The hypoglycemic effect and sensory properties of bread prepared using combinations of Japanese whole rye, whole-wheat and wheat flours


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Abstract

The aim of this study was to produce healthy breads capable of reducing postprandial glycemia. Macronutrient composition, in vivo hypoglycemic effect, microstructure, and sensory properties of the products were investigated. The mixing ratios of the main materials in Japanese wheat flour, whole wheat flour, and whole rye were 100:0:0 (No.1), 20:40:40 (No.2), and 20:0:80 (No.3), respectively. Total dietary fiber level was higher in Nos. 2 and 3 than in No.1 and especially, insoluble fiber level was considerably high in the former two. Glycemic index was lower in Nos. 2 and 3 than in No.1. The closed pore structure was observed in Nos. 2 and 3, in contrast, the opened pore structure was observed in No.1. There were significant differences (p<0.05) of sensory attributes such as bread smell, bread color, brittle, hard, watery and coarse notes among Nos. 1, 2, and 3. Palatability such as overall taste, ease of swallowing, and texture was significantly lower (p<0.05) in No.3 than in No.1 although that of No.2 was almost the same as No.1. These findings suggest that the differences in main raw materials and food structure are important factors in producing breads with hypoglycemic effect and high palatability.

Introduction

Diabetes is one of the most challenging lifestyle diseases facing Japan today. According to the national health and nutrition examination survey in 2012, 9.5 million people showed a strong indication of diabetes and another 11.0 million indicated some potential signs of diabetes (International Diabetes Federation, 2013). Currently, the number of type 2 diabetes in Japan has increased. The reason might be due to an increase in opportunities of high fat meal intake or lack of exercise because westernized diets have been influenced by changes in the social environment prevalent in modern society. Diabetes is a factor of large importance in arteriosclerotic cardiovascular disease and is the cause of critical illness such as cerebral and myocardial infarctions. From the above, diabetes prevention is an important issue in Japan now. It is well-known that in type 2 diabetes, which the beta cells of the pancreas produce insulin but the body is unable to use it effectively because the cells of the body are resistant to the action of insulin. So, it is important for consumers to control blood glucose by food intake due to an improvement in insulin secretion or sensitivity.

On the other hand, consumers have switched from an emphasis on satisfying hunger to an emphasis on the promising use of foods to promote well-being and to help reduce the risk of disease during the past decades although foods were primarily recognized for their essential nutrients for normal body activity and function in the past (Niva, 2007). So, there were some reports concerning development of healthy breads such as whole wheat enriched bread (Dewettinck et al., 2008), high protein soy bread (Islam et al., 2007), whole grain cereal enriched bread (Maneju et al., 2011), wheat bread supplemented with stabilized undefatted rice bran (Ameh et al., 2013) and pseudocereal bread (Chlopicka et al., 2013).

A variety of bakery products such as breads,
doughnuts, and cakes, which have rich dietary fiber and minerals, have also been produced now in Japan because health-consciousness of the consumer has increased. We have previously reported the results of a survey on blood glucose and palatability of doughnuts and cakes mixed with soybean curd refuse which has a rich dietary fiber (Funatsu et al., 2008; Funatsu et al., 2009). Glucose response curves for 120 mins after consumption of soybean curd i.e. tofu refuse enriched products revealed that the addition of the tofu refuse slowed increases in blood glucose levels, and significantly (p<0.05) lowered in the tofu refuse enriched products more than in the control products mixed with only wheat flour. Glycemic index (GI), which was introduced by Jenkins et al. (1981), was also significantly (p<0.05) lower in the former than in the latter. Uebaba et al. (2006) reported that an elevation of postprandial blood glucose level was slower in tofu refuse enriched bread with deep sea water than in the wheat flour bread with deep sea water and GI was about 10% lower in the former than in the latter.

In general, it is presumed that moderating postprandial blood glucose level might be different from the processed food form in the bakery products prepared using a combination of whole wheat flour, whole rye grain, and wheat flour in a same manner as the tofu refuse enriched products because the food compositions in the bakery products are quite different (Standard Tables of Food Composition in Japan, 2015). Funatsu et al. (2015) reported on the effects of doughnuts and cakes prepared using combinations of Japanese whole rye, whole-wheat and wheat flours on blood glucose level and palatability after the products consumption. Blood glucose levels were significantly (p<0.05) lower in whole wheat flour or whole rye doughnuts at 15 mins than in the wheat flour doughnut after 50 g sugar loading in healthy subjects. Acceptability of the whole wheat flour doughnut was higher and that of the whole rye doughnut which was lower compared to the wheat flour doughnut. The hypoglycemic effect and palatability of the cakes were almost the same between the samples. Therefore, it is considered that selection of the processed food form should be needed to utilize a diabetic diet. However, blood glucose level and palatability on breads prepared with different main materials remain to be investigated. In this study, we investigated the effect of breads prepared using combinations of Japanese whole rye, whole-wheat and wheat flours on blood glucose level and palatability after the breads consumption.

