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Original Article

Fortification of iron in brownies with Sinlek brown rice flour

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Abstract

Sinlek, a Thai rice cultivar, has a high iron content with a low glycemic index. Advantageous properties of this rice make it a functional ingredient in food. The research objective was to develop a brownie with iron fortification by substituting Sinlek brown rice flour for wheat flour. Sinlek brown rice flour was mixed with wheat flour at 0-100% (w/w). The Sinlek brown rice flour had high protein (15.83%) and iron (12.96 mg/kg) contents, but low amylose content (11.97%). Water solubility index gradually decreased while water absorption index increased with the substitution level of Sinlek brown rice flour in flour mixes. Brownie with Sinlek brown rice flour and wheat flour at 75:25 ratio gave the highest sensory scores for all attributes. This product has a high nutritional value in its iron content. Therefore, Brownie with Sinlek brown rice flour is a recommended health product for consumers who need high-iron food.

Keywords: Sinlek brown rice, iron fortification, brownies, flour mixes

1. Introduction

Asian Rice (*Oryza sativa* L. spp. *indica*) is known as a staple food of Thai and Asian populations (Kupkan chanakul, Kadowaki, Kubota, & Naivikul, 2018). Therefore, development of rice cultivars with improved nutritional quality and high yield would benefit both farmers and consumers. In Thailand, the Rice Gene Discovery Unit, Rice Science Center at Kasetsart University developed a new crossbreed of rice between Khao Dok Mali 105 (KDML105) and Hom Nil rice, namely Sinlek rice. This new rice cultivar is a source of iron and antioxidants with high contents of these, but is low in amylose content and glycemic index (Chaiyakul, Sukkasem, & Natthapanpaisith, 2016). Iron (Fe) is a useful element, which plays an important role in the body via hemoglobin formation and oxygen transport, and in enzyme catalysis by acting as a cofactor (Abbaspour, Hurrell, & Kelishadi, 2014). Therefore, iron is essential for people of all ages, and consuming rice with high iron content helps increase the iron intake in daily meals. Furthermore, Sinlek rice has many further health benefits, as it has been reported to control blood glucose levels and to reduce total cholesterol levels and the risk of cardiovascular disease in type 2 diabetic patients (Chaiyakul *et al.*, 2016). Considering its health benefits, developing products containing Sinlek rice is an alternative way to address health concerns with food products. Partial or total substitution of brown rice flour in place of wheat flour in bakery products has been reported in several studies, motivated by nutritional values in health promotion and increasing the value of rice (Noorfarahzilah, Lee, Shari

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fudin, Mohd Fadzelly, & Hasmadi, 2014; On-nom, Nualkae kul, Chalermchaiwat, Nitithamyoung, & Murtaza, 2016). Thus, substituting wheat flour with Sinlek brown rice flour is an attractive candidate to develop new appearance and to improve nutritional quality of bakery products. Recent studies have reported that an effective substitution ratio of Sinlek brown rice flour to wheat flour is 40:60 in cookies (Somboon panyakul, Hudthagosol, Meekhruerod, Lasukhang, & Chua duangpuy, 2012), and 20:80 in biscuits (Wonglao, Sricham nong, Hudthagosol, Suttisansanee, & Somboonpanyakul, 2013). However, no prior report has characterized the weight-ratios of Sinlek brown rice flour to wheat flour in brownies. In terms of iron fortification, increasing the substitution by Sinlek brown rice flour to 50% (w/w) of wheat flour is of interest to obtain a high iron functional food product. Therefore, this study characterized the physico-chemical properties of Sinlek brown rice flour when substituted for wheat flour at 0-100%, and tested brownies with total or partial substitution of wheat flour with Sinlek brown rice flour. Characteristics of the iron-fortified brownies in regards to physical properties, sensory evaluation, and chemical composition are reported.

2. Materials and Methods

2.1 Raw materials

Sinlek rice was obtained from Rice Gene Discovery Unit, Rice Science Center, Kasetsart University, Kamphaeng Saen Campus. Wheat flour (All Purpose Flour Brand, United Flour Mill Public Co., Ltd.), baking powder (KCG Corporation), butter (Thai Dairy industry Co., Ltd.), sugar (Thai Roong Ruang Sugar Group), salt (Thai Refined Salt Co., Ltd.), cocoa powder, egg, and almond slices were obtained from a local market.

