椙山女学園大学研究論集　第46号（自然科学篇）2015

Effect of Banana Ripeness on the Quality of Bread made with Banana Flour, and Preparation of Wheat Flour-Free Bread made with Banana Flour

Sachiyo SHITASUE*, Mikiko OGURI*, Akari KAJITA*, Tamio MASE* and Shinobu ISSHIKI*

Abstract

Because of reports that overripe bananas are superior to ripe bananas for breadmaking when mixed with wheat starch, we attempted to prepare wheat flour-free bread using banana flour. Initially, we examined whether ripe bananas or overripe bananas perform better when used in breadmaking. Overripe bananas have lower starch content but higher contents of dietary fiber, soluble protein, α-amylase, and saccharides such as glucose, fructose, and sucrose when compared with ripe banana flours. For these breadmaking experiments, 25% of the wheat flour in the recipe was replaced with flour made from either overripe or ripe banana flours. Bread made from overripe banana flours showed higher specific loaf volume and lower firmness than bread made from ripe bananas. The sensory attributes and palatability of these two types of banana-flour breads were compared using a five-step scoring method. The bread made from overripe bananas was preferred by the panel in all 5 quality categories, color, aroma, flavor, mouthfeel, texture, and total acceptance. For wheat flour-free breadmaking using overripe banana flour, we used corn, rice, dog-tooth violet, and tapioca flours as alternate flours replacing the wheat flour, and the bread made with tapioca flour was preferred by the test panel in all quality terms.

Introduction

Many foods are made from wheat, including bread and other baked foods. However, wheat is also a common allergenic food for many individuals. Wheat allergy typically presents itself as a food allergy, signs and symptoms start within seconds to two hours after eating wheat. Twenty-seven potential wheat allergens have been successfully identified to date (Sotkovský *et al*., 2011). Among these, glutenin, one of the four major classes of seed storage proteins, is a predominant allergen (Akagawa *et al*., 2007). Allergenic prolamin proteins known as gliadins and glutelins which together comprise glutenins in wheat form the class glutens. In the process of breadmaking, the glutenic proteins have a very important role for the development of the gluten network. Gluten

* *School of Life Studies, Department of Human Nutrition*

is also a causative agent of celiac disease. Wheat allergy is frequently confused with celiac disease and Krohn's disease, which can be contrasted with gluten allergy by the involvement of various immune cells and antibody types (Catassi and Yachha, 2009).

Allergic reactions can occur at various times after eating wheat. In some people, allergic reactions occur while exercising after eating wheat. A few people have very severe reactions, or anaphylaxis, after eating wheat. All food allergies are caused by an immune system malfunction. When the immune system identifies certain allergenic proteins as harmful, the body produces immunogloblin E (IgE) antibodies. These IgE antibodies recognize the non-native proteins in the body and signal the immune system to release histamine, which causes a range of allergy symptoms. The primary treatment for wheat allergy is to avoid eating foods that contain wheat.

Presently, many wheat products are available on the Japanese market, such as bread, cakes, and pastries, and so wheat allergy has become a more common risk in the modern Japanese diet. Therefore, making breads using recipes that do not use wheat flour has become much more popular. In most of these recipes in Japan, rice flour is typically substituted for wheat flour. For bread made from mixtures of rice and wheat flour, substitution of wheat flour with up to 30% rice flour has been deemed acceptable without compromising sensory qualities (Watanabe *et al*., 2004; Nakamura *et al*., 2009).

More recently, however, banana flour has also been used for gluten-free breadmaking. Seguchi *et al*. (2012) found that flour made from overripe bananas gave better results than flour from unripe or ripe bananas in breadmaking with wheat starch. They surmised that various enzymes present in overripe bananas contributed positively to baking qualities.

In this report, we investigated how flour made from overripe bananas would perform compared to ripe bananas as a substitute for wheat flour in breadmaking, and developed a recipe for wheat flour-free bread made with banana flour.

