Gluten-Free Rice Breeding Using Compositd Rice Flour and Pre-Germinated Brown Rice Flour for Health Benefits

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Summary Gluten-free rice breeding (RB) is generally prepared by shredding of gluten-free rice bread. It is an alternative product for Celiac patients, who are gluten allergic. The aim of this research was to formulate gluten-free rice bread formula by using composited rice flour (RF) and pre-germinated brown rice flour (PGBRF) from two Thai rice cultivars, Pathum-Thani1 (15% amylose) and Phitsanulok2 (27% amylose) at the ratio of 1:1, by considering the structural properties of bread, health benefit of breeding, and the properties of breeding as coating material of fried frozen croquette (C-) and compared to those of samples prepared from wheat flour. Increasing PGBRF from 0% to 50% in gluten-free rice bread formula increased the puffed cell wall of air cell in gluten-free rice bread. Antioxidative activity of RB containing 50% PGBRF (RB-50%) was 4 and 9 times higher than RB without PGBRF (RB-0%) and wheat breeding (WB), respectively. WB contained 356,289 mg/kg of gluten, while RB contained less than 2 mg/kg of gluten, which could be labeled “gluten-free”. When compared all breeding samples as coating material of C-, oil absorption of C-RB-50% (14.32%) was 3 times lower than that of WB (44.36%). Therefore, the RB-50% had health benefits for consumers who are suffered from Celiac disease and also other health-conscious consumers considering the higher antioxidative properties and lower oil uptake than the consumption of WB.

Key Words pre-germinated brown rice, rice flour, gluten-free, breeding

Breading (or breadcrumb or breader) is a flour-based breadcrumb or cracker meal that plays a major ingredient to coat over moistened or battered food products and create the unique characteristics. Generally, breading is made from wheat-based bread that is shredded and dried (1). However, the number of Celiac patients, who are wheat gluten allergic, has been increasingly reported at a rapid rate around the world (2–4). Many researchers suggested rice flour as gluten-free flour for bread making, but the gluten-free rice bread still exhibited some poor bread characteristics consisting of dense structure, hard texture, and quick retrogradation (5–7). It could be resulted a difficult flake-shredding and hard texture after frying of gluten-free rice breeding. Therefore, the textural properties of gluten-free rice bread should be improved by modifying of rice components, particularly starch and protein.

Germination process has been widely reported to induce chemical changes of rice due to a complex biochemical process of starch, protein and other components being considerably activated, particularly during rice paddy growth at 0.5–7 mm of embryonic growth length (EGL), which is called pre-germination process (8, 9). Pre-germinated brown rice flour (PGBRF) from 24–48 h of germination, which were substituted in the

composited wheat-rice bread formula, were reported to reduce hardness of bread after baking and to reduce staling rate after storage (10). In addition, the significant increasing total phenol content of waxy RD6 rice cultivar from 413.24 μmol gallic acid eq./100 g dw to 579.59 μmol gallic acid eq./100 g dw were found after 96 h of paddy germination, which could be further applied as healthy food ingredients to obtain a higher antioxidative activity of rice-based products (11).

However, the effects of PGBRF substitution in gluten-free rice bread/breading formula on their structural and antioxidative properties are not well understood. Therefore, the objectives of this study were to investigate the effect of PGBRF substitution on the structural, antioxidative properties, and oil absorption of rice breeding (RB) compared to those of wheat breeding (WB) before and after applying as coating material of fried frozen croquette.

Materials and Methods

Materials

Two matured paddy Thai rice (*Oryza sativa* L.) cultivars—Pathum-Thani1 (PTT1; low amylose rice, 15% amylose); and Phitsanulok2 (PNL2; high amylose rice, 27% amylose)—were used after 2 mo of dormancy and then stored at -18°C . Commercial wheat flour (WF; White Swan Bread Flour, United Flour Mill Public Co., Ltd., Thailand) and all ingredients of breeding formula

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were obtained from a local market.

Preparation of composited rice flour (RF) and pre-germinated brown rice flour (PGBRF)

Rice paddy of PTT1 and PNL2 cultivars were dehusked, polished, dry-milled, and sieved through a 100-mesh sieve to obtain dry-milled white rice flour of PTT1 (RF-PTT1) and dry-milled white rice flour of PNL2 (RF-PNL2). RF-PTT1 and RF-PNL2 were composited at the ratio of 1:1 to obtain the composited rice flour (RF).

