Technical paper

Effects of Soymilk on Rising of Gluten-Free Rice Flour Bread

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The use of soymilk instead of water when making gluten-free rice flour bread significantly increases the dough volume after yeast fermentation. The soymilk solids function to increase the dough viscosity, reducing the amount of CO₂ that is released through the dough’s surface. Additionally, more CO₂ is generated due to the sucrose content of soymilk. Dough made with soymilk rises better during baking than does dough made with water. We found that glycinin and β-conglycinin contained in soymilk prevent gas cells from merging or collapsing during yeast fermentation and baking. Therefore, this study suggests that the use of soymilk may affect the rising of gluten-free rice flour bread.

Keywords: gluten-free, rice flour bread, soymilk, dough, rising, glycinin, β-conglycinin

Introduction

The increased incidence of celiac disease (Catassi and Yachha, 2009) and other wheat allergy-related diseases, such as gluten sensitivity (Fasano et al., 2008) and baker’s asthma (Baur and Posch, 1998), has become a global issue in recent years, with α-gliadin exposure identified as one of the causes of these diseases (Morita et al., 2003). For this reason, people suffering from wheat allergy cannot consume wheat flour products, such as bread and wheat noodles, that exploit the properties of gluten.

Technology has been developed in Japan that can create rice flour of similar particle size as wheat flour for the purpose of expanding rice consumption, and bread is now made using rice flour instead of wheat flour. Most bread made with rice flour alone, however, contains added active gluten to aid in bread rising. Thus, there is very little gluten-free rice flour bread that people with wheat allergies can consume.

The addition of water to wheat flour and subsequent kneading causes the gliadin and glutenin within the dough to form an elastic gluten network. This gluten network traps the CO₂ released during yeast fermentation, causing the dough to rise. Oryzenin, the main protein in rice (Juliano, 1985), does not form a gluten network when kneaded with water, resulting in a liquid dough instead of a traditional dough. The rice flour dough cannot properly retain the produced CO₂, which escapes from the dough surface. This means the dough will only rise slightly after yeast fermentation and cannot be baked into bread of acceptable quality.

Most commercial gluten-free bakeries have overcome this issue by adding thickeners such as xanthan gum (Lazaridou et al., 2007), guar gum (Schwarzlaff et al., 1996), hydroxypropyl methylcellulose (Mariotti et al., 2013), and so on. Because these thickeners are not usually used for breadmaking, it is concerned about giving a different texture to bread. From this, it is anticipated that the development of novel formulations of gluten-free rice flour bread that rises well and does not contain thickeners would address a void in the market.

It is known that adding soymilk to baked products in which the three main allergens (wheat, eggs and milk) have been removed, will increase the ability of the product to rise (Jinda, 2007). Additionally, gluten-free products with added soy powder (Shin et al., 2013) or soy proteins (Ranhorta et al., 1975; Crockett et al., 2011) have previously been reported. It is not clear, however, by what mechanism soybean components affect the ability of gluten-
free breads to rise.

The main component of soymilk is soy protein, which is comprised primarily of the storage proteins 11S and 7S globulins. Of these, glycgin (11S) and β-conglycinin (7S), which respectively make up 40% and 28% of the soy protein, both have SH groups and SS bonds. These greatly impact a protein’s ability to be processed and form complex, 3-dimensional structures. Additionally, these proteins will locally denature when subjected to heat or pressure, changing the molecular structure and giving the substance additional functionality, such as oil absorption, foaming, adhesive and spinning capacities, and so on (Liu, 1999).

The aim of this paper, therefore, is to clarify the mechanism by which the use of soymilk in rice flour dough enables gluten-free bread to rise.

Materials and Methods

Bread materials The bread was made using a commercial organic soymilk (MARUSAN-AI Co., Ltd., Aichi, Japan). According to measurements entrusted to the Japan Food Research Laboratories (Tokyo, Japan), the protein, fat, ash and water contents of the soymilk were 4.6, 2.8, 0.5, and 90.7%, respectively. Four other ingredients were used in the recipe: rice flour (Powder rice type D; Niigata Seifun Co., Ltd., Niigata, Japan); cultivars Koshihikari and Koshiibuki; mean particle size, approximately 55 µm; sugar (granulated sugar; Fuji Nihon Seito Corporation, Tokyo, Japan), salt (refined salt; The Salt Industry Center of Japan, Tokyo, Japan), and freeze-dried instant yeast (Nisshin Foods Inc., Tokyo, Japan).

