グルタチオン米粉パンに対するソルガム粉の効果

報　文

Effect of sorghum flour on glutathione-rice bread

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Abstract

Owing to accumulating evidence of the high prevalence of celiac disease and wheat allergy, the development of high-quality gluten-free foods has become an urgent issue in the food industry. We previously reported that glutathione, a natural tripeptide, improves the gas-retaining capacity of gluten-free rice batter. In addition, sensory evaluation revealed that it is better to use "oxidized" glutathione in view of the bread smell. However, several problems remain to be solved before the practical application of glutathione in batter can be achieved. First, salt, a key flavor of bread, hinders the swelling of glutathione-supplemented batter. Second, although purified glutathione may be used as a food additive in the USA and some Asian countries, its use is not permitted in several other countries. In this paper, we sought to overcome these problems. Microstructure analysis revealed that sorghum flour in the batter resulted in thickened breadcrumb walls. Moreover, the swelling of the batter was not reduced by salt. Furthermore, a yeast extract containing a high content of glutathione worked effectively as a substitute for purified glutathione. Thus, the essential ingredients of the bread—rice/sorghum flour, water, yeast extract, dried yeast, and sugar—were all foodstuffs. In conclusion, a basic practical formulation for a glutathione-rice bread has been developed in this study.

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; HPMC, hydroxypropyl methylcellulose

Key words: Bread; Glutathione; Gluten-free; Rice bread; Sorghum

Introduction

The wide prevalence of celiac disease (Mooney and others 2014) and wheat allergy (Mansueto and others 2014) has increased the demand for gluten-free foods (Lamacchia and others 2014). Several approaches have been used to develop gluten-free rice-based breads, as rice flour is naturally gluten-free and has a simple and bland taste. The gluten-free rice breads use synthetic thickeners (Demirkesen and others 2010), transglutaminase (Renzetti and others 2008), and proteases (Renzetti and Arendt 2009; Hamada and others 2013; Kawamura-Konishi and others 2013). In general, rice bread has a palatable chewy texture. However, the quality of these breads has been insufficient to satisfy customers' needs, and thus further research is needed to develop high-quality gluten-free breads.

Recently, we found that glutathione, a naturally occurring tripeptide, improved the gas-holding capacity of

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gluten-free rice batter in the yeast leavening process (Yano 2010). The swelling rate was 2.4 times that of the control bread (without glutathione), and the specific volume was 3.0 cm^3/g. In addition, the use of oxidized glutathione (GSSG) improved the smell of the bread (Yano 2012). However, from the viewpoint of practical applications, this bread prototype required improvement in several areas. First, salt reduced the ability of the batter to swell and rise during fermentation. As saltiness is one of the key flavors of breads (Noort and others 2010), this issue seemed a critical one to be solved. Second, whereas purified glutathione can be used as a food additive in the USA and several Asian countries, its use is not permitted in Europe or Japan. Therefore, the substitution of a yeast extract for the purified glutathione is a critical step before the bread can be used widely. However, although purified glutathione is effective in improving the specific volume of the bread, the use of a yeast extract with high glutathione content has not been successful, presumably as a result of the salt content of the yeast extract.

In this study, in order to enhance the practical utility of gluten-free breads, we sought to overcome these associated problems. In addition, although semisynthetic chemicals such as hydroxypropyl methylcellulose (HPMC) (Barcenas and Rosell 2005) or guar gum (Ribotta and others 2004) are effective for allowing the gluten-free batter to rise during fermentation, some consumers prefer to avoid such non-natural materials in foods (Devcich and others 2007; Varela and Fiszman 2013). We therefore sought to develop a gluten-free bread made without using any non-natural additives.