Pre-germinated paddy (PGP) of two Thai rice cultivars were prepared as described in our previous work (8). The total pre-germination time of PTT1 and PNL2 cultivars were 34 and 52 h, respectively. PGP samples were dehusked, dry-milled, and sieved through a 100-mesh sieve to obtain dry-milled pre-germinated brown rice flour of PTT1 (PGBRF-PTT1) and dry-milled pre-germinated brown rice flour of PNL2 (PGBRF-PNL2). PGBRF-PTT1 and PGBRF-PNL2 were composited at the ratio of 1:1 to obtain the composited pre-germinated brown rice flour (PGBRF).

Preparation of bread, breading and fried frozen croquette samples

Rice bread samples were prepared by using mixture of RF and PGBRF at the ratio of 100:0, 75:25, and 50:50 before shredding to obtain rice breading containing PGBRF at 0% (RB-0%), 25% (RB-25%), and 50% (RB-50%), respectively. Dried ingredients of bread formula consisting of flour (100% baker), sugar (2%), baking powder (2%), and salt (1%) were mixed by hand. The mixture of dried ingredients was added with an activated yeast suspension, which was a mixture of instant dried yeast (3%) and water (160% for dry matter of all RF samples and 80% for dry matter of WF) with a continue mixing by an automatic home bakery machine (KitchenAid) using mixed paddle for 1 min at 100 rpm and then a rice bran oil with 8,000 ppm oryzanol (3%) was added with a continue mixing at 200 rpm 4 min for rice batter and 20 min for wheat dough. The mixture of all ingredients was fermented at 35°C with 85%RH for 45 min before baked at 185°C with 1 L of steamed water for 25 min. After that, the bread samples were cooled and dipped in liquid nitrogen before shredded breading samples. The moisture content of breading sample was adjusted to $29 \pm 2\%$ for semi-dry breading using a hot air oven (35°C for 30 min).

Fried frozen croquette coated by RB (C-RB) and fried frozen croquette coated by WB (C-WB) samples were prepared by coating their breading samples on round mashed potato (8 g, diameter=3.8 cm and thickness=0.5 cm), dipping in liquid nitrogen, and stored at -18°C for a week before deep-frying at 180°C for 5 min.

Analysis of bread, breading and fried frozen croquette samples

Characteristic of air cell (at 100×) and its cell wall (at 500×) in bread samples were observed using scanning electron microscope (SEM: Tabletop Microscope; TM3030Plus, Hitachi, Japan).

After shredded breading samples, the bulk density of breading was determined according to the method described previously (15). The particle size distribution

Fig. 1. Morphological properties of air cell (at 100×, left) and its cell wall (at 500×, right) in rice bread samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (A), 25% (B), and 50% (C) compared to that of wheat bread (D) observed by scanning electron microscope (SEM).

(%) of breading was also determined according to the method described previously (15) with slight modification. Briefly, the particle size distribution of breading was classified into three proportion groups of breading sizes, i.e., coarse breading (larger than 850 μm), medium breading (between 250 μm and 850 μm), and fine breading (smaller than 250 μm) by using sieve no. 20 mesh (850 μm) and sieve no. 60 mesh (250 μm). The pick-up capacity of breading was determined the weight of adhered breading, which coated on round mashed potato. It was calculated as the percent of pick-up capacity based on weight of adhered breading per weight of cylinder mashed potato. Tocopherol derivatives in breading samples were determined using a reverse phase high performance liquid chromatography (RP-HPLC) as described previously (16). Total phenol content (TPC) of breading samples were determined, following a modified Folin-Ciocalteu reagent method as described previously (17) and calculated using Gallic acid in ethanol as standard. Radical-scavenging activity (RSA) of breading sample was determined by following a modified DPPH method and calculated using Trolox as standard (16). In addition, gluten content of breading samples were measured by determining the gliadin content using a monoclonal R5-antibody-based on sand-

wich enzyme-linked immunosorbent assay (ELISA), wheat/gluten (gliadin) ELISA Kit II (Morinaga Institute of Biological Science, Inc.; Yokohama-Shi, Japan) according to the procedure of assay.

