entered the WS fraction and other portion entered the T fraction (Table I). Consequently, the WS and T fractions increased from 5.6 to 13.0% and from 42.2 to 54.2%, respectively. On the other hand, the PS fraction decreased from 42.5 to 24.9% when the protein was added, suggesting that egg white protein increased the binding between PS and T fractions and caused part of the PS fraction move to the T fraction. Heated (120°C, 1 hr) egg white protein, however, did not enter the WS fraction (6.0%), probably due to heat denaturation. Most of the heated egg white protein entered the T fraction (67.8%) and the percentage of PS fraction decreased to 17.3%, indicating that the binding of PS and T fractions was increased further by heat treatment. Heated egg white protein had a marked effect on the binding of PS and T fractions in wheat flour.

Heating Condition 1—Effect of Water Amounts

In this experiment, the amount of water (2.0, 4.0, 6.0, 8.0, and 10 mL/5.0 g of egg white protein) was examined, and the wetted egg white protein was heated at 120°C for 1 hr. The protein and wheat flour was subjected to acetic acid (pH 3.5) fractionation. The results showed a decrease in the percentage of PS fraction from 100% (no addition of water) to 81.4, 69.5, 67.8, 58.5, and 66.9%, respectively, with increase of water. The effect of water in heated egg white protein on the binding between PS and T fractions was marked. An increase of water to 6.0 mL in egg white protein decreased the percentage of PS fraction to 60–70%. Water may soften the protein molecule and could easily change secondary structures of protein by heating. Subsequently, all heating experiments included 6.0 mL of water/5.0 g of egg white protein.

Heating Condition 2—Effect of Heating Time

The effect of the heating (120°C) time (hr) of the egg white protein on the binding between PS and T fractions in the protein and wheat flour was examined. Heating times selected were 1, 2, 7, and 8 hr. When egg white protein was heated, the percentage of PS fraction decreased from 100% in unheated protein and wheat flour to 72.1, 51.8, 47.2, and 33.7%, respectively, due to increased binding between PS and T fractions.

Heating Condition 3—Effect of Heating Temperature

The effect of the heating temperature (120, 160, and 180°C for 1 hr) of egg white protein on the binding between PS and T fractions was examined. The 100% PS fraction at room temperature

TABLE I
Effects of Egg White Protein on Wheat Flour Fractionation by Acetic Acid (pH 3.5)

	Water Solubles		Gluten		Prime Starch		Tailings		Total	
Conditions	g	%	g	%	g	%	g	%	g	%
Wheat flour alone	2.3 ± 0.1[a]	5.6	4.0 ± 0.4	9.7	17.5 ± 0.4	42.5	17.4 ± 0.3	42.2	41.2 ± 0.2	100
With egg white protein (5 g)	5.8 ± 0.6	13.0	3.5 ± 0.5	7.9	11.1 ± 2.0	24.9	24.1 ± 2.1	54.2	44.5 ± 1.6	100
Heated at 120°C for 1 hr	2.8 ± 0.1	6.0	4.1 ± 0.5	8.9	8.0 ± 0.2	17.3	31.4 ± 2.0	67.8	46.3 ± 1.8	100

[a] Values represent means of three replicates ± standard deviation.

decreased to 72.1, 74.8, and 54.1%, respectively, which suggested that the binding between PS and T fractions in the protein and wheat flour was sharply increased at 120°C. The percentage of PS fraction was further decreased at 180°C.

Effect of Amount of Heated (180°C, 1 hr) Egg White Protein on the Decrease of PS Fraction

The effect of various amounts (1.0, 2.0, 3.0, and 4.0 g/50.0 g of flour) of heated (180°C, 1 hr) egg white protein on the binding between PS and T fractions was examined. The results showed that increasing the amount of heated protein decreased the percentage of PS fraction from 100% (no addition) to 81.1, 54.3, 29.1, and 28.0%, respectively, which indicated increased binding between PS and T fractions. The saturation level for the binding of PS and T fractions with heated egg white protein was ≈4 g/50 g of flour.

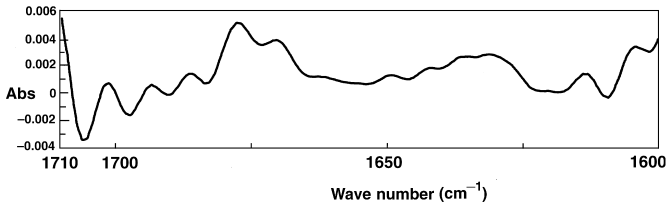

Fig. 1. Original FT-IR spectrum in amide I region of unheated egg white protein powder.

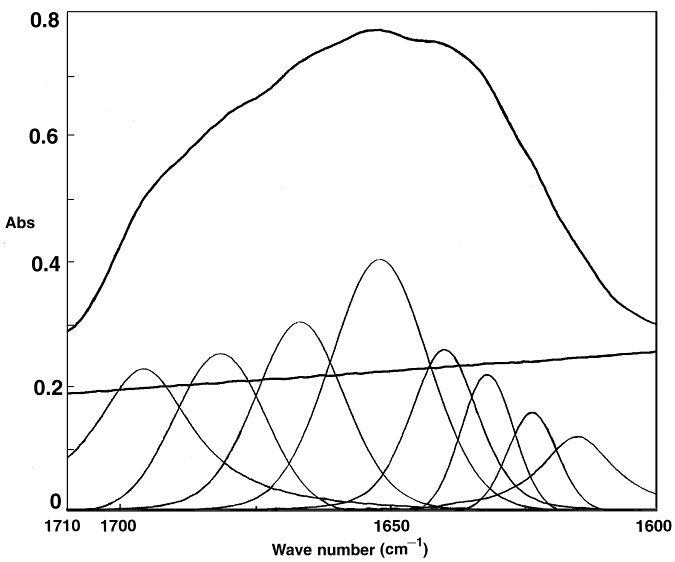

Fig. 2. Deconvoluted FT-IR spectrum and reconstituted spectrum after curve-fitting. Reconstituted spectrum drawn as sums of fitted Gaussian bands.

