JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7949–7954 7949
DOI:10.1021/jf1003946

Improvements in the Bread-Making Quality of Gluten-Free Rice Batter by Glutathione

HIROYUKI YANO*

National Food Research Institute, National Agriculture and Food Research Organization, Kannondai 2-1-12, Tsukuba, Ibaraki 305-8642, Japan

The wide prevalence of celiac disease and wheat allergy has led to a growing demand for gluten-free foods. Rice proteins do not possess the viscoelastic properties typically found in gluten, thus making rice flour unsuitable for the production of yeast-leavened products. In the present study, we found that the addition of glutathione to rice batter improves its gas-retaining properties. Glutathione was found to prevent the formation of the disulfide-linked macromolecular protein barrier, which is reported to confer resistance to the deformation of rice batter in the baking process. Also, glutathione appeared to gelatinize rice starch at lower temperatures. Microstructure analyses of glutathione-added rice bread revealed it to have a perforated structure like wheat bread but with a smoother-looking surface. These data collectively suggest that glutathione facilitates the deformation of rice batter, thus increasing its elasticity in the early stages of bread baking and the volume of the resulting bread.

KEYWORDS: Gelatinization; glutathione; rice bread

INTRODUCTION

Wheat is one of the most-produced cereals along with maize and rice. Wheat gluten demonstrates a unique property to hold the carbon dioxide produced during yeast fermentation, thus making wheat flour the representative ingredient of breads. On the other hand, peptides released from wheat gluten during digestion are responsible for celiac disease, a gluten-sensitive enteropathy in genetically predisposed individuals (*1*). Moreover, allergic sensitization to wheat flour components is one of the most frequent causes of occupational asthma (*2*), and wheat-dependent exercise-induced anaphylaxis is a fatal allergic reaction induced by gliadin and glutenin (*3*). Despite the advances being made in the understanding of these wheat-dependent pathologies as well as the potential development of novel therapies, at present the only safe and effective treatment of the sufferers is to avoid gluten-containing foods. Thus, the demand for gluten-free cereal products, especially for breads, is growing.

Rice is considered a suitable substitute for wheat, as it is available worldwide and is less allergenic. Several efforts have been made to produce gluten-free rice bread. The addition of gums (hydroxypropylmethylcellulose, guar gum, locust bean gum) or emulsifiers increases the batter consistency (*4*). Transglutaminase promotes cross-linking among rice proteins (*5*) while cyclodextrin glycosyl transferase produces cyclodextrin which formed complexes with lipids/proteins (*6*), thus increasing the elastic and viscous behavior of the batters. These additives produce a gluten-like macromolecular network, which improves the rheological properties of the rice batter and increases the volume of the bread. However, most of these additives are still in the investigational stage, and further studies are needed to meet the quality expectations of the gluten-free consumers.

Meanwhile, recent studies opened the door to an alternative approach for the production of gluten-free breads. By investigating the pasting characteristics of rice flour in the presence or absence of dithiothreitol (DTT), Hamaker and Griffin (*7*) pointed the existence of the disulfide-linked protein polymers in or surrounding the native starch granules. Derycke et al. (*8*) have proposed that the disulfide-linked macromolecular proteins, natively present in the rice endosperm or formed/strengthened during cooking, work as a barrier and restrict the heat-induced swelling of rice starch. They have noted that the existence of this barrier affects starch swelling and thus the rheological and cooking properties of rice. To break the barrier, Renzetti and Arendt (*9*) have treated brown rice batters with peptidase and successfully improved the textural and baking properties of the brown rice bread. Peptidase was found to induce the release of low molecular weight proteins from the macromolecular complexes, thus opening up the barrier. The microstructure of the crumb revealed by confocal laser scanning microscopy supported this hypothesis by showing that smaller protein aggregates were more widely dispersed in the dominating starch phase, in comparison with the large aggregates of the control bread. They concluded that a lowered resistance to the deformation of batters positively affects the breadmaking performance of the flour by increasing bread specific volume and decreasing crumb hardness and chewiness. Huttner et al. (*10*) have also provided supporting evidence for the hypothesis by demonstrating that the treatment of oat batters at high hydrostatic pressure causes "pregelatinization" of starch, resulting in a higher batter elasticity. They also mentioned that the higher elasticity increases gas retention and therefore improves texture and volume of bread.

*To whom correspondence should be addressed. Tel: +81-298388051. Fax: +81-298387996. E-mail: hyano@affrc.go.jp.

Published on Web 06/02/2010

In spite of its uniqueness, the barrier theory has not been fully explored for the development of gluten-free rice bread. In this paper, we sought to break the disulfide-linked barrier by the cleavage of its disulfide bonds. Glutathione, a tripeptide and also a safe food additive, was tested for its capacity to increase bread volume.