In order to apply the breeding samples as coating material of croquette product, the appearances of frozen croquette samples before and after frying process were observed and recorded as photographs using a compact camera (Nikon Coolpix S9100, Nikon, Japan). Color values of fried frozen croquette samples were measured using a Mini scan XE (model 45/O-L by Hunter Association Laboratory, United States) according to the method described previously (15). Oil absorption of fried frozen croquette samples were determined according to the method described previously (18). Fried frozen croquette samples were drained to remove the oil by leaving them on a metal grid for 10 min, and then weighed (A). Fried croquette samples were then wrapped with oil absorbent towel, which was weighed (B), for 1 h to absorb the oil. After that, the cooking paper with absorbed oil was weighed (C). The oil absorption was calculated using the following equation;

$$\text{Oil absorption} = \left(\frac{C-B}{A} \right) \times 100$$

After removing absorbed oil from fried frozen croquette samples, the breeding part was separated from croquette samples, and then ground with a blender. Furthermore, the oil in ground fried breeding was continually extracted with a modified Soxtec apparatus of Approved Method 30–20 with petroleum ether (a range of boiling point on 35–60°C) (19).

Experimental design and statistical analysis

Complete randomize design (CRD) was applied to determine all properties of samples. All sample measurements were expressed as a mean $\pm$ standard deviation (SD). Data were analyzed using the SPSS 11.0 software (SPSS Inc., Chicago, IL, USA) for one-way ANOVA. The Tukey's multiple comparison test was used for detection the statistically significant difference among the investigated groups of samples with a 95% confidence interval.

Results and Discussion

Bread characteristics

SEM micrographs show the differences in morphological properties of all rice bread samples and wheat bread (Fig. 1). All bread samples contained continuous air cells with solid cell wall. Rice bread (Fig. 1A–C) contained smaller air cells with thicker cell wall than those of wheat bread (Fig. 1D). In particular, PGBRF substitution in rice bread formula affected cell wall characteristic. The cell wall of rice bread containing 25% PGBRF (Fig. 1B) and rice bread containing 50% PGBRF (Fig. 1C) showed the increasing of roughness with puff surface compared to rice bread without PGBRF (Fig. 1A). The morphology structures of wheat bread continually contained air cell with thin cell wall. The cell wall in wheat bread samples had rough surface with small puff surface. The increasing of puff cell wall RB with PGBRF was probably due to the partial degradation of rice starch molecules (9). This could be related to a lower

paste viscosity during rice batter proofing and also in the early stages of baking, which might have facilitated batter expansion. Additionally, the preserved batter elasticity and the increased paste stability during baking insured the structural integrity of the bread to be cell wall of air cell. In addition, the reduction of disulfide-linked macromolecular protein during pre-germination could also enhance the forming of rice bread structure (8). The existence of disulfide-linked protein polymer was surrounding starch granules (20). Disulfide-linked macromolecular proteins are natively present in rice endosperm or formed and strengthened during cooking, work as a barrier and restrict the heat-induced swelling of rice starch and thus the rheological and cooking properties of rice resulting in a barrier to form the structure of rice bread (13, 14, 21).

Breeding properties

Subsequently, breeding samples were prepared by shredding of bread samples. The physical properties as bulk density, particle size distribution, and pick-up capacity of rice breeding (RB) samples were evaluated and compared to those of wheat breeding (WB). They are shown in Table 1. Bulk density of WB was found to be 226.9 kg/m³, which was significantly ($p < 0.05$) lower than those of all RB samples at the range of 439.2–459.1 kg/m³. Normally, bulk density of breeding is known to range from 200 kg/m³ to 600 kg/m³ as more to less porosity (22). Our results showed that bulk densities of all breeding samples were within normal range of bulk density. In addition, coarse breeding with larger particles than 850 μm showed a main proportion of particle size for all breeding samples, which ranged from 77.6% to 84.4%. Fine breeding with smaller particles than 250 μm was hardly found ranging from 2.8% to 3.3%. Pick-up capacity of WB was found to be 45.00% which was not significantly ($p \geq 0.05$) different from those of RB (45.63–46.38%). When compared among RB samples, the substitution of PGBRF between 0% and 50% in rice bread formula did not affect bulk density and pick-up capacity of shredded RB, but it significantly affected increasing proportion of coarse breeding and decreasing proportion of medium breeding, particularly at 50% PGBRF substitution. This led to the change in proportions of particle size of RB-50% samples, which were close to the proportions of particle size in WB.

Antioxidative properties of all rice breeding (RB) and wheat breeding (WB) are shown in Table 2. The radical-scavenging activity (RSA) and total phenol content of RB was significantly higher than that of WB. Moreover, the RB-25% and RB-50% showed significant increase of both antioxidative properties. The highest RSA and total phenol content were found in RB-50% at 110.28 μmol Trolox eq./100 g dw and 155.30 μmol Gallic acid eq./100 g dw, respectively. 25% and 50% PGBRF substitution significantly increased the total phenol contents of RB at 5 times and 7 times, respectively. The contents of four tocopherol derivatives of RB were significantly increased when PGBRF substitutions in formula, especially alpha- and gamma-tocopherol. These results confirmed the antioxidative potential of RF particularly

Table 1. Bulk density, proportions of particle size, pick-up capacity of rice breeding (RB) samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (RB-0%), 25% (RB-25%), and 50% (RB-50%) and compared to that of wheat breeding (WB).