Materials and Methods

Sample preparation

Wheat flour (Haruyutaka Blend), whole wheat flour (Komugi Zenryufun), and whole rye (Raimugi Zenryufun) were purchased from Ebetsu Flour Milling Co. Ltd., Japan. All the flour used is from domestic production. The initial moisture contents (w/w) of Japanese whole rye, whole-wheat and wheat flours were 11.9%, 13.4% and 13.0%, respectively. The breads for the present study were mixed bread which was prepared with 20% wheat flour, 40% whole wheat flour, and 40% whole rye flour (No.2), and enriched whole rye which was prepared with 20% wheat flour and 80% whole rye bread (No.3). The wheat bread (100% wheat flour bread) was used as a control product (No.1). Table 1 shows the proximate compositions of three bread products. Water and sugar levels were higher in No.1 than in Nos. 2 and 3 while ash and dietary fiber levels were on the reverse. There were some notable differences in soluble and insoluble fibers levels between the samples. In particular, the insoluble fiber level was 3 times higher in Nos. 2 and 3 than in No.1.

In general, Japanese people prefer to use wheat bread due to its delicious appearance when it's rising. However, the Japanese people do not like rye bread because it does not rise. Whole-wheat bread also rises like wheat bread. Due to the results of the preliminary examination describe below in Figure 1 as well as taking into account the above reasons, we chose the mixing ratios of the three main materials. The dough was made from 48.5% each powder (Nos.1-3), 2.9% sugar (ST, Dai-Nippon Meiji Sugar Co., Ltd., Tokyo, Japan), 1.7% defatted milk powder (Skim milk, Morinaga Milk Industry Co., Ltd., Tokyo, Japan), 1.0% salt (Akou Natural Salt, Akou Co., Ltd., Akou, Japan), 2.4% margarine (Risublue 700, Adeka Co., Ltd., Tokyo, Japan), 1.7% dry yeast (Saf-instant, Lesaffre Co. Ltd., France), and 41.8% water. The dough was mixed for 20 mins with a mixer (SS-161, Kanto Kongoki Industrial Co., Ltd., Tokyo, Japan). After a floor time of 20 mins at 30°C, the dough was divided into 1,000 g pieces. The divided pieces were manually molded and fermented for 50 mins at 30°C.
in a dough conditioner (PSDC2-32F2, Kyoritsu Plant Industry Co., Ltd., Osaka, Japan). The loaves were baked at 230°C for 50 mins in a deck oven (PIA-222B, Fujisawa-Maruzen Co., Ltd., Tokyo, Japan). After cooling at room temperature, the loaves were stored in a freezer (FPC-K1H, Fukushima Industry Co., Ltd., Osaka, Japan) at -20°C until use.

Proximate analysis of bread products

Proximate compositions of three kinds of bread products were carried out using explanation of analytical manual of standard tables of food composition in Japan (Food constituent committee of Resources Council of Science and Technology Agency, 1997). Nitrogen-protein conversion coefficient was made use of 6.25. Carbohydrate was determined by subtracting the sum of moisture, ash, protein and lipid levels from 100. Protein, lipid and carbohydrate of energy conversion coefficient were 4 kcal/g, 9 kcal/g, and 4 kcal/g, respectively.

Blood glucose levels

Subjects were comprised of 10 healthy students (male: 5, female: 5) aged 18-23 years who had never been diagnosed with diabetes. A fasting blood glucose level in the subjects was 89.0±7.6 mg/dL, and all of the subjects were determined to be normal type based on the diagnostic criteria for diabetes (<110 mg/dL) (Seino et al., 2010). The measurement of blood glucose level was approved by the Ethics Committee of Rakuno Gakuen University (Approval# 08-2). In accordance with the principle of the Declaration of Helsinki, subjects were fully informed of the main purpose of study, the safety of test samples, and how to measure blood glucose levels before written consent was obtained.

In this study, a cross over test with the breads by the same subjects was conducted and blood glucose levels were measured after more than 3 days had passed from the previous measurement. Subjects were instructed not to engage in strenuous exercise and alcohol intake on the day before an experiment. Only cold and warm water was allowed from 9 p.m. on the day before an experiment to the start of the experiment on test-day. Intake of all food and drink was prohibited in a same manner as the above period. In all experiments, the total weight of carbohydrate included in each test sample was 50 g.

