Note

Role of Wheat Protein Fractions in the Expansion of Yakifu (Baked Gluten Product)

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Received June 2, 2000; Accepted January 15, 2001

The relationship between wheat flour protein and the expansion of *yakifu*, one of the traditional foods in Japan, was studied. Wheat flour proteins were fractionated by their solubility in various solvents. The protein fraction (PF) and wheat starch were blended into reconstructed flours (RFs). In RF 1, the protein content was equal to that of hard wheat flour, while in RF 2 part (10%, 20%, or 30%) of the protein originally included in soft or hard wheat flour was replaced with a PF extracted from each flour. Among RF 1, those blended with water-soluble and NaCl-soluble fractions did not produce substantial gluten doughs and therefore failed to form well expanded structures when baked. A smooth and well homogenized gluten dough was prepared with 70% ethyl alcohol-soluble and HCl-soluble fractions although the acetic acid-soluble, acetic acid-insoluble, and HCl-insoluble fractions produced a tough and coarse gluten dough. The specific volume of *yakifu* was greater when smooth and well homogenized gluten doughs were baked than when tough and coarse ones were. In RF 2, the specific volume gradually increased with the increase of the HCl-soluble fraction ratio, while it remained almost constant with the increase of the HCl-insoluble fraction ratio. These results demonstrated the primary role of the 70% ethyl alcohol-soluble fraction and HCl-soluble fraction, that is, the gliadin-rich fraction, in the expansion of *yakifu*.

Keywords: *yakifu*, flour, gluten, glutenin, gliadin

Yakifu, one of the traditional foods in Japan, is a baked product of gluten with a honeycomb structure similar to that of bread although its texture is harder and the moisture content is lower than bread. While most breads are made from wheat flours with additional ingredients, *yakifu* is made from gluten and flour without any additives. Gluten, a residual by-product of wheat starch manufacturing, is blended with flour in an approximately 1 : 1 ratio and kneaded to form a sticky and elastic dough. This dough is called gluten dough throughout this report because it has a higher gluten content than doughs from flour for breadmaking. The dough must have a uniform network structure of gluten with starch granules uniformly interspersed among them (Murase *et al.*, 1996). The expansion of *yakifu* is thought to be a function of gluten, and its specific volume reflects the protein content of gluten dough (Murase *et al.*, 1997; Murase *et al.*, 2000). In bread-making, the importance of protein content (Aikten & Geddes, 1934) and the special role of high molecular weight glutenin have been established (Cressey *et al.*, 1987; Lawrence *et al.*, 1987; Lorenzo & Kronstad 1987; Payne *et al.*, 1987). The protein fraction is thought to play a similar role in the expansion of *yakifu*, but the relationship between the protein composition of flour and the specific volume of *yakifu* has not been examined. In this study, wheat flour proteins were fractionated depending on their solubility in various solvents, and the effect of the fractions on the expansion of *yakifu* was studied.

Materials and Methods

Samples Two types of commercial flour (hard wheat, protein concentration 13.7%; Central Milling Co., Ltd. (Chita), and

soft wheat, protein concentration 8.7%; Nisshin Flour Milling Co., Ltd., Tokyo), and frozen gluten (Yokoo Shoten Co., Ltd., Tatsuno) were purchased. Wheat starch was graciously donated by Glico Eiyou Shokuhin Co., Ltd (Takatsuki).

Protein fractionation Wheat proteins were fractionated by two methods. One was the method described by Chen and Bushuk (1970), in which water-soluble (WP), NaCl-soluble (SP), 70% ethyl alcohol-soluble (EAP), 0.05 M acetic acid-soluble (AAP), and acetic acid-insoluble fractions (AAIP) were obtained by lyophilization. The other was the method of MacRitchie (1985): dilute hydrochloric acid-soluble and fractions insoluble at pH 5.3 were separated and lyophilized after neutralization and designated the HCl soluble-fraction (HCIP) and HCl-insoluble fraction (HCIIP), respectively. All fractions were analyzed for NaCl concentration and used to prepare reconstructed flours 1 and 2 (RF 1 and RF 2).