Breadmaking procedure The dough for the gluten-free rice bread was made with the following ingredients: 50 g of rice flour (baker’s percentage of 100%), 55 g of water, soymilk, various soymilk:water ratios or protein solutions described below (110%), 1 g of sugar (2%), 0.625 g of salt (1.25%), and 0.625 g of yeast (1.25%). After adjusting the temperature of the ingredients to a dough temperature of 10°C, the ingredients were dispensed into a bowl with ice water and mixed for 5 min with an electric mixer (THM 26M; Tescom Co., Ltd., Tokyo, Japan) at a mixing rate of 800 rpm. The resulting dough was poured in 40 g portions into paper muffin molds (diameter, 59 mm; height, 45 mm) and yeast-fermented at 38°C with 50% RH for 80 min in an incubator (SIB-35; SANSYO Co., Ltd., Tokyo, Japan). After the yeast fermentation, the dough was baked at 170°C in a household electric oven (RE-WB30-S; Sharp Corporation, Tokyo, Japan) for 20 min. After baking, the bread was removed and cooled in an incubator (KCL-1000; Tokyo Rika Kikai Co., Ltd., Tokyo, Japan) at 20°C with 60% RH for 2 h.

Rate of dough volume increase A 50 g portion of dough was poured into a graduated cylinder (200 mL) and yeast-fermented at 38°C with 50% RH for 80 min. The rate of volume increase (%) of the dough was calculated by the following formula: \(\frac{\text{volume after fermentation (mL)} - \text{volume before fermentation (mL)}}{\text{volume before fermentation (mL)}} \times 100\).

CO₂ concentration A 40 g portion of dough in a paper muffin mold was placed in a desiccator (diameter, 185 mm; depth, 200 mm) with a CO₂ monitor (pSENSE-RH EQC; Sakaki Corporation, Osaka, Japan) and yeast-fermented at 38°C with 50% RH for 80 min. The concentration of CO₂ (ppm) emitted in the desiccator was calculated by the following formula: \{concentration after fermentation (ppm) – concentration before fermentation (ppm)\}. The ingredients of the culture solution for the measurement of CO₂ concentration were as follows: 1 g of sugar, 0.625 g of salt, 0.625 g of yeast, and 55 g of water, soymilk or protein solution. After all of the ingredients were mixed by the method described earlier, 21 g of the culture solution was poured into a paper muffin mold, placed in a desiccator with a CO₂ monitor and yeast-fermented at 38°C with 50% RH for 80 min. The concentration of CO₂ (ppm) emitted in the desiccator was calculated by the formula described earlier. In addition, the dough and culture solution made with 50 g of water, the same water content as the soymilk, were measured for the CO₂ concentration emitted in the desiccator.

Sucrose content of soymilk The sucrose contained in soymilk was extracted using 10 volumes of 80% ethanol, and then the extract obtained after centrifugation was filtered through a 0.22 µm membrane filter. The sucrose content of the filtrate was analyzed using an HPLC system (JASCO Corporation, Tokyo, Japan) equipped with RI detector (RI-2031 Plus). The analytical conditions were as follows: column, CAPCELL PAK NH₂ (Shiseido Co., Ltd., Tokyo, Japan); solvent, 75% acetonitrile; flow rate, 1 mL/min; column temperature, 40°C.

Dough viscosity The dough used for viscosity measurement was prepared with all of the above-described ingredients except the yeast. The shear measurement of the dough was performed with a corn plate type rotational viscometer (Haake VT550; Thermo Fisher Scientific Inc., MA, US) under the following measurement conditions: plunger, PK1(1°); shear rate, 0 – 100 sec⁻¹; measurement time, 100 sec; stage temperature, 38°C. Viscosity at 100 sec⁻¹ was read from the rheogram.

Specific volume Each loaf of bread was weighed and then measured for volume using a rapeseed displacement method (Campbell et al., 1984). Specific volume (cm³/g) as the ratio of the volume (cm³) and the mass of the bread (g) was calculated.