Materials and Method

Experimental materials Wheat flour (Camellia brand), and dry yeast (Super Camellia brand) were purchased from Nisshin Flour Milling Co., Ltd., Tokyo, Japan. Giant Cavendish bananas grown in the Republic of the Philippines were purchased from Mitsukoshi Department Store, Nagoya, Japan, and designated as ripe bananas (RBs). Overripe bananas (ORBs) were prepared from ripe bananas by allowing them to stand at room temperature for 7 days. All chemicals used were of analytical grade.

Preparation of banana flour and extract Bananas used for these experiments were sliced and freeze-dried. The freeze-dried bananas were milled into flour using a blender (Wonder Blender WB-1; Osaka Chemical Ltd., Amagasaki, Japan) and the flour was passed through a 500 μm sieve. The flour was stored at −5°C and used within 3 days. The flour was used for assay of total starch or dietary fiber. To assay another banana flour properties, 2.5 g of freeze-dried banana flour was mixed with 50 mL of distilled water and extracted with a homogenizer (Homojinaizer; IKA Japan Co., Ltd., Higashiosaka, Japan). The resulting mixture was centrifuged at 10,000 rpm for 15 min.

The resulting supernatant solution was used to assay soluble protein, saccharides, α-amylase activity, and antioxidative activity.

Moisture content The moisture content of intact bananas was calculated as weight loss after drying at 135°C using a moisture meter (MF-50; A & D Co., Tokyo, Japan).

Soluble protein content Soluble protein content was measured using a Bradford assay with the Bio-Rad protein assay reagent (Bio-Rad Laboratories. Richmond, CA) according to the manufacturer's protocol, using bovine serum albumin as standard.

Total starch content Starch content was determined using a Total Starch Assay Kit (Megazyme International, Ltd., Wicklow, Ireland) according to the manufacturer's protocol.

Saccharide assay Glucose, fructose and sucrose levels were measured with the F-kit D-glucose/fructose/sucrose (J. K. International Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol.

Reducing sugar assay Reducing sugars were measured by the Somogy-Nelson method (Aoyagi *et al*., 2011).

Antioxidative activity A spectrophotometric assay for 1,1-diphenyl-2-picrylhydrazy (DPPH)-radical scavenging activity was used to determine the antioxidant activity (Kogure *et al*., 1999) in banana flour samples. The sample solution (0.3 mL) dissolved in 80% ethanol was added to a reaction mixture containing a solution of DPPH in ethanol (400 μM, 0.3 mL), MES buffer (200 mM, 0.3 mL) and 20% ethanol (0.3 mL). After 20 min, the absorbance of the reaction mixture was measured at 520 nm. The DPPH scavenging activity was estimated as the decrease in the absorbance at 520 nm, and expressed as mmol-Trolox equivalent per 100 g of dry weight sample using the standard curve for Trolox.

Assay of dietary fiber The dietary fiber content was measured using a Dietary Fiber Assay Kit (Wako Junyaku Co. Ltd., Osaka, Japan) according to the manufacturer's protocol.

α-Amylase activity α-Amylase activity was measured using an α-Amylase Assay Kit (Kikkoman biochemifa Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol.

Breadmaking The control tests for breadmaking were carried out using the no-time straight dough method typically used for standard white bread production according to the following formula: wheat flour (135 g), banana flour (45 g), butter (6 g, Snow Brand Milk Products Co., Ltd., Tokyo, Japan), sucrose (18 g, Nissin Sugar Manufacturing Co., Ltd., Tokyo, Japan), NaCl (3 g, Hakata Salt MFG Inc., Imabari, Japan), dry yeast (3.6 g), and water (96 mL). For wheat flour-free breadmaking, 135 g of the wheat flour was replaced with 135 g of various other starch flours. The ingredients were mixed and fermented in an automatic breadmaker (SD-BT50; Matsushita Electric Industrial Co., Ltd. Tokyo, Japan) using the standard bread-dough preparation conditions described in the manufacturer's manual. The dough was allowed to rest at 28°C for 10 min, and was then punched lightly 7 times. The dough was divided into 42 g portions that were rounded by hand and proofed for 30 min. Each dough portion was put into a microwave oven fermenter (RO-EL2; Mitsubishi Electric Industrial Co., Ltd., Tokyo, Japan) for a second fermentation at 35°C for 30 min and was baked at 200°C for 10 min.