SDS-PAGE SDS-PAGE was carried out at pH 8.3 with slight modification of the method of Laemmli (1970).

Yakifu baking Wet gluten (400 g) and flour or RF (400 g) were mixed with water (20 ml) and kneaded to prepare gluten dough. RF 1 was prepared by mixing PFs and wheat starch to give the same protein content as that of soft or hard wheat flour. In RF 2, part (10%, 20% or 30%) of the protein originally contained in the flour was replaced with HCIP or HCIIP, keeping the total protein content constant. Freshly prepared gluten dough was sealed in a polyethylene bag to prevent surface drying and kept in the refrigerator until use to slow aging (Murase *et al.*, 1997). Gluten dough was cut into small pieces, and the pieces were arranged on the floor of an oven preheated to an appropriate temperature. Ten milliliters of water was sprayed over the pieces, and the lid of the oven was closed to start baking; baking was contin-

ued for 11 min. During baking, the temperature of floor and ceiling of the oven was kept at 200°C and 230°C, respectively. Baked products were kept at room temperature for longer than 24 h. The volume of baked products (*yakifu*) was measured by the rapeseed displacement method. The volume was expressed as specific volume (cm³/g). All the data were statistically calculated to obtain standard deviations.

Scanning electron microscopic observation of fine structure of gluten dough and yakifu Small pieces of gluten dough fixed in 2% glutaraldehyde solution (0.1 M phosphate buffer, pH 7.0) were dehydrated and critical-point-dried in carbon dioxide. The *yakifu* was kept in a vacuum desiccator overnight and then sliced with a razor to observe the inner structure. Observation was made with a scanning electron microscope (Nihon Denshi Co., Ltd., model 8032, Tokyo).

Results and Discussion

The SDS-PAGE patterns of PFs are shown in Figs. 1-I and 1-II. Among proteins fractionated by the method of Chen and Bushuk (1970), WP lacked high molecular weight proteins, and SP lacked both high and low molecular weight proteins (Fig. 1-I). They seemed to be grouped into two main classes. One included EAP (Fig. 1-I, L4) and HCIP (Fig. 1-II, L5), and the other included AAP, AAIP (Fig. 1-I, L5, L6) and HCIIP (Fig. 1-II, L3, L6). The specific volumes of expanded products when these PFs were supplemented as RF 1 are shown in Table 1. Gluten doughs supplemented with EAP and HCIP gave a bigger specific volume, implying that the gliadin fraction plays the main role in expansion of *yakifu*. The differences of moisture and protein content between dough for breads and gluten dough for *yakifu* are great, and therefore their elasticity differs. In breadmaking, gas produced by yeast fermentation plays the main role in forming gas cells in the dough, and at that stage of processing, dough expands greatly. But, in *yakifu* baking, steam forms small bubbles in the gluten dough and expands it. In this case, a higher visco-elasticity is desirable, suggesting that a higher gliadin con-

Table 1. Effects of fractionated wheat proteins on specific volumes of *yakifu*.

Fraction	Specific volume (cm ³ /g)
Water-soluble	4.03 (0.43) ^{a)}
NaCl-soluble	4.34 (0.52)
70% ethyl alcohol-soluble	8.29 (1.03)
0.05 M acetic acid-soluble	5.38 (0.76)
0.05 M acetic acid-insoluble	5.23 (0.65)
HCl-soluble	7.38 (0.46)
HCl-insoluble	3.83 (0.31)

^{a)}standard deviation ($n=10$)

Each protein fraction and wheat starch were blended into reconstituted flour (RF 1) with the same protein content as that of hard wheat flour. RF 1 (400 g) and wet gluten (400 g) were mixed with 20 ml of water and kneaded into a gluten dough. The gluten dough was baked at 200°C for 11 min with 10 ml of water sprayed in the oven. The specific volume was 6.56 (0.57) when hard wheat flour was used for gluten dough.

tent is preferable for gluten dough. As mentioned previously, well expanded *yakifu* could be obtained by baking only a well kneaded gluten dough, in which starch granules were uniformly dispersed among the network structure of the gluten.