Fractionation of glycgin and β-conglycinin from soybean seeds and preparation of protein solution Soybean (Glycine max L., cultivar Hukuyutaka) seeds grown in Saga Prefecture were powdered with a grinder mill (IFM-720G-W/Y; Iwatani Corporation, Tokyo, Japan) and then soaked in hexane at 20°C for 3 h. The residue was filtered and air-dried (defatted soybean powder). Glycgin and β-conglycinin were fractionated from the defatted soybean powder by the method described by Thanh and Shibasaki (1976). The glycgin fraction precipitated at pH 6.4 and the β-conglycinin fraction precipitated at pH 4.2 were respectively suspended in 60 mM Tris-HCl buffer (pH 6.4) and adjusted to pH...
7.8 with sodium hydrate. These solutions were then dialyzed against water at 4°C for 48 h and dried with a freeze dryer (FDU-810; Tokyo Rika Kikai Co., Ltd., Tokyo, Japan). Protein solutions were then prepared by dissolving the freeze-dried glycinin or β-conglycinin fractions in water of sufficient quantity to equal the protein content of the soymilk (4.6 g/100 g).

**Image analysis of gas cells in the bread** The baked loaf was cut into two after cooling and sectional images were taken with a digital camera. The gas cells in the images were scanned into a multiplex high-speed image processor (LUZEX FS; Nireco Corporation, Tokyo, Japan) using a 3CCD color video camera module (XC-003; SONY Corporation, Tokyo, Japan) and then processed into binary images. After processing, the total area of the gas cells in a measurement field of 7,782 mm² was obtained as a circle-equivalent diameter.

**Measurement of SH groups** Measurement of SH group content in soy protein followed the method of Andrews et al. (1995). After respective dissolution of freeze-dried glycinin and β-conglycinin (2.5 mg) in water (1 mL), 0.45 mL was collected in a microtube to which 2-mercaptoethanol (0.15 mL) was added and mixed. To this mixture, 1 mM of ethylenediaminetetraacetic acid, 30 mM of 3-morpholinepropanesulfonic acid buffer (pH 7.0, 3 mL), and 0.6% 4-chloro-7-nitro-2,1,3-benzoxadiazole-dimethyl sulfoxide solution (60 µL) were added. After reaction in darkness for 30 min, absorbance was measured at 420 nm. From a calibration curve prepared by employing 0.5 M cysteine solution, the SH group content (mM) in the soy protein solution was determined, and the SH group content (µmol/g) contained in the soy protein was calculated.

**Statistical analysis** All measurements were performed in triplicate and each value was expressed as the mean ± standard deviation (SD). Differences among samples were determined using the Student’s t-test. A p value of less than 0.05 was assumed to indicate statistical significance.

**Results and Discussion**

The effect of soymilk on dough volume after fermentation The first characteristic studied was the impact of soymilk on bread volume after yeast fermentation of rice flour dough. Dough was made with solutions where the water was progressively replaced with soymilk (water:soymilk ratios of 100:0, 75:25, 50:50, 25:75 and 0:100), and the volume increase (%) after fermentation was measured for each combination (Fig. 1). The volume increase was calculated as the proportion of soymilk in each combination. A significant positive correlation (r = 0.983, p < 0.05) was indicated.

Doughs made with water and soymilk were placed into clear glass containers and allowed to ferment so that the gas cells could be observed. Only small amounts of gas cells could be seen with the dough made with water. We also observed the dough splash on the glass walls as the gas cells moved up through the dough and broke through the surface. On the other hand, as shown within the dotted line in Fig. 2, numerous gas cells were observed in the dough made with soymilk, and the surface of the dough rose significantly.

These experiments revealed that using soymilk instead of water when making rice flour dough results in a 2.5 times greater volume after fermentation, due to the many gas cells that are retained in the dough.
viscosity of the dough would be expected to increase. Increased dough viscosity helps prevent gas cells from rising through the dough or combining, and therefore helps to contain the gas cells within the dough. We measured the viscosity of dough made with both water and soymilk (without added yeast) and fermented under identical conditions.

The viscosity of the dough made with soymilk was significantly greater than that of the dough made with 55 g of water (Fig. 3). The viscosity of the dough made with 50 g of water, the same water content as the soymilk, was also significantly higher than that of the dough made with 55 g of water. However, there was no significant difference in viscosity between the dough made with soymilk and that made with 50 g of water. Additionally, the volume increase after fermentation in the dough with 50 g of water was 266.5 ± 7.1%, much higher than the dough made with 55 g of water (121.7 ± 3.6%, Fig. 1).