The swelling mechanism of glutathione-rice bread appears to be different from that of wheat bread (Yano and others 2013). In the case of glutathione-rice bread, the batter is liquid and it becomes meringue-like in fermentation. Thus, the walls of the bubbles in the rising batter should be more fragile than those in wheat dough. It has been reported that a mixture of cereal flours improves the quality of gluten-free bread (Sciarini and others 2010). Moreover, among cereal flours, sorghum flour is frequently used for gluten-free foods such as bread (Schober and others 2007; Onyango and others 2011). Thus, in this study, we investigated the effects of sorghum flour on the properties of gluten-free bread, such as the rising of the batter during fermentation in the presence of salt and the fineness of the breadcrumbs. The replacement of purified glutathione with yeast extract was also studied.

Materials and Methods

Materials

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). Sorghum flour (12.4% moisture, 0.6% ash, 9.6% protein, 1.5% lipid, and 73.6% starch) was purchased from Nakano Sangyo, Co., Ltd. (Kagawa, Japan). Dried yeast (Nisshin Super Camellia) was obtained from Nisshin-foods Co., Ltd. (Tokyo, Japan). Purified glutathione was a product of Nacalai Tesque (Kyoto, Japan). The GSSG-containing (18%, w/w) yeast extract, YH-D18, was a gift from Kohjin Life Sciences Co., Ltd. (Tokyo, Japan).

Bread making

Breads were baked in a SPM-KP1 commercial bread maker (Sanyo Electric, Osaka, Japan) as described in a previous paper (Yano 2010) with some modifications. In summary, 160 g of a rice/sorghum flour mixture, 140 g of distilled water, and 0 to 5 g of glutathione 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 2.5 g of baker's yeast (Nisshin Flour Milling Inc., Tokyo, Japan) were added and the batter was mixed for 20 min. Then, 200 g of the batter was transferred to a square pan case with an 800 mL capacity. Subsequent fermentation (40°C for 60 min) and baking (180°C for 24 min) were done using an EMO-C16C electric oven (Sanyo Electric, Osaka, Japan) by following the supplier's recommendations.

Specific volume

The loaves were measured 3 h or later after baking. The volume, weight, and specific volume were determined using a laser volume measurement unit (Selnac-WinVM2100A; ASTEX, Tokyo, Japan) according to the instruction manual.

Microstructure analysis of rice bread

Microscopic observation of the breadcrumbs was conducted as described previously (Yano 2010). Samples were taken from the center of the bread and placed on a cryo-specimen holder (JEOL, Tokyo, Japan). They were then cryo-fixed in slush nitrogen and transferred to the cryo unit in a frozen state, where they were fractured and sublimed. The morphologies of the bread samples were observed with a scanning electron microscope (SEM; JSM-5310LV Low Vacuum SEM; JEOL) at 20 kV. The magnification used was × 100.

Sensory evaluation

Sensory evaluation of the bread was conducted in a sensory panel room by an analytical panel consisting of 12 assessors. Bread that contained the yeast extract YH-D18 and bread that did not (control) were subjected to paired comparison tests. First, each panel member compared the breads in terms of the appearance of the crumb structure (coarse/fine), and scored the fineness of the YH-D18-containing bread relative to that of the control bread on a 7-point scale: +3, much finer; +2, finer; +1, slightly finer; 0, neither; -1, slightly coarser; -2, coarser; -3, much coarser. Then, the panel masticated the breads and evaluated the texture of the YH-D18-containing bread relative to that of the control on a 7-point scale: +3, much better; +2, better; +1, slightly better; 0, neither; -1, slightly worse; -2, worse; -3, much worse.

Results and Discussion

Effect of sorghum flour on the glutathione bread

First, we investigated the effect of sorghum flour on the glutathione-rice batter in terms of the swelling of the bread. The total amount of the flour was 160 g with various rice/sorghum flour ratios. The amount of GSSG and distilled water was 0.5 g and 140 g, respectively. Figure 1 shows the specific volume and a cross-section of the breads. The specific volume was the highest (3.79 ± 0.11 cm^3/g) and the crumbs appeared finest when the rice/sorghum ratio was around 130/30. Raising the portion of sorghum flour further did not improve the specific volume or crumb appearance (data not shown).