MATERIALS AND METHODS

Baking Procedure. Breads were baked in a commercial bread maker, SPM-KP1 (Sanyo Electric, Osaka, Japan). Formulation of the batter and the baking condition were determined following the supplier's recommendations. Rice flour (10.7% moisture, 0.3% ash, 6.2% protein, 0.9% lipid, and 81.9% starch) was obtained from Namisato Co., Ltd. (Tochigi, Japan). Distilled water and rice flour, both 280 g, and glutathione (Sigma-Aldrich, St. Louis, MO), 0−5 g, were mixed by kneading paddles for 20 min in a bread bin of the bread maker. The batter was left overnight at room temperature. Then, 15 g of sugar and 4 g of bakery yeast (Nisshin Flour Milling Inc., Tokyo, Japan) were added to the batter, which was mixed for 20 min. Subsequent fermentation was allowed to occur for 50 min with increasing temperature to 38 °C, followed by baking at 140 °C for 35 min. The increase of the bread volume was calculated using a ratio of the height of glutathione-added breads to the height of control bread, because the base area of the breads was identical.

Microstructure Analysis of Rice Batter/Bread Using a Low-Vacuum Scanning Electron Microscope. Each sample was withdrawn from the center of the batter or bread and was placed on the cryospecimen holder (JEOL, Tokyo, Japan). The samples were cryofixed in slush nitrogen, then transferred to the cryounit in the frozen state, where they were fractured and sublimed. The morphology of the bread samples was observed with a scanning electron microscope (SEM) (JSM-5310LV low-vacuum SEM; JEOL) at 20 kV. The magnification was 30× to 1500×.

Rapid Viscosity Analyzer Analysis of the Slurry. The consistency curve was determined with a rapid viscosity analyzer (RVA) (Model 3D; Newport Scientific, Warriewood, Australia) (*11*) with 3.5 g suspensions of rice flour in 25 mL of distilled water. To 3.5 g of rice flour was added 0, 12.5, or 25 mg of glutathione. The temperature profile used was as follows: holding at 50 °C for 70 s, heating to 93 °C in 4 min, holding at 93 °C for 7 min, cooling to 50 °C in 4 min, and holding at 50 °C for 3 min. The paddle speed was 960 rpm for the first 10 s to homogenize the sample, which was then adjusted to 160 rpm. The consistency measurements of a selected set of samples were carried out in triplicate.

Analysis of the Major Protein Structure of the Rice Bread. The nonreducing/reducing sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (SDS−PAGE) was carried out as reported previously (*12*). The SDS-soluble protein was extracted from the batters (0.1 g) with 1 mL of a reductant-free Laemmli sample buffer (*13*). The sample was taken in the early stage of baking, i.e., 1 h after the baking program started, and the temperature of the batter was 4.0 ± 0.1 °C. After homogenization in a centrifuge tube, the sample was centrifuged at 10000*g* for 10 min. Then, 10 μL of the supernatant was subjected to electrophoresis in the first dimension. After electrophoresis, a narrow lane was cut from the gel and was immersed in the Laemmli sample buffer with 100 mM DTT for 10 min; it was then subjected to the second SDS−PAGE. After electrophoresis, the gel was incubated overnight at room temperature in 20% methanol containing 5% acetic acid and 0.025% Coomassie brilliant blue R-250 (CBB) and finally was destained with a solution of 20% methanol and 5% acetic acid until the protein bands were visible.

Investigation of the Effect of Glutathione on the Sulfhydryl Groups of Rice Protein. Fluorescence labeling of the protein was conducted according to the previous report (*14*). The SDS-soluble protein was extracted from the batters (1 g) in the early stage of baking with 10 mL of a reductant-free Laemmli sample buffer containing 2 mM monobromobimane, which then was incubated for 20 min at room temperature. Then, the extract was centrifuged at 10000*g* for 10 min, and the supernatant was filtered through a 0.45 μm centrifugal filter (Ultrafree CL; Millipore, Bedford, MA) at 5000*g* for 30 min. The filtrate was desalted with a centrifugal filter (Microcon YM-10; Millipore) at 14000*g* for 30 min. Finally, the concentrate was dissolved in a Laemmli sample buffer containing 100 mM DTT, and 10 μL of the solution was subjected to SDS−PAGE. The resultant gel was stored in 30% methanol/5% acetic acid solution and was examined under an FAS2513 365 nm UV light (Toyobo, Tokyo, Japan) to detect monobromobimane-labeled proteins. The gel was then stained with CBB as mentioned above.