To begin with, subject’s fasting blood glucose level (0 min) was measured on test-day. Subsequently, test samples were consumed within 10 mins, and subjects consumed 50 g sugar equivalent of bread and 200 mL of water alternately. The recommended usage frequency was as follows. One bite-size portion size was approximately 15 g, which was chewed 45-50 times. After consumption of test samples, subjects rested until the completion of the 120-min blood glucose level measurement. The blood sugar response curve was expressed as relative value (%) compared to the fasting blood glucose level i.e. the relative blood glucose level (%) was calculated by dividing each glucose level by each fasting blood glucose level (set at 100%) due to there were considerable individual differences in the fasting glucose levels (68-103 mg/dL) in this study.

Using a small blood glucose level measurement device (NEO SUPER, Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan), subject’s blood glucose levels were measured before (0 min) and 15, 30, 45, 60, 90, and 120 mins after the consumption of the test sample. Puncture needle devices (MS-GN4530, Telmo Co., Ltd., Tokyo, Japan) were prepared for each individual subject.

According to the method recommended by the Food and Agriculture Organization of the United Nations and the World Health Organization, the GI of each test sample containing 50 g of carbohydrate was calculated by dividing the area under the blood glucose response curve by the area under the curve (set as 100) to 50 g/150mL glucose solution (Tolerant 50, Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan).

Scanning electron microscopic observation

Smaller sized bread products (About 15 cm³) were dried in a desiccator for three days. After dehydration, each sample was cut into small pieces (5 mm×5 mm×2 mm) with a single-edged razer blade and the shaped samples were adhered to aluminum-made stage with carbon paste. These samples were conducted to Pt-Pb vapor deposition coating using an ion sputtering device. After coating, the microstructure of the samples was observed at 10 kV of accelerating voltage using a scanning electron microscope (S-2460, Hitachi Co., Ltd., Tokyo, Japan).

Measurement of density

The inside of each bread sample was hollowed out using cylindrical stainless ring (diameter: 1.2 cm, height: 1.0 cm, cubic capacity: 1.130 cm³). The density (ρ, g/cm³) was obtained by dividing weight (g) by cubic capacity (cm³). All of the experiment was conducted in duplicate and data was expressed as mean.

Sensory evaluation

Sensory panels (age: 21.1±1.1, male: 6, female:
20) selected Rakuno Gakuen University students who had no wheat allergy. The sample size and a recommended mastication frequency were about 15 g and 45-50 times. The panelists selected 10 sensory notes related to bread quality characteristics such as bread color, bread smell, sweet, salty, sour, bitter, coarse, ease of swallowing, hard, brittle, and watery notes and evaluated these using the three kinds of bread products (Nos. 1-3). No.1 was used as a control. Each attribute was evaluated on a 7-point scale of sensory attribute (bread color: +3, very dark, 0, same, -3, very light; bread smell, sweet, salty, sour, bitter: +3, considerably strong, 0, same, -3, considerably weak; coarse, hard, brittle, and watery: +3, considerably high, 0, same, -3, considerably low) compared to the control (0). On the other hand, in the case of the preference test, the panelists mentioned above evaluated external appearance, overall smell, texture, ease of swallowing, and overall taste of the three kinds of bread products (Nos. 1-3). Each attribute was evaluated on a 7-point scale of preference (+3: more agreeable, 0: same, -3: more disagreeable) compared to the No.1 (0).

Statistical analysis
Statistical analysis was performed using JMP 8.02 (SAS Institute Inc., NC, USA). Sensory properties were analyzed using dunnett’s test. In all analyses, statistical significance was set at 5% (p<0.05).

Results and Discussion

Moderating postprandial blood glucose level after mixed bread and whole rye enriched bread consumption
To begin with, a preliminary examination was conducted using a combination of main materials such as wheat flour, whole wheat flour, and whole rye to select breads which have a moderating postprandial blood glucose level after the bread consumption (Figure 1). In a single dose oral administration, the rate of increase in relative blood glucose level up to 30 mins did not decline with increment of whole wheat flour level after 50g sugar loading in subjects compared to the wheat bread (Figure 1 A, B) while the rate declined with increment of whole rye level (Figure 1C). However, the rate of decrease in relative blood glucose level from 30 mins to 90 mins was significantly slower (p<0.05) in 40% whole grain rye bread and 60% whole wheat flour (— — ) than in wheat bread (— ). From the above results, mixing ratios of main materials in the mixed bread and enriched grain rye bread were 40% whole rye, 40% whole wheat flour, and 20% wheat flour, and 80% whole rye and 20% wheat flour, respectively, to effectively control the level of sugar in the blood after bread consumption.