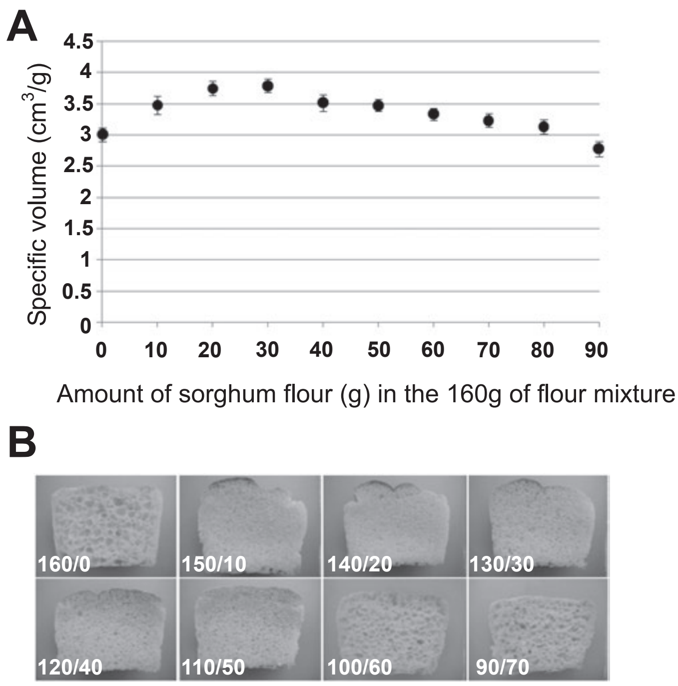

Fig. 1 Effect of adding sorghum flour to glutathione bread

A, The specific volume of breads plotted against the amount of sorghum flour. *B*, Cross-sections of some of the breads. The total amounts of sorghum/rice flour, distilled water, oxidized glutathione, dried yeast, and sugar were 160, 140, 0.5, 2.5, and 15 g, respectively. The standard fermentation and baking conditions were 40°C for 60 min and 180°C for 24 min, respectively. Each data point represents the mean ± SD of three independent experiments.

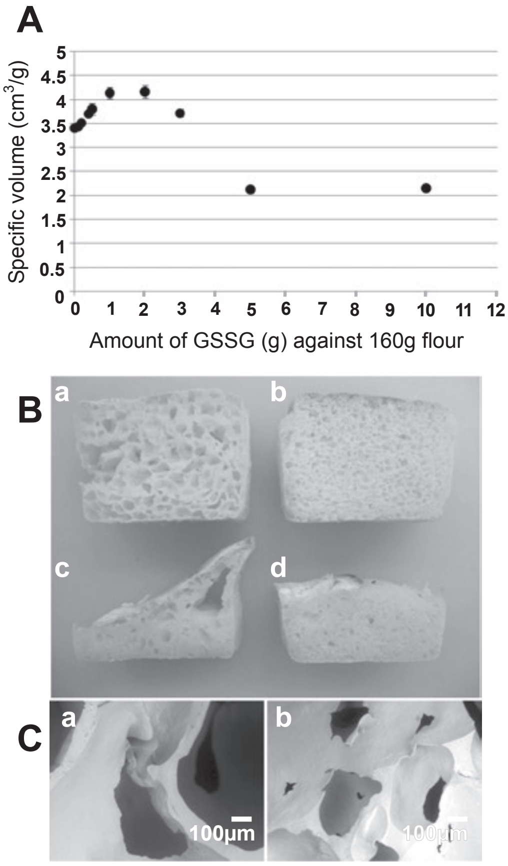

Fig. 2　Effect of oxidized glutathione on the sorghum/rice bread

A, The specific volume of breads plotted against the amount of oxidized glutathione (GSSG) added. Each data point represents the mean ± SD of three independent experiments. *B*, Cross-sections of some of the breads. The amounts of GSSG were 0, 2, 5, and 10 g for *a*, *b*, *c*, and *d*, respectively. *C*, Low-vacuum scanning microscopic analysis of the control (*a*) and GSSG-added breads (*b*). The amounts of sorghum flour, rice flour, distilled water, dried yeast, and sugar were 30, 130, 140, 2.5, and 15 g, respectively. The standard fermentation and baking conditions were 40°C for 60 min and 180°C for 24 min, respectively.