Measurement and evaluation of bread properties The bread was allowed to stand at 23°C for 1 h before the specific loaf volume (SLV) was determined by the rapeseed displacement method. The SLV (mL/g) was calculated by using the following equation:

$$SLV = \text{volume of bread (mL)}/\text{weight of bread (g)}.$$

Firmness was determined with a texturometer (TDU-1; Yamaden Co., Ltd., Tokyo, Japan) by measuring the compressive force of ultrasonic waves on bread slices (20×20×20 mm). Inner cubes of bread were compressed using a plunger (Φ16 mm) at a speed of 5 mm/s with 2 mm clearance.

Sensory evaluation The sensory characteristics of the breads were evaluated by a panel of 12 female students (age 22±1 year) of Sugiyama Jogakuen University, who were trained to recognize bread qualities and flavor. For each sample, the color, aroma, flavor, texture, mouthfeel, and total acceptance were scored on 5-point scale, 1 being lowest and 5 being highest. Scores for each sample were totaled and the means±SD were calculated.

Statistics Data were analyzed using the t-test or one-way ANOVA, following Tukey's multiple comparison test using SPSS Statistics version 19 (IBM, Chicago, US). *P*-values of <0.05 were considered significant.

Results and Discussion

The moisture contents of ORB and RB were 79.4% and 68.4%, respectively. Measurement of the moisture content in this research was computed from the reduction of the weight by dryness as described previously. The moisture contents of ORB was higher than that of RB. It is thought that non-volatile components changed to volatile components in the process of overripening.

The properties of RB and ORB flour are shown in Table 1. ORB flour had high levels of total dietary fiber and lower levels of starch. The importance of dietary fiber in human nutrition (Kay 1982) has been extensively researched. High dietary fiber content is expected to improve the nutritional quality of breads supplemented with ORB flour.

Table 1 Saccharides content of ripe banana, and overripe banana flour (g/100 g. DW).

Banana	Total-sugar	Total-starch	Reducing sugar	Glucose	Fructose	Sucrose	Fiber
Ripe	69.86	16.67	14.69	6.90	8.05	41.19	10.9
Overripe	62.89	2.11	49.61	24.9	25.48	20.68	15.1

The starch content of ORB was very low (2.11%) in contrast to the starch content of RB (16.67%). Nevertheless, despite the low starch content of ORB, the amounts of total sugars and reducing sugars in ORB were higher than in RB. Glucose, fructose, and sucrose contents of ORB were also high. This is likely because starch, oligosaccharides, and polysaccharides in RB are hydrolyzed by enzymes during the 7 days of overripening.

α-Amylase activity of banana flour was assayed using a commercial assay kit following the manufacturer's protocol (Table 2). Kojima (2000) had previously reported that amylase activity of

ORB was higher than that of RB. α-Amylase activity is known to affect the volume of bread after yeast growth and subsequent CO_2 production has occurred, and also helps prevent the degradation of bread upon the release of oligosaccharides.

Antioxidant compounds in food are thought to play an important role in protecting health. Furthermore, polyphenol content is also related to antioxidative activity in a variety of foods (Yamada *et al*., 2008). The antioxidative activity of RB flour was 1.3-fold higher than that of ORB (Table 2). Mase (2012) reported the antioxidant activity of unripe banana to be 2-fold higher than that of RBs; therefore, RBs contain high levels of polyphenols that decrease during banana ripening.

Table 2 Properties of ripe banana, and overripe banana flour.

Banana	α-Amylase activity* (U/mL)	Antioxidative activity (mmol Trolox eq./100 g. DW)
Ripe	0.056 ± 0.03	1.005
Overripe	0.107 ± 0.04	0.760

* Values are means ± SD, n = 3.

The soluble protein contents of ORB and RB were 1331 and 393 (mg/100 g DW), respectively. During overripening, either several proteins must be synthesized, or insoluble proteins are converted to soluble proteins.