From these results it is presumed that replacing water with soymilk reduces the water content and introduces solids to the dough, thereby increasing the viscosity of the dough. Moreover, the volume after fermentation is increased over that when water is used.

Next, the effect of increased dough viscosity on the dough’s ability to contain gas cells was investigated. Dough was respectively prepared with soymilk, 55 g or 50 g of water and then poured into a muffin mold. The dough was allowed to ferment in a desiccator installed with a CO₂ monitor. CO₂ concentration was measured within the desiccator following fermentation to determine the amount of CO₂ escaping from the dough surface during yeast fermentation (Fig. 4, Dough). Results showed that CO₂ concentration was significantly lower in dough made with soymilk or 50 g of water (p < 0.05), indicating that the amount of CO₂ escaping through the dough surface was lower when soymilk or 50 g of water was used.

The soymilk used in this study contained 3.57 ± 0.31 mg/mL of sucrose. Yeast exhibit invertase activity (Phaff et al., 1966). This indicates that more CO₂ may be generated when making dough with soymilk than with water. To eliminate the influence of viscosity on the results, a culture solution was made without rice
flour and yeast-fermented, and then CO₂ concentrations were measured (Fig. 4, Culture solution). The concentration of CO₂ emitted from the culture solution made with soymilk was 1.3 times that emitted from culture solutions made with 55 g and 50 g of water (p < 0.05).

These results indicate that the reason dough made with soymilk has a greater volume after yeast fermentation compared to dough made with 55 g of water is because of the reduced water and higher solids content of soymilk, which leads to greater dough viscosity and less CO₂ escaping from the dough surface, as well as increased CO₂ production due to the sucrose content of soymilk.

In the next investigation, breads were baked from dough respectively made with soymilk, 50 g or 55 g of water to compare the rising of the different doughs. Bread made with soymilk rose better than bread made with 55 g of water, with a rounded bread surface and a greater specific loaf volume (p < 0.05, Fig. 5). As described above, dough made with 50 g of water had a greater volume after yeast fermentation than the dough made with 55 g of water; however, after baking, the center was sunken (as can be seen in the dotted line), with the bread rising only about as much as the bread made with 55 g of water.

The interior of both breads made with water reveal squashed and filled in gas cells. Many fine gas cells could be identified in the bread made with the soymilk, notably, in a honeycomb structure unique to good quality bread.

These results showed that when baking bread with rice flour, gas cells are not properly retained even when the amount of added water is reduced to increase dough viscosity. This indicates that bread made with soymilk instead of water rises better as a result of the components of soymilk.

**Influence of soy globulin** One of the main components of soybeans is soy globulin. Native soy globulin exists as a globular protein with a higher order structure through hydrogen or hydrophobic bonds. Heating, agitation or other physical processes that go into the production of products like soymilk cause part of the globular protein to break apart, creating hydrophilic and hydrophobic domains, and leaving a portion of the molecular surface exposed. As a result, during the fermentation of dough

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**Fig. 4.** Concentrations of CO₂ emitted from the surface of dough and culture solution made with water and soymilk. Dough was respectively prepared with soymilk, 55 g or 50 g of water. CO₂ concentrations were measured in a desiccator following fermentation to determine the amount of CO₂ escaping from the dough surface during yeast fermentation. Means carrying different letters are statistically significant (p < 0.05).

**Fig. 5.** Specific loaf volume and vertical cross-sectional image of bread made with different water contents (A, 55 g added; B, 50 g added) or soymilk (C). The dotted line drawn on (B) indicates sinking. Means carrying different letters are statistically significant (p < 0.05).
made with soymilk and the concomitant CO$_2$ production, the hydrophobic regions of the protein orient towards the CO$_2$ and the hydrophilic regions orient towards the water content. In this way, the protein molecules may create a membrane. It is assumed that these membranes solidify when heated, preserving the gas cells trapped within, and allowing the dough made with soymilk to rise better than the dough made with water.

To verify this hypothesis, glycinin and β-conglycinin, the main components of soy globulin, were isolated from soybeans. Per 100 g of soymilk, 4.6 g of proteins are contained; thus, appropriate amounts of the isolated proteins were mixed to generate protein solutions for use instead of soymilk in breadmaking.