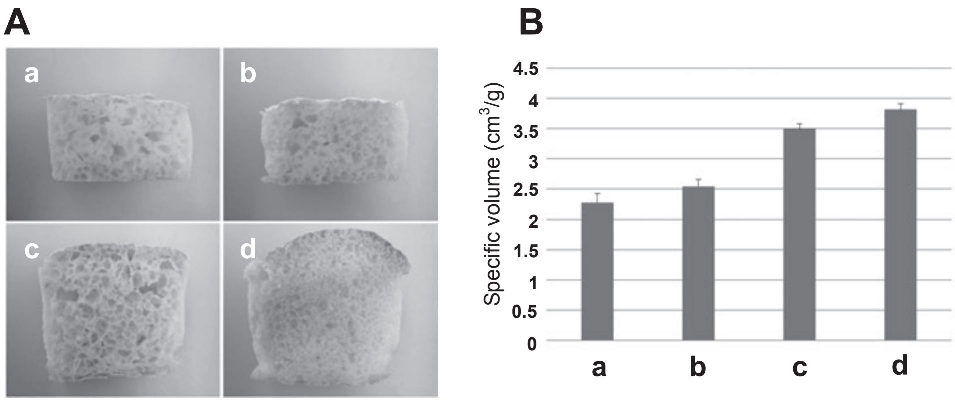

Fig. 3　Effect of sorghum flour on the salt tolerance of the rice bread containing glutathione

A, Cross-sections of the relevant rice breads. *a*, Rice bread; *b*, Oxidized glutathione (GSSG)-containing rice bread; *c*, Sorghum/rice bread; *d*, GSSG-containing sorghum/rice bread. *B*, The specific volume of each bread. Each data point represents the mean ± SD of three independent experiments. The amount of GSSG for *b* and *d* was 1 g. The amount of rice flour was 160 g for *a* and *b*. The amounts of sorghum flour and rice flour were 30 and 130 g, respectively, for *c* and *d*. The amounts of salt, distilled water, dried yeast, and sugar were 2.4, 140, 2.5, and 7.5 g, respectively, for *a* to *d*. The standard fermentation and baking conditions were 40°C for 60 min and 180°C for 24 min, respectively.

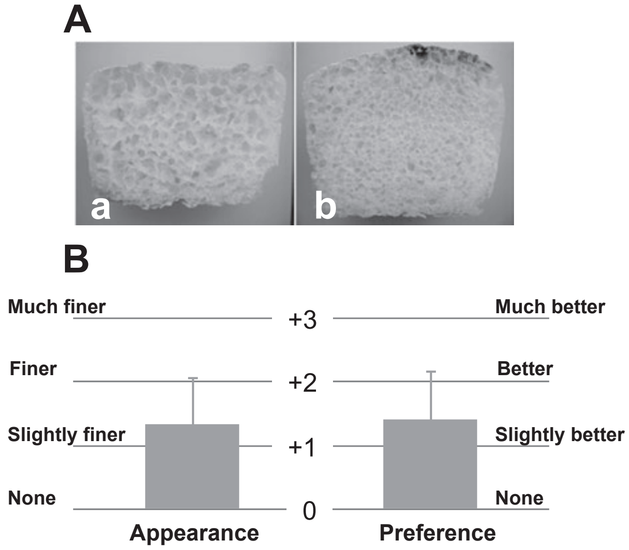

Fig. 4 Effects of a yeast extract with a high content of oxidized glutathione on the sorghum/rice bread