Figure 2 shows the effect of three bread products on relative blood glucose (A) and GI (B) levels after loading of 50 g of sugars in healthy subjects. The blood glucose level of No.1 was significantly increased (p<0.05) up to 30 mins after bread consumption and significantly decreased (p<0.05) from 30 to 90 mins, and thereafter it was on a gradual downward trend (Figure 2 A). On the other hand, the blood glucose level was lower at 30 mins in Nos. 2 (145±19%) and 3 (146±15%) than in No.1 (157±22%) after bread consumption. The relative blood glucose level was also lower at 90 mins in Nos. 2 (113±7%) and
3 (113±13%) than in No.1 (123±15%) after bread consumption. However, the rate of decrease in the relative blood glucose level from 30 mins to 60 mins after bread consumption was lower in No.2 than in Nos.1 and 3. The rate of decrease in the relative blood glucose level of No.2 was significantly decreased (p<0.05) from 60 to 90 mins after bread consumption. The relative blood glucose level was lower at 120 mins after bread consumption in No.3 than in Nos. 1 and 2. GI level was 12.2% and 24.5% less in Nos. 2 (76.7±42.6) and 3 (65.6±36.0) than in No.1 (86.8±37.9), respectively (Figure 1B). The falling rate of GI was higher than that of tofu refuse enriched bread in this study, suggesting that the differences between the two might be caused by the main materials and mixture (Uebaba et al., 2006).

According to the international table of glycemic index and glycemic load values (Foster-Powell et al., 2002), GI levels of white-wheat-flour, whole meal (whole-wheat) wheat-flour and whole-meal rye breads were 69-73, 52-78 and 41-66, respectively. GI levels are slightly higher in No.1 than in the white-wheat-flour breads although GI levels of Nos. 2 and 3 were almost the same as those of the whole-meal-wheat-flour and whole-meal rye breads in this study. This could be due to the differences in the ingredient compositions and insulin sensitivity and insulin response of the subjects because GI of white Turkish bread, which is classified into spelt wheat bread, is almost the same as No.1 (87) (Foster-Powell et al., 2002) and there is a hyperbolic relationship between insulin sensitivity and insulin response in African, Caucasian, and East Asian normal glucose tolerance (NGT) cohorts (Kodama et al., 2013). It is currently under consideration that the reason why individual variations of blood glucose levels and GI values (i.e. there was no significant differences (p>0.05) of them between the samples) are relatively large in this study might be due to the fact that East Asians have a limited innate capacity of insulin secretion (Fujimoto, 1996).

There were some reports that postprandial elevation of blood glucose level was restrained by augmentation of insulin release in the case of food intake containing not only carbohydrate but also protein compared to food intake containing only the former (Nuttall et al., 1984; Spiller et al., 1987; van Loon et al., 2000). Schenk et al. (2003) reported that the differences in GI between foods such as breakfast cereals might be caused by the differences in glucose uptake from the blood by tissue. This phenomenon may be also related to the argumentation of insulin release when protein ingests with carbohydrate.

According to the macronutrient composition of three bread products, the protein level was slightly lower in No.3 (9.0 g) than in Nos. 1 (10.2 g) and 2 (10.5 g) although the total dietary fiber level was 3 times higher in Nos. 2 (8.8 g) and 3 (8.9 g) than in No.1 (2.7 g) (data not shown). It is conceivable that these tendencies might be caused by proximate compositions of the main materials (Standard tables of food composition in Japan, 2015). However, the protein levels in the three bread products in this study were different from those in breakfast cereals (Schenk et al., 2003). So, it is considered that differences in GI levels between the samples in this study cannot be fully explained by the scientific hypothesis of Schenk et al. (2003).

Insoluble cereal fibers are associated with a reduction in the risk of type 2 diabetes attributed to a decreased insulin demand and a lower glycemic response (Salmeron et al., 1997). Samra and Anderson (2007) reported that the insoluble fiber found in a high-fiber ready-to-eat breakfast cereal suppresses appetite, lowers food intake, and improves glucose response to a meal consumed 75 mins later. Both Nos.2 and 3 compare to No.1 have a rich insoluble fiber and it reduced glycemic response to a meal consumed 90 mins later in this study (Table 1 and Figure 1). However, the details of this are currently under consideration because the insoluble fiber level per serving size differed considerably in the breakfast cereal (33 g) for Nos. 2 (7.0 g) and 3 (7.9 g).