The fine structures of gluten dough prepared from RF supplemented with WP, SP, HCIP, and HCIIP are shown in Fig. 2. When WP was used, the gluten dough was soft and sticky but not elastic. SP-supplemented gluten dough was hard but not elastic. In both cases, gluten did not form a network structure, so starch granules were not dispersed uniformly in the gluten. HCIP produced a soft, elastic, and sticky gluten dough. Gluten formed a network structure, and starch granules were dispersed uniformly within this network structure. HCIIP gave a hard and elastic gluten dough whose microstructure was rather similar to that of SP gluten dough. These textural features of gluten dough seemed to reflect the characteristics of gliadin and glutenin proteins (Khatkar & Schofield, 1997). The most distinct differences among SEM observations were cell formations in the gluten dough. The microstructure of the dough supplemented with HCIP was

Fig. 1. SDS-PAGE patterns of fractionated flour proteins. (I) Hard wheat flour was fractionated according to the method of Chen and Bushuk (1970). L1: hard wheat flour, L2: water-soluble fraction (WP), L3: NaCl-soluble fraction (SP), L4: 70% ethyl alcohol-soluble fraction (EAP), L5: 0.05 M acetic acid-soluble fraction (AAP), L6: 0.05 M acetic acid-insoluble fraction (AAIP), and L7: molecular weight standard. (II) Soft and hard wheat flour were fractionated according to the method of MacRitchie (1985). L1: soft wheat flour, L2: HCl-soluble fraction (HCIP), L3: HCl-insoluble fraction (HCIIP), L4: hard wheat flour, L5: HCl-soluble fraction (HCIP), L6: HCl-insoluble fraction (HCIIP).

Fig. 2. Scanning electron micrographs of gluten dough with RF 1. Gluten doughs were prepared at a ratio of wet gluten to reconstructed flour of 1 to 1. RF 1 was prepared by blending wheat starch and A: WP, B: SP, C: HCIP, and D: HCIIP.

smooth, and cell structures probably attributable to starch granules were distributed homogeneously, meaning that the gluten and starches were mixed well (Fig. 2C). In the case of other fractions, the cells were not distributed uniformly. Furthermore, the network structure of gluten was not developed with WP and SP. Neither WP nor SP have gluten-forming functions, and instead seem to interfere with the development of a network structure. This was also the case in gelatinized starch (Murase *et al.*, 2000). The number of cells larger than 10 μm in the major axis were compared in the field of Fig. 2, and was calculated to be 28 for WP, 49 for SP, 86 for HCIP and 60 for HClIP. The average major axes were 18.65, 18.01, 16.07 and 15.84 μm , respectively. Thus, gliadin- and glutenin-rich fractions produced more and smaller cells than WP and SP did, implying the former fractions contribute to the formation of the dough structure. Furthermore, the cell wall was thin and continuous only in HCIP-gluten dough. These differences in the microstructures of gluten dough might result from different degrees of expansion and specific volume. When these gluten doughs were baked, WP and SP did not have a honeycomb structure, the specific volumes were small and the texture of the *yakifu* was hard. In the other fractions, *yakifu* had a honeycomb structure, and the biggest specific volume was obtained with EAP for RF. AAP and AAIP gave smaller specific volumes than EAP did. In the case of HCl fractionation, HCIP produced a bigger specific volume than HClIP did.

In general, EAP and HCIP fractions are classified as gliadin, whereas AAP, AAIP, and HClIP fractions are classified as glutenin. Glutenin protein is said to play a main role in formation of bread loaf volume (Ng & Bushuk, 1988). The same protein was also expected to play an important role in *yakifu* baking because *yakifu* has a honeycomb structure similar to bread, although the detailed procedures of baking bread and *yakifu* are different. Gliadin protein played the main role in the case of *yakifu*, as shown in Table 1. AAP, AAIP, and HClIP, the glutenin-rich fractions, gave smaller specific volumes than did AAP and HCIP, the gliadin-rich fractions, although the specific values of the volumes varied. This suggests two possibilities. First, the differences in

extraction methods affected the proteins extracted, which can be supported by the SDS-PAGE pattern in Fig. 1. The second is the contribution by synergisms of the two proteins. Gliadin plays the primary role in *yakifu* expansion, but the activity would be emphasized by forming gluten through cooperation with glutenin.