2.2 Preparation of Sinlek brown rice flour

Sinlek rice was dry milled and then sieved through 100 mesh screen to obtain Sinlek brown rice flour. The flour was kept dry in sealed polyethylene bags and stored at 4ºC until use. Chemical compositions of flours (moisture, protein, fat, ash, fiber and carbohydrate) were determined accordingly to the methods of Association of Official Analytical Chemists (AOAC, 2005). Iron content of the flour was determined with an Atomic Absorption Spectrophotometer (AAS) (N066-1298, USA) according to the method of Association of Official Analytical Chemists (AOAC, 2006).

2.3 Properties of Sinlek brown rice flour and wheat flour mixes

Sinlek brown rice flour and wheat flour were mixed at the ratios 0:100, 25:75, 50:50, 75:25 or 100:0 % (w/w). Each flour mix was blended and sieved through a 60 mesh screen to get a homogeneous flour mix. The flour mix was determined for amylose content by spectrophotometer (UV-1601, Shimadzu, Japan) at 620 nm according to the method of Juliano (1971). Color coordinates of the flours were determined using a Handy colorimeter (NR-3000, Nippon Denshoku, Japan) in the CIE color scale as *L* (lightness), *a* (redness) and *b* (yellowness). The total color differences (ΔE) were calculated as follows (Varavinit & Shobsngob, 2000): $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$ using the 100% wheat flour as reference.

Water solubility index (WSI) and water absorption index (WAI) of each flour mix were determined according to the method of Bryant, Kadan, Chamapagne, Vinyard, & Boykin (2001) with slight modifications as follows. The flour sample (2.5 g) was suspended in 30 ml distilled water and shaken at room temperature for 30 min. After that, the sample tube was centrifuged at 2,200 rpm for 10 min. The supernatant was removed with a pipette to a pre-weighed aluminum dish and dried at 105ºC until constant weight. The WSI is expressed as a percentage, representing the difference in weight of the aluminum dish relative to the original dry weight of flour. WAI was calculated by weighing the sediment after centrifugation and dividing it by the original dry weight of flour.

Pasting properties of each flour mix were analyzed using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Australia) according to the method of American Association of Cereal Chemists (AACC, 2000). The flour sample (3.0 g, 12% moisture basis) was transferred into an aluminum cup and mixed with 25 mL distilled water. For the temperature profile, the flour suspension was heated to 50ºC for 1 min, and then the temperature was raised from 50 to 95ºC within 3 min 48s. The temperature was held at 95ºC for 2 min 30s, cooled from 95 to 50ºC in 3 min 48s and held at 50ºC for 2 min. Pasting temperature, peak viscosity, trough, final viscosity, breakdown and setback from trough were determined from the pasting curve using Thermocline v.2.3 software.

2.4 Preparation of brownies

A control formulation for brownie was prepared according to Dech-Kunchorn (2013) with some modifications. The formulation, given in % weight relative to wheat flour, consisted of 100% wheat flour, 190% butter, 119% whole egg, 110% sugar, 24% cocoa powder, 0.9% baking powder and 0.7% salt. Five formulations of brownies were prepared, with the mixes of Sinlek brown rice flour and wheat flour at the ratios 0:100 (control), 25:75, 50:50, 75:25, or 100:0 % (w/w). The other ingredients were fixed in weight across all formulations. For brownie preparation, flour, baking powder and cocoa powder were sieved, then mixed with melted butter, sugar, salt and eggs. The mixture was stirred until a well combined batter was obtained. After that, the brownie batter was poured in a baking pan and spread evenly. The brownie batter or the batter with almond slices (24%) on top was baked in an oven at 180ºC for 30 min. Finally, the brownie was removed from the baking pan and cooled for 1 h before placing in plastic boxes at a room temperature. Three replications of each brownie formulation (treatment) were prepared.

2.5 Physical properties of brownies without almond slices

Color values of the top surfaces of brownies (without almond slices) were determined with a Handy colorimeter in CIE color scale and the total color difference (ΔE) was calculated from the formulation of brownie at 100% wheat flour as reference. For texture analysis, ten replications of each brownie were tested with a texture analyzer (TA-XT2i, Stable Micro System, UK). The sample was cut to 1 x 1 x 1 cm size and compressed with a 100 mm aluminum probe. The texture profile analysis was performed with a double-cycle program at a speed of 1 mm/sec to 50% original height, and waiting for 5s before starting the second compression (Matos, Sanz, & Rosell, 2014). Hardness, springiness, cohesiveness and chewiness were recorded from the texture profile analysis.