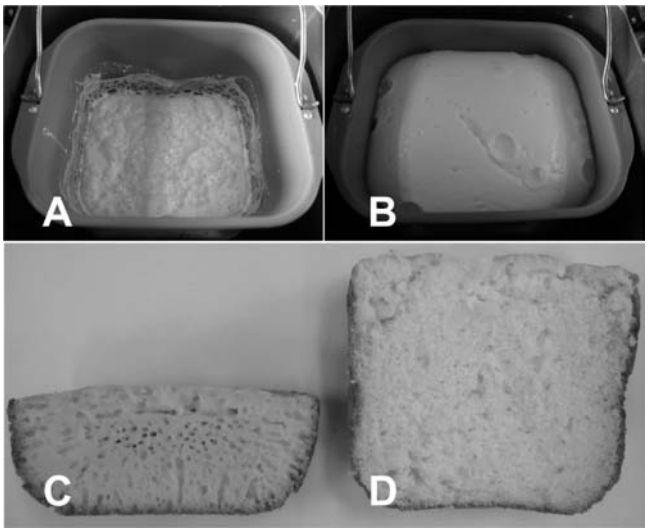

Figure 1. Pictures of rice batters in the early stage of the baking process (**A**, control; **B**, glutathione-added) and the breads (**C**, control; **D**, glutathione-added). Glutathione was added at 0.75 g against 280 g of rice flour.

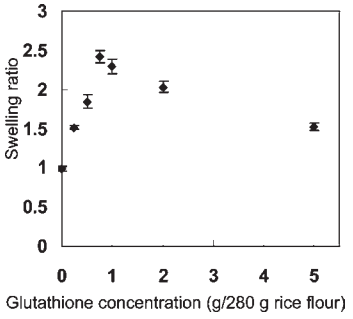

Figure 2. Swelling ratio of rice batters against the concentration of glutathione. The value of each data point represents mean ± SD of three independent experiments.

RESULTS

Glutathione Improved the Gas-Retaining Properties of Rice Batter in the Yeast-Leavening Process. First, we investigated the effect of glutathione on the gas-retaining properties of rice batter in the yeast-leavening process. Water and glutathione were added to rice flour in a commercial bread machine, which was mixed by kneading paddles and left overnight. The rice batters were quite liquid and more resembled a cake batter than wheat dough. Sugar and dry yeast were then added to the batter, which was mixed and subjected to the baking process. **Figure 1** compares the control (**A**) and the glutathione-added rice batter (**B**) in the early stage of the baking process. As the temperature of the bread pan rose, the glutathione-added rice batter swelled. In contrast, the control batter did not rise and was bubbling because it could not hold the fermentation gas. The rate of swelling of the bread increased to 2.4 as the amount of added glutathione increased to 0.75 g against 280 g of rice powder (**Figure 2**). The addition of more glutathione gradually decreased the volume of bread. The swelling profile against the amount of glutathione did not change significantly when the flour/water (w/w) ratio was between 280 g/280 g and 320 g/240 g (data not shown). The results suggest that glutathione