The dough made with the glycinin and β-conglycinin solutions rose just as well as the dough made with soymilk (Fig. 6). The ratio of volume increase after yeast fermentation, viscosity and CO$_2$ emissions were all measured as described above, with no significant difference in the results between both protein solutions (Table 1). These results clearly show that soy globulin is one of the reasons why the volume of yeast-fermented dough made with soymilk is greater than that made with water.

The two types of dough made with the glycinin and β-conglycinin solutions were then baked into bread (Fig. 7). Bread made with dough of both protein solutions rose better than dough made with 55 g of water (Fig. 5, (A)). There was, however, a noticeable difference between the specific loaf volume ( $p < 0.05$) and gas cells of the different proteins, with a higher proportion of smaller gas cells in the bread made with glycinin dough. The comparative sizes of gas cells in the different breads were then determined using image analysis.

### Table 1. Viscosity and volume increase after yeast fermentation of dough made with protein solutions, and CO$_2$ emission from the surface of dough and culture solution.

<table>
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<tr>
<th></th>
<th>Glycinin</th>
<th>β-Conglycinin</th>
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<tbody>
<tr>
<td>Volume increase after fermentation (%)</td>
<td>223 ± 7</td>
<td>222 ± 2</td>
</tr>
<tr>
<td>Viscosity (mPa s)</td>
<td>250 ± 36</td>
<td>210 ± 14</td>
</tr>
<tr>
<td>Concentration of CO$_2$ emitted from dough (×10$^3$ ppm)</td>
<td>1.50 ± 0.07</td>
<td>1.63 ± 0.04</td>
</tr>
<tr>
<td>Culture solution</td>
<td>6.19 ± 0.23</td>
<td>6.05 ± 0.24</td>
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The approximate diameters of gas cells in bread made with the glycinin solution were predominantly in the 0.5 ~ 4.0 mm range, while the bread made with the β-conglycinin solution had fewer gas cells in that range than glycinin solution and some gas cells with diameters greater than 4.0 mm (Fig. 8). The maximum approximate diameter of gas cells was also greater for bread made with β-conglycinin solution than glycinin solution. There were also fewer gas cells overall in the bread made with the β-conglycinin solution.

These results indicate that the β-conglycinin membrane is weaker than the glycinin membrane, and that some of the gas cells created in the β-conglycinin membrane may join together during the fermentation or baking process to form fewer, larger bubbles. This is assumed to be due to the difference in the amount of SH groups between glycinin and β-conglycinin, as reported by...

The SH group contents in purified glycinin and β-conglycinin were 63.7 ± 9.0 µmol/g and 17.7 ± 0.9 µmol/g, respectively. According to Saio et al. (1971), the SH group content in soy protein increased with heating up to 70°C, and decreased thereafter. The rate of increase and decrease is alleged to be faster for glycinin than for β-conglycinin. The increase and subsequent decrease of SH groups is thought to occur because of SS bond formation. Superior rising of bread prepared with glycinin solution compared to that prepared with β-conglycinin solution was assumed to result from SS bond formation during the early heating stage for glycinin, which resulted in the formation of a strong gas membrane.

Based on the above results, it is proposed that the improved rising and the honeycomb structure created in gluten-free rice bread made with soymilk is mainly due to the stable membrane created by glycinin.

**Conclusions**

The use of soymilk instead of water when making gluten-free rice flour bread significantly improves the dough volume increase after yeast fermentation. This is because the solids contained in soymilk increase dough viscosity, reducing the amount of CO$_2$ that is released through the surface of the dough. The increased amount of CO$_2$ that is generated in the dough also improves the dough volume increase. Additionally, dough made with soymilk rises better during baking compared to dough made with water. This is because glycinin, one of the soy globulins contained within soymilk, creates a stable membrane that prevents gas cells from merging or collapsing during fermentation and baking.

The results of this research will enable the preparation of gluten-free rice flour bread that rises properly, without the use of thickeners. These experiments, however, only report on bread baked in muffin molds. Form and appearance are also important characteristics when selecting food. If gluten-free rice flour bread with soymilk can be made into larger products (i.e., full-sized bread loaves), it would create a highly appealing product that meets a market need. Future experiments involve the study of gluten-free rice bread baking with larger volumes of dough.

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**References**