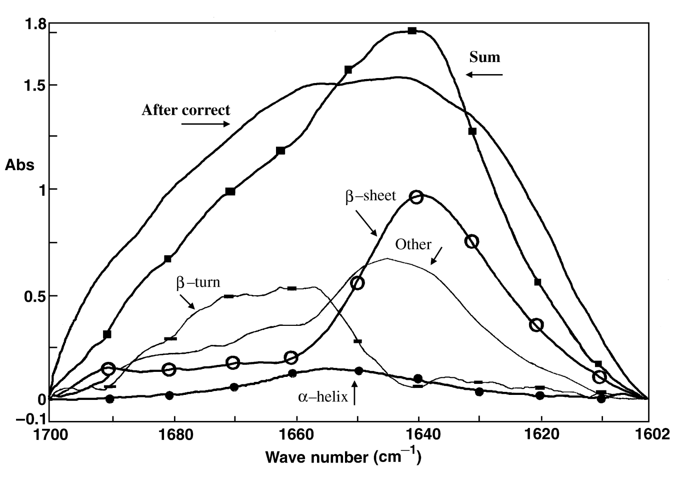

Fig. 3. Positions and relative areas of bands fitted to Fourier-deconvoluted FT-IR spectra of unheated egg white protein powder. Each band was assigned to a secondary structure component according to previous data.

FT-IR-480C Spectroscopy and Decrease of PS Fraction

The FT-IR spectra of the egg white protein with and without heating were measured in the powdery state. The measured spectra in the amide I region were carefully deconvoluted as described above. Band-fitting with Gaussian band shapes was performed on the deconvoluted spectra to estimate the content of the various secondary structures. The original and Fourier-deconvoluted amide I bands, curve-fitted with sums of Gaussian bands of the unheated sample powder are shown in Figs. 1 and 2, respectively. Figure 3 and Table II show the relative areas of the bands assigned to the structural components. Heated egg white protein (at 120°C for 1–8 hr) was also analyzed by FT-IR-480C spectroscopy. The relative contents of secondary structural components are shown in Table II. α-Helix content in the egg white protein decreased from 9.0 to 3.0% at 120°C, whereas β-sheet content increased from 37.0 to 41.0%. However, β-turn content and other structures were almost unchanged. Other heated egg white proteins treated at various temperatures for 1 hr produced the same decrease in α-helix and increase in β-sheet structures and no changes in β-turn or other structures (Table III). When these results were compared with the previous decrease in PS fraction in the heated egg white protein and wheat flour, changes in α-helix and β-sheet structures were closely related to the interaction between PS and T fractions. The relative coefficients between decrease in PS fraction in heated (at 120°C for various times) egg white protein and wheat flour, and decrease in α-helix and increase in β-sheets of the same heated egg white protein were r = 0.9402 and –0.9629, respectively. Also, the decrease in the percentage of PS fraction and the effects on the secondary structures of the protein at various temperatures (120, 160, and 180°C) for 1 hr were compared. The relative coefficients between decrease in PS fraction in heated egg white protein and wheat flour, and decrease in α-helix and increase in β-sheets of the same heated egg white protein were r = 0.7859 and –0.7171, respectively. These findings suggested that changes in secondary structures of heated protein were related to the binding of PS and T fractions in fresh wheat flour and subsequently related to improved pancake springiness by aging or heat-treatment of wheat flour (Seguchi 1990b; Seguchi et al 1998).

TABLE II
Effect of Heating (120°C) Time on Secondary Structures of Egg White Protein

Conditions	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Other (%)
Unheated	9.0	37.0	26.0	28.0
Heated at 120°C				
1 hr	4.7	39.3	27.0	29.0
2 hr	3.3	40.7	27.0	29.0
7 hr	4.0	40.0	27.0	29.0
8 hr	3.0	41.0	27.0	29.0

TABLE III
Effect of Heating Temperature on Secondary Structures of Egg White Protein

Conditions	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Other (%)
Unheated	9.0	37.0	26.0	28.0
Heated 1hr				
120°C	4.7	39.3	27.0	29.0
160°C	2.3	42.7	27.0	28.0
180°C	3.7	41.3	27.0	28.0

CONCLUSIONS

Secondary structures such as α-helix and β-sheets of egg white protein are changed by heating and the addition of protein to a fresh wheat flour could bind PS and T fractions. Those results suggested that heating may change the protein structure from hydrophilic to hydrophobic (Seguchi et al 1998) by changing the buried hydrophobic sites of protein to a naked state. Some dynamic changes of egg white protein would occur in wheat protein by aging at room temperature for longer times, binding the PS and T fractions in wheat flour and improving pancake springiness.

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