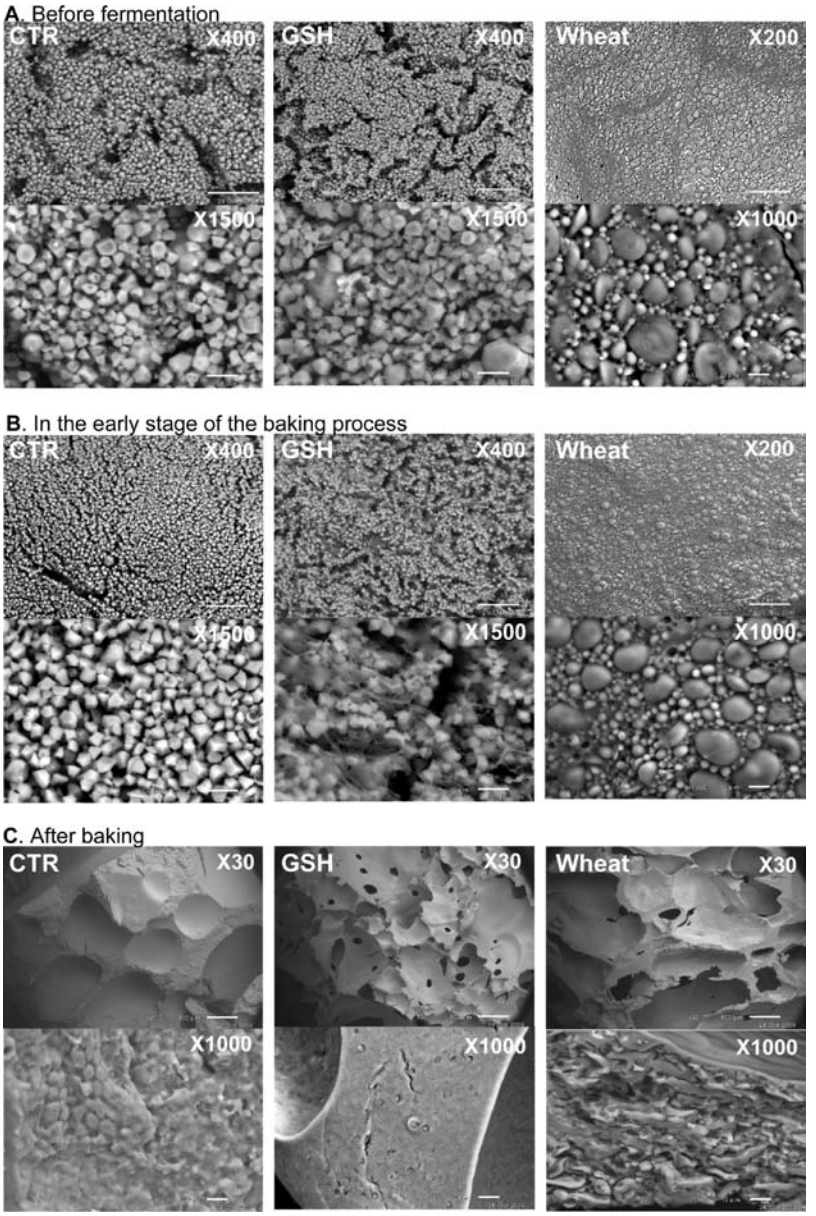

Figure 3. Low-vacuum scanning microscopic analysis of control rice (CTR), wheat, and glutathione-added (GSH) rice batter. Glutathione was added at 0.75 g against 280 g of rice flour. (**A**) Before fermentation; (**B**) in the early stage of the baking process; (**C**) after baking.

rendered the rice batter capable of retaining the fermentation gas and increased the bread volume (**Figure 1C**,**D**). In contrast, addition of salt canceled the effect of glutathione. When 0.1, 0.25, and 0.5 g of sodium chloride were added to the batter with 0.75 g of glutathione, the relative bread volumes were reduced from 2.4 to 2.0, 1.4, and 1.2, respectively.

Microstructure Analysis of Rice Batter/Bread Using a Low-Vacuum SEM. Next, the microstructure of the batter or bread was investigated using low-vacuum SEM. **Figure 3** shows the microstructure of the control (CTR) and glutathione-added (GSH) rice bread as well as wheat bread. Panels **A**, **B**, and **C** show their microstructures before fermentation, in the early stage of baking, and after baking. There is no apparent difference between the microstructures of glutathione-added and the control rice batter before fermentation (**A**). In contrast, in the early stage of baking, deformation of starch was observed in the case of the glutathione-added sample (GSH in **Figure 3B**), while the appearance of the control starch in the early stage of baking (CTR in **Figure 3B**) was not different from the starch before fermentation (CTR in **Figure 3A**). After baking, both the glutathione-added bread and wheat bread had perforated structures (**C**), showing their gas-retaining capacity in the fermentation process. However, the glutathione-treated bread had a smooth-looking surface without visible traces of starch granules, while both the control rice/wheat breads appeared to have rougher surfaces. The results suggest the possibility that the gas-retaining mechanism of the glutathione rice bread was different from that of the wheat bread.

Deformation of glutathione-added batter in the early stage of baking and the smooth-looking surface of the bread both suggest that the addition of glutathione facilitated gelatinization of rice starch.

RVA Analysis of the Slurry. To investigate the impact of glutathione on the rice starch gelatinization, the glutathione-added and the control rice batters were subjected to consistency measurements by RVA (**Figure 4**). The whole profile (**A**) and a close-up of this profile in the range of 140−260 s (**B**) are shown. The peak/end viscosity and the temperature at 100 cP/400 cP are summarized in the **Table 1**. The results show that addition of

glutathione made rice starch gelatinize at a lower temperature than the control batter. In addition, glutathione lowered the consistency of the batter after gelatinization. The results correlated with the RVA measurements by Derycke et al. (*8*), which have shown that DTT decreases the consistency development onset temperature of rice flour. Addition of salt canceled the effect of glutathione (**Figure 4**).

Analysis of the Major Protein Structure of the Rice Batter. We also investigated whether glutathione changed the structure of rice protein. Van der Borght (*15*) reported that 2% SDS extracted 64% of the rice endosperm protein. So the SDS-soluble protein was subjected to the nonreducing/reducing SDS−PAGE (**Figure 5**). In the unique electrophoretic system, a protein molecule without a disulfide bond migrates on the diagonal line of the gel because the molecular size (thus, the migration distance) of the protein is unchanged in the first nonreducing and the second reducing gels. In contrast, a protein with intermolecular disulfide bonds migrates to below the diagonal line of the gel because the cleavage of the disulfide bonds decomposes the protein into its respective subunits with smaller molecular weights, thus making their migration distances longer in the second dimension. The major storage protein of rice is glutelin, which is composed of two polypeptide chains, namely, the acidic and the basic chains. The two chains are connected by an intermolecular disulfide bond. Thus, the acidic (spot 1) and the basic (spot 2) chains align on the perpendicular line (**Figure 5**) (*12*). In the case of untreated rice bread, the glutelin formed intermolecular disulfide bonds other than the native ones and produced macromolecules (arrows) (**A**). In contrast, in the case of glutathione-treated rice bread, glutelin did not form extra intermolecular disulfide bonds (**B**). The data suggest that addition of glutathione to rice batters prevented the formation of macromolecular glutelin complexes.