Breeding samples	Bulk density ¹ (kg/m ³)	Proportions of particle size ¹ (%)			Pick-up ¹ (%) ^{ns}
		<250 μ m	250–850 μ m	>850 μ m	
RB-0%	439.2 $\pm$ 14.1 ^a	3.1 $\pm$ 0.2 ^b	19.3 $\pm$ 0.6 ^a	77.6 $\pm$ 0.8 ^b	45.63 $\pm$ 3.47
RB-25%	447.6 $\pm$ 8.1 ^a	3.3 $\pm$ 0.3 ^a	15.0 $\pm$ 0.9 ^b	81.7 $\pm$ 1.8 ^a	46.13 $\pm$ 3.27
RB-50%	459.1 $\pm$ 18.3 ^a	3.1 $\pm$ 0.2 ^b	13.0 $\pm$ 1.1 ^c	83.9 $\pm$ 1.5 ^a	46.38 $\pm$ 2.93
WB	226.9 $\pm$ 4.9 ^b	2.8 $\pm$ 0.4 ^c	12.8 $\pm$ 0.7 ^c	84.4 $\pm$ 1.4 ^a	45.00 $\pm$ 4.97

¹ Results are expressed as mean $\pm$ SD ($n=6$). Means with different lowercase letters in the same column are significantly different ($p<0.05$). ^{ns} refers to not significantly different ($p\geq0.05$).

Table 2. Antioxidative properties of rice breeding (RB) samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (RB-0%), 25% (RB-25%), and 50% (RB-50%) and compared to that of wheat breeding (WB).

Breeding sample	Total Phenol Content ¹ (μ mol gallic acid eq./100 g dw)	Content of tocopherol derivatives ¹ (μ g/g dw)				Radical-scavenging activity ¹ (μ mol Trolox eq./100 g dw)
		Alpha	Beta	Gamma	Delta	
RB-0%	17.99 $\pm$ 0.67 ^c	0.07 $\pm$ 0.01 ^c	0.02 $\pm$ 0.00 ^c	1.78 $\pm$ 0.09 ^b	0.00 $\pm$ 0.00 ^b	27.67 $\pm$ 0.56 ^c
RB-25%	90.53 $\pm$ 0.63 ^b	0.76 $\pm$ 0.02 ^b	0.07 $\pm$ 0.01 ^b	1.88 $\pm$ 0.10 ^b	0.07 $\pm$ 0.01 ^a	69.56 $\pm$ 0.61 ^b
RB-50%	155.30 $\pm$ 0.76 ^a	1.39 $\pm$ 0.03 ^a	0.11 $\pm$ 0.01 ^a	2.57 $\pm$ 0.13 ^a	0.09 $\pm$ 0.01 ^a	110.28 $\pm$ 0.29 ^a
WB	12.40 $\pm$ 0.53 ^d	0.01 $\pm$ 0.00 ^d	0.02 $\pm$ 0.00 ^c	0.89 $\pm$ 0.16 ^c	0.00 $\pm$ 0.00 ^b	12.23 $\pm$ 0.23 ^d

¹ Results are expressed as mean $\pm$ SD ($n=3$). Means with different lowercase letters in the same column are significantly different ($p<0.05$).

PGBRF to be the functional ingredients as rancidity prevention for dried food as breeding and fried food as fried croquette and also prevent many oxidative stress-linked diseases, e.g., cancer, immunodeficiency, and inflammatory response.

In addition, the gluten contents of all RB samples were less than 2 mg/kg, while that of WB was 356,289 mg/kg (Table 3). It indicated that all RB samples can be claimed to be gluten-free RB followed by the regulation of the International Codex Alimentarius Commission standard, in which, the gluten content of gluten-free products have to be less than 20 mg/kg in total based on the food ready for consumption (23). Therefore, all RB samples are the alternative gluten-free products for Celiac patients, who are gluten allergic.