A, Cross-sections of the control (*a*) and yeast extract-containing (*b*) sorghum/rice breads. *B*, Sensory test of the breads with respect to crumb appearance (*left*) and preference (*right*). The results of the sensory tests (i.e., the difference in the crumb fineness and preference of the bread containing YH-D18 compared with the control bread) are shown. The ingredients of the breads were sorghum flour (30 g), rice flour (130 g), water (140 g), sugar (15 g), dried yeast (2.5 g), and butter (2 g). Two grams of the yeast extract YH-D18 had been added to the batter in panel *b* only. The standard fermentation and baking conditions were 40°C for 90 min and 180°C for 24 min, respectively.

Effect of oxidized glutathione on the sorghum/rice bread

Next, we investigated the effects of GSSG on the sorghum/rice batter in terms of the specific volume and crumb coarseness/fineness of the bread. The amount of rice/sorghum flour was 130 g/30 g, as this ratio provided the highest specific volume in the previous experiment (Fig. 1). Figure 2A shows the specific volume of breads plotted against the concentration of glutathione. Interestingly, the specific volume was relatively high (3.40 ± 0.04 cm^3/g) even in the absence of GSSG. The maximum specific volume, around 4.1 cm^3/g, was obtained with addition of 1 to 2 g of GSSG against 160 g of the flour mixture. When the amount of GSSG was 3 g or higher, the specific volume decreased. Figure 2B shows a cross-section of the rice/sorghum bread with and without GSSG. The crumbs appeared coarse in the absence of GSSG but became finer in its presence. The low-vacuum scanning microscopic analysis (Fig. 2C) of the control (a) and GSSG-added (b) breads suggested that the bubble wall was thicker in the absence of GSSG and thinner in its presence. Thus, it was speculated that the combined use of sorghum/rice flour strengthens the framework of the bubbles by thickening their walls.

The enhancing effect of the use of mixed flours on the volume of gluten-free bread has already been reported (Sanchez and others 2002; Sciarini and others 2010). The mixture of sorghum/rice flour should have a similar effect. On the other hand, glutathione seems to enhance the fineness of the crumb structure. Although the swelling mechanism of the glutathione bread has not yet been elucidated, we hypothesize that glutathione affects the structure of the batter proteins by reducing the protein disulfide bonds (Yano and others 2013). Hamaker and Griffin (1993) have proposed that an endosperm matrix protein, or possibly a specific starch-granule-associated protein, exerts influence on the gelatinization behavior of rice starch granules. In short, the barrier theory postulates that disulfide-bound protein polymers in or surrounding the native starch granule affect the viscoelastic properties of the cooked grain and flour. When the protein disulfide bonds are disrupted, rice starch granules swell to a larger size, thereby increasing the paste viscosity. Therefore, as we discussed earlier, the addition of glutathione may cleave the disulfide bonds of the barrier protein, thereby increasing the batter viscosity as well as the gas-holding capacity in fermentation

(Yano 2010). Meanwhile, in their report on protease-treated rice batter, Hamada and others (2013) considered that a partially denatured protein, possibly glutelin, may work as a linker to join starch granules. These aggregates of starch granules, which have a lot of space within them, have a lower specific gravity and result in slower sedimentation. Hamada and others (2013) concluded that the aggregates may have caused a change in the batter rheology, particularly its viscosity and sedimentation behavior. Our own ongoing studies, as well as those of other researchers, will reveal the relations between the barrier protein and starch granule, as well as the swelling mechanism of the gluten-free bread.