Investigation of the Effect of Glutathione on the Sulfhydryl Groups of the Rice Protein. Next, to compare the status of the sulfhydryl groups of proteins in the control and the glutathione-added rice batter, free sulfhydryl groups of the SDS-soluble protein were labeled fluorescently with monobromobimane (**Figure 6**) (*14*). Densitometry analyses showed that the total fluorescence and the amount of protein in the glutathione-added batter was 2.77- and 1.14-fold, respectively, compared to those of the control batter. Thus, glutathione increased the free sulfhydryl group/protein ratio 2.4 times against the control.

DISCUSSION

The present findings confirm and extend earlier hypothetical mechanisms correlating the disulfide-linked protein barrier with the breadmaking performance of gluten-free rice batter. Derycke et al. (*8*) reported that the protein barrier is strengthened by additional cross-linking among protein during cooking. Meanwhile, Ohno et al. (*16*) reported that formation of the intermolecular disulfide bonds proceeds in aged rice. So in some cases, the disulfide-linked polymer may exist in the flour as a preformed aggregate and may also work as a barrier. Renzetti and Arendt (*9*) have broken the barrier with peptidase and improved the bread quality by increasing the specific volume, while decreasing the crumb hardness and chewiness. In contrast, in the present study,

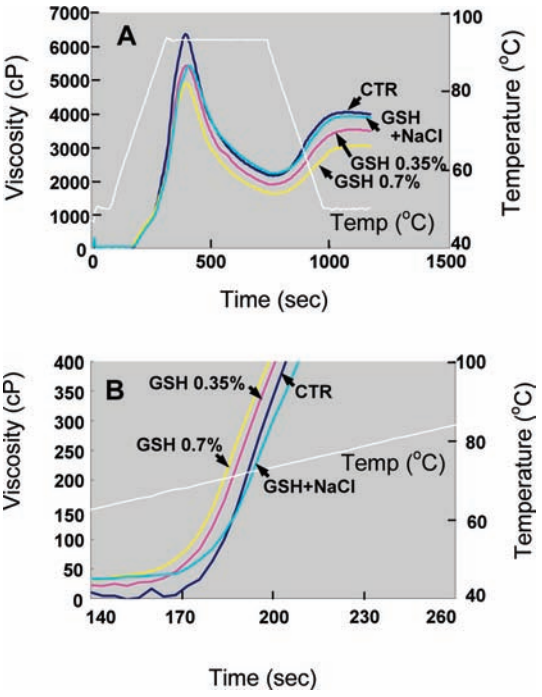

Figure 4. Rapid viscosity analyses of rice flour. Key: navy blue line, control rice flour; pink and yellow lines, addition of 0.35% and 0.7% glutathione against rice flour (w/w), respectively; light blue line, addition of both 0.7% glutathione and 0.7% sodium chloride against rice flour (w/w).

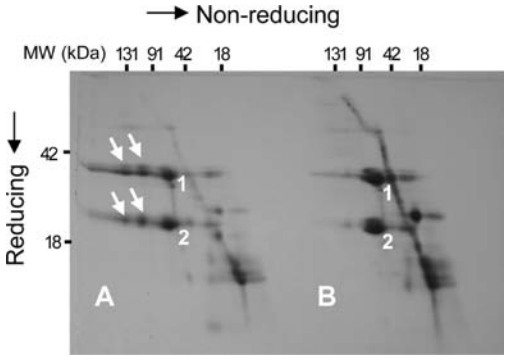

Figure 5. Nonreducing/reducing SDS−PAGE of protein taken from the control (**A**) and glutathione-added (**B**) rice batters in the early stage of the baking process. Glutathione was added at 0.75 g against 280 g of rice flour. Arrows: Protein subunits obtained after reduction of disulfide-linked polymers.

Table 1. Effect of Glutathione and Sodium Chloride on the Rapid Viscosity Analyses of Rice Flour[a]

			temp (°C) at	
	peak viscosity (cP)	end viscosity (cP)	100 cP	400 cP
control	6348 ± 22	4103 ± 34	70.60 ± 0.35	74.60 ± 0.04
+0.35% GSH	5543 ± 131[b]	3540 ± 114[b]	69.68 ± 0.06	73.85 ± 0.04[b]
+0.7% GSH	5002 ± 83[b]	3094 ± 56[b]	68.88 ± 0.06[b]	73.25 ± 0.04[b]
+0.7% GSH, 0.7% NaCl	5589 ± 201	3978 ± 154	70.37 ± 0.06	74.90 ± 0.41

[a] Mean values ± standard errors of three replicates. Abbreviations: GSH, glutathione; NaCl, sodium chloride. [b] Asterisks indicate values differing significantly ($p < 0.05$) from the value for control.