In breadmaking, about 2% NaCl is blended with flour, and a greater amount of NaCl is reported to reduce the volume of the loaf (Linko *et al.*, 1984). The specific volume of *yakifu* is also dependent on the amounts of NaCl added (Murase *et al.*, unpublished data). More than 1% of NaCl severely inhibits the expansion of *yakifu*. MacRitchie (1985) reported that his fractions included NaCl, but the amount was too small to reduce the loaf volume of the bread. Our fractionates included 1.66% of NaCl

Fig. 4. Effect of replacement of proteins with PF. Part (10%, 20%, or 30%) of protein originally contained in flours (hard wheat flour and soft wheat flour) were replaced with HCIP or HClIP from each flour, keeping the protein concentration constant. ○: hard HCIP, ●: hard HClIP, △: soft HCIP, ▲: soft HClIP.

Fig. 3. Scanning electron micrographs of *yakifu*. Gluten doughs prepared as described in the legend of Fig. 2 were baked at a floor temperature of 200°C for 11 min with 10 ml of sprayed water. Two portions of *yakifu* (center (upper range) and just the inner part where *yakifu* started to expand (lower range)) were observed.

for HCIP and 0.21% for HCIIP. This proportion of included NaCl did not result in the reduction of specific volumes of *yakifu*, because the NaCl concentration of gluten doughs replaced by those supplemented flours were so small.

Scanning electron microscopic (SEM) observations of *yakifu* are shown in Fig. 3. Although a honeycomb structure was produced in dough near the floor of the oven for all samples, they were neither homogeneous nor continuous when SP and HCIIP were used as the protein source for RF. In the case of WP, the partition walls of the honeycomb were thick, and the number of cells formed was small. These observations were also true for the honeycomb structures at the center part of *yakifu*. Only when HCIP was used were a uniform and regular honeycomb structure and therefore continuous, thin partition walls formed. Other fractions gave irregular structures and discontinuous, thick partition walls. This suggests that the capability of capturing evaporating steam in gluten dough during baking differs among the doughs supplemented with the groups of proteins. Gliadin-rich gluten dough captured steam as fine bubbles and these grew to form a honeycomb structure with continued heating.

Next, the effect of gliadin-to-glutenin ratio in the flour was examined with RF 2. The results are shown in Fig. 4. When the gliadin-to-glutenin ratio was increased by replacing the protein originally contained in the flour with HCIP, the specific volume of *yakifu* increased, but when the gliadin-to-glutenin ratio was decreased by replacing it with HCIIP, almost no change in specific volume was observed. This means that among wheat proteins, gliadin works substantially to expand *yakifu*, proving the ability to capture steam as fine bubbles, while in bread-making glutenin works. The reason almost no change in specific volume was observed when the gliadin-to-glutenin ratio was lowered could be attributed to the importance of protein content in the expansion of *yakifu*, forming the important protein complex gluten.

In this study, flour proteins were fractionated with ethyl alcohol, acetic acid, HCl, and NaOH. If some of these chemicals remained in the fractionated proteins, they may have affected the expansion of gluten dough. The pH's of gluten doughs prepared with RF 1 and RF 2 ranged between 6.03 to 6.12, and these values were close to the pHs of gluten doughs prepared with original flours. Although MacRitchie's methods used HCl and NaOH, the NaCl detected in the fractionated proteins was slight, and this amount of NaCl did not influence the expansion of *yakifu*.

Factors important in breadmaking are (1) the absence or presence of certain subunits of high molecular weight glutenin, (2) the gliadin-to-glutenin ratio, (3) the molecular weight distribution

and (4) the protein concentration. In this study, we confirmed that some of these factors were also true for *yakifu* baking.

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