The SLV of breads made with banana flour are shown in Fig. 1. Bread made with ORB flour had higher SLV than did bread made with RB flour. This is likely because ORB flour contains many fermentable saccharides that yield CO_2 gas when fermented by yeast. On the other hand, RB flour contains degraded starch instead of fermentable saccharides like ORB flour. Degraded starch results in reduced bread volume and softness (Takahashi *et al*., 2011). The firmness of each type of bread is shown in Fig. 1. Bread made from ORB had lower firmness than did bread made from RB, probably because the higher levels of fermentable sugars in ORB result in higher levels of CO_2 in

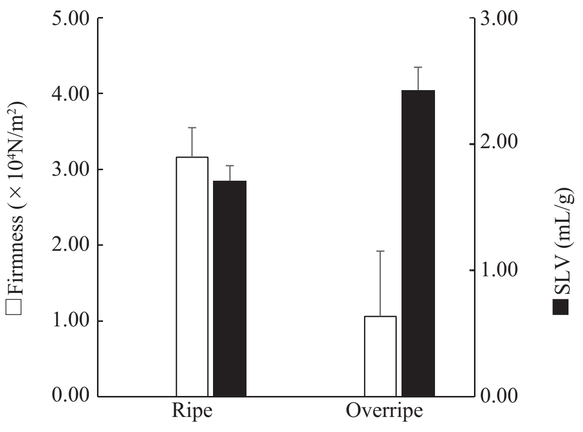

Fig. 1 SLV and firmness of banana flour bread.
Values are mean ± SD, n = 3.

the dough after fermentation by yeast. In general, the SLV of breads is affected by the amount of CO_2 produced by yeast and the amount of CO_2 retained in the dough by gluten. The softness of ORB bread was also maintained for longer periods of time than was the softness of RB bread (data not shown). It could be that the oligosaccharides produced by h-amylase prevent the further degradation of bread starch.

Sensory evaluation of breads The sensory attributes and palatability of two types of banana flour bread were compared using a 5-step scoring scale. The bread made from ORB was preferred by the panel for all 5 qualities of color, aroma, flavor, mouthfeel, texture, and total acceptance (Fig. 2).

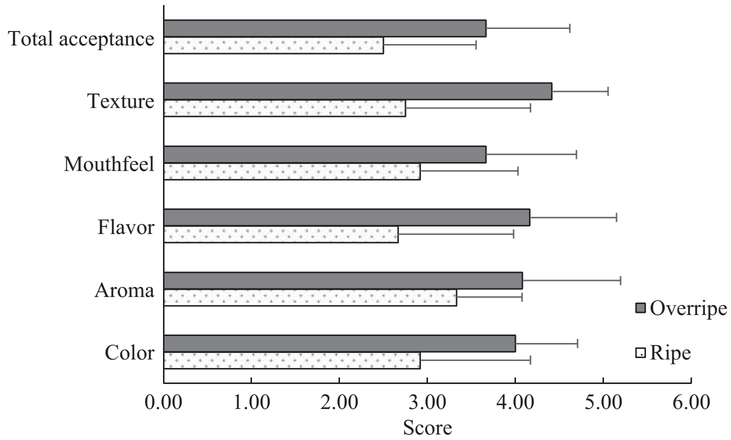

Fig. 2 Sensory evaluation of banana-flour breads.
Values are means ± SD, n = 12.

For a wheat flour-free bread recipe based on ORB flour, we replaced the wheat flour with corn, rice, dog-tooth violet, or tapioca flour. The wheat-free breads were prepared by the same breadmaking method as above, with one of each possible alternative flour replacing wheat flour. Among all of the substitute flours tested, tapioca was preferred by the panel for all 5 qualities (Fig. 3). The bread made with ORB flour combined with tapioca was also judged to be excellent in terms of SLV and firmness (data not shown).

Conclusions

The results of this study show that ORB flour performs better than RB flour as a partial replacement for wheat flour in banana-flour bread. Bread made from ORB flour was preferred by the panel members for all of the qualities judged, color, aroma, flavor, mouthfeel, texture, and total acceptance. The panel also found that tapioca was a satisfactory replacement for wheat flour for the preparation of wheat-flour free bread using ORB flour. Thus, the wheat flour-free bread prepared for this study would likely be a helpful and palatable replacement gluten-free bread for those who