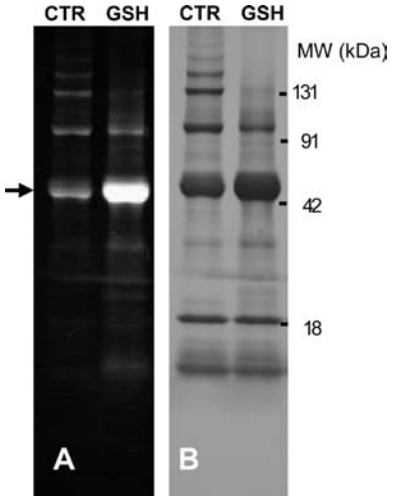

Figure 6. Effect of glutathione on the sulfhydryl groups of rice protein in the early stage of the baking process. Glutathione was added at 0.75 g against 280 g of rice flour. Key: CTR, control; GSH, glutathione-added batter. (**A**) Protein labeled with monobromobimane visualized by UV absorption. (**B**) Protein stained with Coomassie blue. Arrow: Original glutelin molecule.

glutathione hindered the formation of macromolecules by cleaving or preventing the intermolecular disulfide cross-linking among glutelins (**Figure 5**). That resulted in a lowered resistance to the deformation of batter in the early stage of the baking process. Hamaker and Griffin (*7*) reported that when rice slurries were cooked under negligible shear stress and measurements were made under low-shear conditions, viscosity and gel consistency increased in the presence of DTT. In contrast, viscosity decreased when the reducing agent was added before cooking, and only moderate shear stress was applied. Thus, the direction of the viscosity change appeared to be dependent on the amount of shear present during the cooking process as well as during the viscosity measurement (*7*). Our bread data support their hypothesis. When the strength of the shear stress was negligible, the starches without interference of the disulfide-bound protein barrier swelled to a larger size, thereby increasing the bread volume. Conversely, in the absence of the rigidity conferred by the barrier, the swollen starch granules broke apart easily when shear stress was high, thus resulting in rapid breakdown in the RVA profile (**Figure 4**, **Table 1**). As far as we know, this is the first report to show a direct contribution of glutathione to the swelling of gluten-free rice bread. Meanwhile, there have been other reports on the work of redox agents, including glutathione, to promote gluten-based protein network during wheat breadmaking (*17*, *18*). However, in the case of the rice batter, glutathione appears to have cleaved the disulfide bonds of the protein macromolecules, rather than promoting the formation of the gluten-like protein network. The intermolecular disulfide bond of glutelin polymers was cleaved to yield the original glutelin molecule (**Figure 5**). Also, the ratio of free sulfhydryl groups/protein increased in the case of glutathione bread (**Figure 6**). At present, we are not sure why glutathione worked differently in the two cases. The concentration of added glutathione in the present rice study was more than 10 times as high as it was in Lagrain's wheat study (*17*). Also, the action of glutathione might be protein-specific. Further investigations of the glutathione-added rice bread are in progress in our laboratory for further elucidation of the swelling mechanism.

The basic ingredient composition of the glutathione rice bread is simple: rice flour, water, sugar, yeast, and glutathione. Addition of salt (sodium chloride) inhibited the swelling of the glutathione-added batter. Also, while addition of glutathione promoted gelatinization of the rice starch, the subsequent addition of salt canceled the effect (**Figure 4**). These observations support the above hypothetical mechanism, because salt is reported to decrease the water uptake during the soaking/heating of rice grain (*19*) and also has been reported to raise the gelatinization temperature of rice starch (*20*). Meanwhile, a high intake of salt increases blood pressure and the risk of cardiovascular disease (*21*). The sodium content of bread is a growing public concern (*22*), because bread is consumed as a staple food worldwide. However, salt is generally considered indispensable for wheat bread. Recently, Miller and Hoseney (*23*) summarized the role of salt in baking. In a flour−water system, the gluten protein has a net positive charge which keeps the protein chains from interacting with each other and allows the gluten to hydrate faster, resulting in a weaker dough. The addition of salt shields the charges, allowing the proteins to interact with each other, thus making the flour hydrate slower, resulting in a stronger dough. While Lynch et al. (*24*) have successfully halved the amount of the salt in bread from the current usage level (1.2%) to 0.3−0.6% without any detrimental effect on the texture of bread, salt remains essential in making gluten-based wheat breads. In contrast, rice breads, which do not require any additional salt, may be beneficial to public health by lowering the intake of salt from bread, which is estimated to constitute approximately one-sixth of the daily salt intake (*25*).

The studies presented here are preliminary ones to show the impact of glutathione on the swelling of rice bread. We have conducted a tasting experiment of the glutathione bread and confirmed that addition of glutathione and absence of salt did not affect the taste negatively (data not shown). Cysteine had a similar bread-improvement effect, but the bread had a sulfurous odor (Yano, unpublished results). While no significant detrimental characteristics have been found in the glutathione-added rice bread thus far, studies regarding improvements in bread quality as well as the baking procedure are ongoing in our laboratory.