Effect of sorghum on the salt tolerance of glutathione-rice bread

One of the major challenges in developing glutathione-rice bread is that salt reduces the swelling of the batter in fermentation (Yano 2010). This is an important problem to overcome, since the taste of salt is a key factor in the preference of bread (Miller and Hoseney 2008). We therefore investigated whether the addition of sorghum flour to the glutathione-rice batter would enable the batter to rise during fermentation, even in the presence of salt. Figure 3A shows a cross-section of the relevant rice breads that contained 2.4 g of salt in 160 g of sorghum/rice flour. Figure 3B shows the specific volume of each type of bread. Without sorghum flour, the specific volumes of the rice bread were 2.28 ± 0.15 cm^3/g and 2.54 ± 0.12 cm^3/g in the absence (a) or presence (b) of GSSG, respectively. On the other hand, addition of sorghum flour improved the swelling of the batter during fermentation, increasing the specific volume of the breads to 3.49 ± 0.09 cm^3/g and 3.82 ± 0.09 cm^3/g in the absence (c) or presence (d) of GSSG, respectively. The crumbs appeared finer in the presence of GSSG (compare Fig. 3A c and d), even in the presence of salt. Conclusively, the addition of sorghum flour to the glutathione-rice batter was effective in making the bread swell and the crumb structure finer, even in the presence of salt.

Substitution of a yeast extract with high GSSG content for purified GSSG

Next, we investigated whether purified GSSG could be replaced with a yeast extract with a high concentration of GSSG. Although purified glutathione can be used as a food additive in the USA and several Asian countries, it cannot be used as a food material in Europe and Japan. Glutathione is produced on an industrial scale by yeast fermentation (Li and others 2004). Although the commonly used yeast extracts contain only a negligible amount of reduced glutathione (GSH) or GSSG, specific yeast extracts with a high content of GSH or GSSG glutathione are commercially available. In many countries, it is permissible to use such yeast extracts as an ingredient in foods. In the following experiment, we used the yeast extract YH-D18, which contains approx. 18% (w/w) of GSSG.

Figure 4A shows cross-sections of sorghum/rice breads with a rice/sorghum ratio of 130/30. No GSSG had been added to the bread in panel *a* (control), whereas 2 g of yeast extract YH-D18 had been added to the bread in panel *b*. Rice batter with YH-D18 did not swell in the absence of sorghum flour (data not shown). The specific volume of the control and the YH-D18-containing bread was 3.34 ± 0.08 cm^3/g and 3.74 ± 0.09 cm^3/g, respectively. The results were consistent with the observed effects of purified GSSG addition, which also increased the specific volume of sorghum/rice bread (Fig. 2A). It also appeared that the addition of the yeast extract made the breadcrumbs finer, as shown by the addition of purified GSSG (Fig. 2B).

Next, the breads were subjected to a comparative sensory test by a panel of 12 trained tasters employed at the Japan Food Research Laboratories (Tokyo, Japan), a foundation that offers analytical services on food products. Figure 4B shows the difference in the crumb fineness/coarseness between the bread containing YH-D18 and the control bread without YH-D18 as judged by the panel, with the results being scored on a 7-point scale (-3 to +3). The crumbs of the bread containing YH-D18 were recognized as being finer than those of the control bread. The difference was 1.33 ± 0.75 ($p < 0.01$) between "finer" and "slightly finer." Moreover, when masticated, the bread containing YH-D18 was judged as being preferable (1.42 ± 0.76; $p < 0.01$) to the control bread; that is, between "better" and "slightly better." The sensory tests thus conclusively demonstrated a clear effect of YH-D18 addition on the fineness of the breadcrumbs. It was speculated that this difference was attributable to the GSSG contained in the yeast extract YH-D18, since the results were consistent with those obtained using purified GSSG, as shown in Figures 2 and 3. Thus, a yeast extract with a high content of GSSG or GSH may be useful for modifying the disulfide structure of food proteins. As disulfide bonds play critical roles in food chemistry (Yano 2014), this finding may expand the use of yeast extract in the food industry. Meanwhile, as the yeast extract contains other components, such as proteins, amino acids, lipids, and carbohydrates, some of these components might have exerted a secondary action on the swelling during fermentation or on the crumb fineness. Therefore, further studies will be needed to confirm a causal relationship between the GSSG in YH-D18 and the improvement of the bread quality.