Fried frozen croquette characteristics

In order to apply the breeding samples as coating material of croquette product, the coating characteristics of all frozen croquette coated by RB (C-RB) samples were similar to that of frozen croquette coated by WB (C-WB) before and after frying processes as shown in Fig. 2. It was due to the fact that all breeding samples were semi-dry breeding, which contained suitable moisture around 30% for food coating. All C-RB samples and C-WB were fully covered and adhered to the surface of croquette substrate with less falling out after frozen process and frying process. In addition, the PGBRF substitu-

Table 3. Gluten content of rice breeding (RB) samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (RB-0%), 25% (RB-25%), and 50% (RB-50%) and compared to that of wheat breeding (WB).

Breeding sample	Gluten content (mg/kg)
RB-0%	<2
RB-25%	<2
RB-50%	<2
WB	356,289

tion in rice bread formula led to the increase in yellowness and redness of C-RB and the decrease in lightness, which was closely similar to that of C-WB (Table 4). The increasing yellowness of RB after PGBRF substitution was probably due to the PGBRF containing sugar, which was the product of partial degradation of starch during pre-germination (9).

Furthermore, oil absorption and oil content of fried frozen croquette samples are shown in Table 5. The oil absorption at 44.36% and oil content at 28.63% of C-WB were significantly ($p<0.05$) higher than those of all C-RB samples at the ranges of 14.32–22.98% for oil absorption and 10.58–14.56% for oil content.

Fig. 2. Appearance of frozen croquette coated by RB (C-RB) samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (C-RB-0%; A), 25% (C-RB-25%; B), and 50% (C-RB-50%; C) and compared to frozen croquette coated by WB (C-WB; D) before (left) and after (right) frying.

The 25–50% substitutions of PGBRF in RB formula significantly decreased both oil absorption and oil content of their C-RB-0%. It clearly indicated that using of RB samples, particularly RB-50%, as coating material of fried food is more health benefit than using of WB, because of lower oil absorption.

In conclusion, the increasing percent substitution with PGBRF up to 50% in rice bread formula led to improve the structural properties of rice bread and physical properties of RB, and it had also significant effects on increasing antioxidative properties of RB. The highest antioxidative activity of RB-50% was 4 times higher than the RB without PGBRF and 9 times higher than the WB. The gluten contents of all RB samples were less than 2 mg/kg, which were not considered an allergen to Celiac patients and could be labeled “gluten-free product”. Furthermore, RB-50% was also shown a good coating material of croquette product to provide the desired golden color after frying process. In addition, C-RB-50% also provided the health benefits as 3 times lower oil absorption than C-WB. Therefore, the RB-50% was not only a gluten-free RB for Celiac patients, but it was also a better breeding for health-conscious consumers considering the higher antioxidative properties to

Table 4. Color of fried frozen croquette coated by RB (C-RB) samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (C-RB-0%), 25% (C-RB-25%), and 50% (C-RB-50%) and compared to that of fried frozen croquette coated by WB (C-WB).

Croquette sample	Color parameter ¹		
	L*	(+) a*	(+) b*
C-RB-0%	60.54±2.04 ^a	6.57±0.32 ^b	22.61±1.29 ^d
C-RB-25%	54.93±1.97 ^b	13.92±0.24 ^a	25.98±0.96 ^c
C-RB-50%	49.92±1.57 ^c	13.95±0.71 ^a	32.75±1.08 ^a
C-WB	51.76±1.98 ^c	13.86±0.96 ^a	30.02±1.54 ^b

¹ Results are expressed as mean±SD (n=10). Means with different lowercase letters in the same column are significantly different ($p<0.05$).

L*=Lightness, (+) a*=redness, and (+) b*=yellowness.

Table 5. Oil absorption and oil content of fried frozen croquette coated by RB (C-RB) samples varying in substitution percentage of pre-germinated brown rice flour (PGBRF) at 0% (C-RB-0%), 25% (C-RB-25%), and 50% (C-RB-50%) and compared to that of fried frozen croquette coated by WB (C-WB).

Croquette sample	Oil absorption (%)	Oil content (%)
C-RB-0%	22.98±0.19 ^b	14.56±0.98 ^b
C-RB-25%	16.94±0.14 ^c	11.80±0.70 ^c
C-RB-50%	14.32±0.11 ^d	10.58±0.77 ^c
C-WB	44.36±0.52 ^a	28.63±0.38 ^a

¹ Results are expressed as mean±SD (n=9). Means with different lowercase letters in the same column are significantly different ($p<0.05$).

prevent some disease, which related to oxidative stress. In addition, the RB-50% was a better coating material of croquette product as lower oil absorption than WB.

Disclosure of State of COI

The authors declare that they have no conflicts of interest.

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Human and Animal Rights

This article does not contain any studies with human or animal subjects.

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