Accumulating evidence suggests that disulfide bonds contribute to the protease resistance (*26*) and allergenicity (*27*) for some allergens. Disulfide bonds often make a protein more stable to digestion in the gastrointestinal tract, thereby enabling some protease-resistant fragments to react with the gut immune system intact. Thioredoxin, a widely distributed oxidoreductase (*28*), has been reported to lessen the allergenicity of milk (*29*) and wheat (*30*) by cleaving the disulfide bonds of the allergenic proteins. Moreover, thioredoxin has been reported to reduce the immunoreactivity of gliadin, while the rheological properties of the dough are not affected (*31*). On the other hand, there is an unsettled dispute regarding whether thioredoxin itself is an allergen (*32*−*34*). In contrast, if glutathione cleaves the disulfide bonds of the macromolecular network, it may also be used industrially to cleave the disulfide bonds of cereal allergens in food processing, thus eventually making it possible to produce less allergenic foods. As glutathione is a ubiquitous thiol-containing tripeptide, there is a lesser possibility for it to function as an allergen.

ABBREVIATIONS USED

CBB, Coomassie brilliant blue R-250; DTT, dithiothreitol; RVA, rapid viscosity analyzer; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide slab gel electrophoresis; SEM, scanning electron microscope.

ACKNOWLEDGMENT

We acknowledge Professor Bob B. Buchanan, University of California, and Dr. Noriaki Aoki, National Institute of

Crop Science, Japan, for helpful comments on the manuscript. Dr. Keitaro Suzuki, National Food Research Institute, Japan, is also acknowledged for helpful discussions on the RVA data. We also acknowledge Dr. Shigeru Kuroda, Bio-oriented Technology Research Advancement Institution, Japan, for encouragements. The low-vacuum SEM analyses and RVA measurements were carried out with the help of JEOL, Ltd. (Tokyo, Japan), and the Industry Technology Institute, Miyagi Prefectural Government (Japan), respectively.

LITERATURE CITED

(1) Green, P. H.; Cellier, C. Celiac Disease. *New Engl. J. Med.* **2007**, *357*, 1731–1743.

(2) Brant, A. Baker's asthma. *Curr. Opin. Allergy Clin. Immunol.* **2007**, *7*, 152–155.

(3) Matsuo, H.; Kohno, K.; Niihara, H.; Morita, E. Specific IgE determination to epitope peptides of omega-5 gliadin and high molecular weight glutenin subunit is a useful tool for diagnosis of wheat-dependent exercise-induced anaphylaxis. *J. Immunol.* **2005**, *175*, 8116–8122.

(4) Demirkesen, I.; Mert, B.; Sumnu, G.; Sahin, S. Rheological properties of gluten-free bread formulations. *J. Food Eng.* **2010**, *96*, 295–303.

(5) Renzetti, S.; Bello, F. D.; Arendt, E. K. Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. *J Cereal Sci.* **2008**, *48*, 33–45.

(6) Gujral, H. S.; Guardiola, I.; Carbonell, J. V.; Rosell, C. M. Effect of cyclodextrinase on dough rheology and bread quality from rice flour. *J. Agric. Food Chem.* **2003**, *51*, 3814–3818.

(7) Hamaker, B. R.; Griffin, V. K. Effect of disulfide bond-containing protein on rice starch gelatinization and pasting. *Cereal Chem.* **1993**, *70*, 377–380.

(8) Derycke, V.; Veraverbeke, W. S.; Vandeputte, G. E.; De Man, W.; Hoseney, R. C.; Delcour, J. A. Impact of proteins on pasting and cooking properties of nonparboiled and parboiled rice. *Cereal Chem.* **2005**, *82*, 468–474.

(9) Renzetti, S.; Arendt, E. K. Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure. *J. Cereal Sci.* **2009**, *50*, 22–28.

(10) Huttner, E. K.; Dal Bello, F; Poutanen, K.; Arendt, E. K. Fundamental evaluation of the impact of high hydrostatic pressure on oat batters. *J. Cereal Sci.* **2009**, *49*, 363–370.

(11) Toyoshima, H.; Okadome, H.; Ohtsubo, K.; Suto, M.; Horisue, N.; Inatsu, O.; Narizuka, A.; Aizaki, M.; Okawa, T.; Inouchi, N.; Fuwa, H. Cooperative test on the small-scale rapid method for the gelatinization properties test of rice flours with a rapid-visco-analyser (RVA). *Nippon Shokuhin Kagaku Kogakukaishi* **1997**, *44*, 579–584.

(12) Yano, H.; Wong, J. H.; Cho, M. J.; Buchanan, B. B. Redox changes accompanying the degradation of seed storage proteins in germinating rice. *Plant Cell Physiol.* **2001**, *42*, 879–883.

(13) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

(14) Yano, H.; Wong, J. H.; Lee, Y. M.; Cho, M. J.; Buchanan, B. B. A strategy for the identification of proteins targeted by thioredoxin. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4794–4799.