Preservation of intact botanical structure of cereal grains and food processing such as baking have been shown to lower the insulin response (Heaton et al., 1988) and reduce the digestibility of starch (Englyst and Cummings, 1985), respectively. These results indicate the importance of preserved food structure and resistant starch for reduced hydrolysis. Juntunen et al. (2003) reported that a lower insulin secretion after ingestion of rye bread than after the ingestion of wheat bread is not explained by the quantity of rye fiber in the bread but may be explained by differences in the structural properties of the 2 breads. The structures of the continuous matrix and starch granules differed between rye and wheat breads after baking. In rye bread, a continuous phase was formed by closely packed starch granules, whereas in wheat bread the starch granules were entrapped in an extensible gluten network that formed the continuous phase. Therefore, particle size before swallowing was much higher for rye than wheat breads and the rate of hydrolysis was slower in the former than in the latter (Brand et al., 1990). Furthermore, in the wheat bread, starch remained inside the granule, became gelatinized, and was more accessible to hydrolysis by amyloytic enzymes. In contrast, in rye breads, amylose leached out and coated the starch granules,
which made the starch resistant to hydrolysis after cooling. The coating amylose on the surface of starch granules has also been suggested to retard the hydrolysis of amylopectin, the other main constituent of starch (Slaughter et al., 2002). Differences in GI between the No.1 and No.3 in this study might be caused by differences in the structural properties of the wheat and whole rye breads.

Figure 3 shows the scanning electron microscopic observation of three bread products. The opened pore structure was observed in No.1 while the closed pore structure was observed in Nos. 2 and 3. The structure might be less porous and firmer in No.3 and 2 than in No.1 due to the density was higher in No.3 (0.46 cm³/g) and 2 (0.37 cm³/g) than in No.1 (0.22 cm³/g). Pomeranz et al. (1983) reported that starch had a much greater role in rye than in wheat bread, especially than in wheat bread baked from relatively high-protein flour and major contributor to the structure of the rye bread was expanded starch. Although the expanded starch was also observed in No.3 in this study, the density level of the starch was lower in No.2 than in No.3. The reason why the density level of the starch between No.2 and 3 might be caused by the amount and component of pericarp-aleurone particles because they were affected to a coherent and continuous rye bread structure (Pomeranz et al., 1983).

According to a study on the differences in mastication and initial starch hydrolysis rate of rye (whole meal rye, endosperm rye, and endosperm rye with gluten) and wheat breads (Pentikainen et al., 2014), rye breads were degraded to smaller particles during mastication and in vitro starch hydrolysis rate was slower in the rye bread boluses than in wheat bread boluses. Nordlund et al. (2016) reported that relationships were shown between chemical and structural properties of rye and wheat breads, their in vitro gastric disintegration and in vivo insulin, but not glucose responses.

There is some possibility of the differences in size and distribution of particles from the rye and wheat breads after chewing because the chewing time and frequency per about 15 g of samples were slightly different, the former (44±10 sec, 47±12 times) from the latter (48±12 sec, 51±14 times) in a follow-up study (data not shown). Further study is needed to investigate the relationship between postprandial glycemia after bread consumption and mastication or chewing in detail.

Differences in sensory properties among white, mixed and whole rye enriched breads

In sensory attributes, bread smell, bread color, brittle, hard, and coarse notes were significantly (p<0.05) stronger and watery note was significantly (p<0.05) lower in Nos.2 and 3 than in No.1 (Figure 4A). Bitter note was significantly (p<0.05) stronger in No.3 than in No.1. There were no significantly differences in sweet, salty and sour notes between the samples. On the other hand, in sensory palatability, overall taste, ease of swallowing, and texture were significantly (p<0.05) lower in No.3 than in No.1 although there were no significant differences in external appearance and smell notes between the samples (Figure 4B). It is considered that palatability might be almost the same between Nos. 1 and 2 due to there being no significant differences in overall taste, ease of swallowing, and texture notes between Nos. 1 and 2. In doughnuts, overall acceptability of whole wheat flour enriched product was higher and that of whole rye enriched product was lower compared to wheat flour product because coarse and brittle notes were higher and fluffy note was lower in whole rye product than in wheat flour product.
Conclusion

The present study suggests that a possible role for the two types of breads i.e. the mixed bread (whole rye flour: whole wheat flour: wheat flour=40:40:20, w/w) and whole rye enriched bread (whole wheat flour: whole wheat flour: wheat flour=80:0:20, w/w) in the diabetic preventive diets of healthy subjects. There were considerable differences of proximate composition, food structure, and sensory properties between the 2 breads and wheat flour bread. In comparison with the 2 breads, sensory palatability was better in the mixed bread than in whole rye enriched bread. Therefore, to produce the healthy bread with high palatability, it would be necessary to consider not only the type of cereal but also the food structure.

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