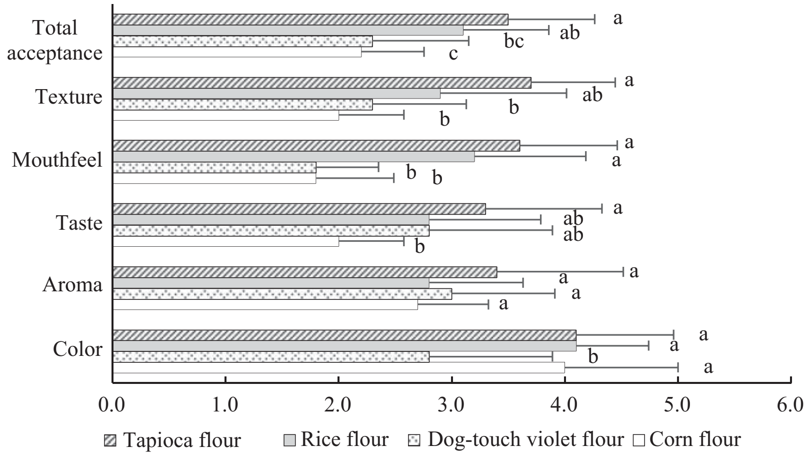

Fig. 3 Sensory evaluation of breads made with overripe banana compared with various those using other flours as substitutes for wheat flour.

Within a category, different alphabets indicate statistical differences ($p<0.05$). Values are means ± SD, n = 12.

are gluten sensitive.

References

Akagawa M, Handoyo T, Ishii T, Kumazawa S, Morita N, Suyama K. (2007). “Proteomic analysis of wheat flour allergens”. *J. Agric. Food Chem*. **55** (17): 6863–70.

Aoyagi Y., Arita M. (2011). Shokuhingaku jittken, 101−105, Kenpaku-sha.

Catassi, C. and yachha, S. K. (2009). The epidemiology of cell disease. In “The science of gluten-free foods and beverages,” ed. By E. K. Arendt and F. D. Bello. AACC International, Inc. St. Paul, Minnesota pp. 1−11.

Kay Ruth McPherson. (1982). Dietary fiber. *J. Lipid Research*, **23**, 221−242.

Kojima, A. (2000). Living thing experiment with banana cell. *J. Hokkaido Education Research Institute*, 82−85.

Kogure, K., Goto, S., Abe, K., Ohiwa, C., Akasu, M. and Terada, H. (1999). Potento antiperoxidation activity of the bisbenzylisoquinline alkaloid cepharanthine: The amino moiety is responsible for its pH-dependent radical scavenge activity. *Biochem. Biophys. Acta*., **1426**, 133−142.

Mase, T., Sato, Y., Miyazaki, S., Kato, Y. and Isshiki, S. (2012). Quality evaluation of bread containing unripe apple or banana flour. *J. Sugiyama Jogakuenn Univ*., **43**, 47−52.

Nakamura, S., Suzuki, K. and Ohtsubo, K. (2009). Characteristica of bread prepared from wheat flours blended with various kinds of newly deveroped rice flours. *J. Food Sci*., **74**, 121−130.

Seguchi, M., Ozawa, M. and Nakamura T. (2012). Preparation of gluten-free bread with banana. *Proceedings of the 59th Annual Meeting of The Japanese Society For Food Science and Technology*. 172.

Sotkovský P., Sklenář J., Halada P., Cinová J., Setinová I., Kainarová A., Goliáš J., Pavlásková K., Honzová S., Tučková L., A new approach to the isolation and characterization of wheat flour allergens.

(2011) *Clin Exp Allergy*. Jul; 41(7): 1031–1043.

Takahashi, K., Okunishi, T., Suzuki, K. and Yukizaki, C. (2011). Processing suitability evaluation method for rice bread and evaluation of rice flour produced in miyazaki. *J. Food Sci. Technol*., 58, 55–61.

Watanabe, M., Maeda, T., Tsukahara, K., Kayahara, H. and Morita, N. (2004). Application of pregerminated brown rice for breadmaking. *Cereal Chem*., **81**, 450–455.

Yamada, J., Igarashi, E. and Matsuda, H. (2008). Comparison between the Radical Scavenging Activity of Arabushi-dash and Karebushi-dashi. *J. Cookery Sci. Jpn*., **41**, 134–137.