(15) Van der Borght, A.; Goesaert, H.; Veraverbeke, W. S.; Delcour, J. A. Fractionation of wheat and wheat flour into starch and gluten: Overview of the main processes and the factors involved. *J. Cereal Sci.* **2005**, *41*, 221–237.

(16) Ohno, T.; Tomatsu, M.; Toeda, K.; Ohisa, N. Texture of cooked rice prepared from aged rice and its improvement by reducing agents. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 2912–2920.

(17) Lagrain, B.; Thewissen, B. G.; Brijs, K.; Delcour, J. A. Impact of redox agents on the extractability of gluten proteins during bread making. *J. Agric. Food Chem.* **2007**, *55*, 5320–5325.

(18) Joye, I. J.; Lagrain, B.; Delcour, J. A. Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking—A review. *J. Cereal Sci.* **2009**, *50*, 11–21.

(19) Ito, J.; Kasai, M.; Kainuma, Y.; Hatae, K. The effect of the various seasonings on cooking properties of rice (Part 1). *J. Jpn. Soc. Food Sci. Technol.* **2004**, *51*, 531–538.

(20) Ito, J.; Kasai, M.; Kainuma, Y.; Hatae, K. The effect of the various seasonings on cooking properties of rice (Part 2). *J. Jpn. Soc. Food Sci. Technol.* **2004**, *51*, 539–545.

(21) Cook, N. R.; Cutler, J. A.; Obarzanek, E.; Buring, J. E.; Rexrode, K. M.; Kumanyika, S. K.; Appel, L. J.; Whelton, P. K. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: Observational follow-up of the trials of hypertension prevention (TOHP). *Br. Med. J.* **2007**, *334*, 885–888.

(22) Castanheira, I.; Figueiredo, C.; André, C.; Coelho, I.; Silva, A. T.; Santiago, S.; Fontes, T.; Mota, C.; Calhau, M. A. Sampling of bread for added sodium as determined by flame photometry. *Food Chem.* **2009**, *113*, 621–628.

(23) Miller, R. A.; Hoseney, R. C. Role of salt in baking. *Cereal Foods World* **2008**, *53*, 4–6.

(24) Lynch, E. J.; Bello, F. D.; Sheehan, E. M.; Cashman, K. D.; Arendt, E. K. Fundamental studies on the reduction of salt on dough and bread characteristics. *Food Res. Int.* **2009**, *42*, 885–891.

(25) Sharp, D. Labeling salt in food: If yes, how? *Lancet* **2004**, *364*, 2079–2081.

(26) Yano, H.; Kusada, O.; Kuroda, S.; Kato-Emori, S. Disulfide proteome analysis of buckwheat seeds to screen putative allergens. *Cereal Chem.* **2006**, *83*, 132–135.

(27) Yano, H. Disulfide-related proteomic studies on food allergens. *Exp. Rev. Proteomics* **2009**, *6*, 563–571.

(28) Schürmann, P.; Buchanan, B. B. The ferredoxin/thioredoxin system of oxygenic photosynthesis. *Antioxid. Redox Signaling* **2008**, *10*, 1235–1274.

(29) del Val, G.; Yee, B. C.; Lozano, R. M.; Buchanan, B. B.; Ermel, R. W.; Lee, Y. M.; Frick, O. L. Thioredoxin treatment increases digestibility and lowers allergenicity of milk. *J. Allergy Clin. Immunol.* **1999**, *103*, 690–697.

(30) Buchanan, B. B.; Adamidi, C.; Lozano, R. M.; Yee, B. C.; Momma, M.; Kobrehel, K.; Ermel, R.; Frick, O. L. Thioredoxin-linked mitigation of allergic responses to wheat. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 5372–5377.

(31) Waga, J.; Zientarski, J.; Obtułowicz, K.; Bilo, B.; Stachowicz, M. Gliadin immunoreactivity and dough rheological properties of winter wheat genotypes modified by thioredoxin. *Cereal Chem.* **2008**, *85*, 488–494.

(32) Weichel, M.; Glaser, A. G.; Ballmer-Weber, B. K.; Schmid-Grendelmeier, P.; Crameri, R. Wheat and maize thioredoxins: a novel cross-reactive cereal allergen family related to baker's asthma. *J. Allergy Clin. Immunol.* **2006**, *117*, 676–681.

(33) Limacher, A.; Glaser, A. G.; Meier, C.; Schmid-Grendelmeier, P.; Zeller, S.; Scapozza, L.; Crameri, R. Cross-reactivity and 1.4-A crystal structure of *Malassezia sympodialis* thioredoxin (Mala s 13), a member of a new pan-allergen family. *J. Immunol.* **2007**, *178*, 389–396.

(34) Buchanan, B. B.; Frick, O. L. Thioredoxin and food allergy. *J. Allergy Clin. Immunol.* **2007**, *119*, 513–514.

Received for review January 29, 2010. Revised manuscript received April 19, 2010. Accepted April 24, 2010.