Collectively, the studies performed in this and previous reports have completed the basic formulation for a glutathione-based gluten-free bread. This bread can be made using foodstuffs only; that is, rice flour, sorghum flour, yeast extract with a high content of glutathione, salt, dry yeast, sugar, and water. No specific apparatus is required other than a commercially available home bakery and electric oven. Semisynthetic chemicals such as HPMC or guar gum are not needed. Collaborative studies with bread makers to improve the quality of the bread and thus enhance its practical applicability as a food are ongoing in our laboratory. Hamaker and others (1987) reported that although cooked sorghum protein is less digestible than other cooked cereal proteins, cooking sorghum in the presence of reducing agents such as 2-mercaptoethanol, dithiothreitol, sodium bisulfite, and l-cysteine increased the protein digestibility to a level comparable with other cereals. Thus, studies on the effect of glutathione on the nutritional value of the bread should also be conducted. Finally, it will be necessary to verify whether the bread has reduced malignancy in celiac and wheat allergy patients.

Conclusions

Addition of sorghum flour improves the swelling of glutathione-rice batter during fermentation. Bread making with the improved batter is possible even in the presence of salt. The basic ingredients are all foodstuffs, and no food additives or synthetic bread enhancers such as HPMC or guar gum are needed. The bread can be made with or without salt, so it can be a suitable food for renal patients or individuals with high blood pressure. Although the bread is at present just palatable, we are currently working with bread makers to improve the quality of the bread. Additionally, the elucidation of the swelling mechanism, which is also ongoing in our laboratory, will help to expand the use of glutathione in the food industry.

Acknowledgments

We thank Professor Bob B. Buchanan, University of California, for his helpful discussions and encouragement. The low-vacuum SEM analyses and the sensory tests were carried out with the help of JEOL, Ltd. (Tokyo, Japan) and the Japan Food Research Laboratories (Tokyo, Japan), respectively. Yeast extract YH-D18 was gifted by Kohjin Life Sciences Co., Ltd. (Tokyo, Japan). This work was supported by a Grant-in-Aid for Scientific Research (C) (25450193 to H. Y.).

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グルタチオン米粉パンに対するソルガム粉の効果

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要旨

セリアック病や小麦アレルギーの蔓延を示す報告が累積されつつあることから、高品質のグルテンフリー食品の開発は食品工業における緊急課題の一つである。著者らは天然トリペプチドであるグルタチオンがグルテンフリー米粉生地のガス保持能を高めることを既に報告した。また、パンの香りの点から、酸化型グルタチオンを使用することが望ましいことを官能評価により明らかにした。しかしながら、グルタチオンを利用した生地を実用化するためには解決すべきいくつかの課題が残されている。まず、パンの味に重要な食塩の添加が、グルタチオンを配合した生地の膨らみを抑制することである。次に、米国やアジアのいくつかの国々で食品添加物として認められている精製グルタチオンが、他国では認められない場合があるということである。本報では、これらの課題の克服を試みた。グルタチオンを添加した米粉生地にソルガム粉を添加するとクラム膜が厚くなることが微細構造解析により示された。また、この生地では食塩を添加しても膨らみが低下しなかった。さらに、高濃度のグルタチオンを含む酵母エキスがこの生地では精製グルタチオンの代用として効果的に作用した。そこで、このパンに必須な原料は米粉/ソルガム粉、水、酵母エキス、ドライイースト、砂糖であり、すべて食品でつくることができる。結論として、グルタチオンパンの実用的な基本処方が本研究で開発された。.

キーワード：グルタチオン、グルテンフリー、米粉パン、ソルガム