2.6 Sensory evaluation of brownies with almond slices

Sensory evaluation of the brownies prepared from five different flour mixes of Sinlek brown rice flour and wheat flour were conducted by 50 untrained panelists. The 9-point hedonic scale was used, where 9 = like extremely and 1 = dislike extremely. The five sensory attributes for brownies were color, flavor, softness, juiciness, and overall acceptance. All samples were prepared in one size and were presented on white dishes with random 3-digit code labels, and served with drinking water for mouth rinse between samples. The brownie samples for sensory evaluation were stored for one day at room temperature after baking.

2.7 Chemical composition analysis of brownies with almond slices

The brownie sample with the highest sensory scores was determined for chemical composition. Moisture, fat, protein and carbohydrate contents were analyzed according to the methods of AOAC (2005). Iron content was analyzed by AAS according to the method of AOAC (2006).

2.8 Statistical analysis

All experiments were performed in triplicates, and data from three samples were analyzed by one-way ANOVA. A completely randomized design (CRD) was used in preparations and property determinations of the flour mixes and brownies. A randomized complete block design (RCBD) was applied in the sensory evaluation. The SPSS software (Version 22; SPSS Inc.; Chicago, IL, USA) was used, and differences in means were subjected to Duncan's multiple range test with threshold level p=0.05.

3. Results and Discussion

3.1 Sinlek brown rice flour properties

Sinlek rice was milled using the dry milling method, in which rice grains are ground without water. The dry-milled rice flour had high protein, lipid and ash contents but low carbohydrate content, when compared with wet-milled rice flour (Leewatchararongjaroen & Anuntagool, 2016). Furthermore, antioxidants in rice grains can be lost in the wet milling process because a group of flavonoids and anthocyanins are water-soluble (Žilić, Kocadağli, Vančetović, & Gökmen, 2016). Therefore, dry milling was used in this study to obtain highly nutritional flour. After the milling, the chemical composition was determined, and the results show that Sinlek brown rice flour had 10.13±0.04% moisture, 15.83±0.26% protein, 1.86±0.16% fat, 1.46±0.04% ash, 1.52±0.23% fiber and 69.20±0.25% carbohydrate on dry basis. These results slightly differ from a previous report, which showed less protein (10.54%) but more fat (2.05%), ash (1.95%), fiber (2.28%), and carbohydrate (72.09%) (Wonglao *et al.*, 2013); this is probably because of rice source and the milling process used for the brown rice. However, Sinlek brown rice flour had considerably higher protein and lower carbohydrate than wheat flour (all purpose flour), in which these contents were 12.58-12.9% and 77.31-85.0% on dry basis (Islam, Taneya, Shams-Ud-Din, Syduzzaman, & Hoque, 2012; Itthivadhana pong & Sangnark, 2016). In this study Sinlek brown rice flour had an iron content of 12.96 mg/kg, which is below the 21-28 mg/kg in a previous report (Chaiyakul *et al.*, 2016). Generally, low iron content is found in common rice cultivars such as KDML105 and RD6 (<10 mg/kg). High iron contents have been found in some local rice cultivars such as CMU122 and CMU123 (> 16 mg/kg), which are from the highlands of Thailand (Prom-u-thai & Rerkasem, 2001).

3.2 Flour mix properties

The properties of flour mixes between Sinlek brown rice flour and wheat flour at the ratios 0:100, 25:75, 50:50, 75:25, or 100:0 % (w/w) are shown in Table 1. Sinlek brown rice flour at 100% had the lowest amylose content (11.97 ± 0.11%), whereas wheat flour had the highest content (20.24 ± 1.29%). The amylose content of dry-milled rice flour is significantly lower than that in wet-milled rice flour because of the purity of starch. Components such as soluble protein, sugars and non-starch-bound lipids get eliminated from wet-milled flour, resulting in higher amylose content than in dry-milled flour (Suksomboon & Naivikul, 2006). The amylose content plays an important role in starch digestion; low amylose rice is digested more easily than high amylose rice (Falade & Christopher, 2015).

The color coordinates lightness (L), redness (a) and yellowness (b) of the flour mixes are shown in Table 1. The flour mixes with high percentages of Sinlek brown rice flour displayed lower L values, but higher a and b values than the others. Regarding the total color difference (ΔE) to pure what flour, it increased with substitution level of Sinlek brown rice flour, as could be expected. Regarding the flour preparation, Sinlek brown rice flour, prepared by dry milling of brown rice, contains pericarp, seed coat and embryo. Therefore, the color of flours or products became darker with increasing Sinlek content, due to the pigments in the flour.

Water solubility index (WSI) and water absorption index (WAI) are important functional properties of flours in bakery product applications. The WSI and WAI of the flour mixes (Table 1) show that WSI gradually decreased, while WAI increased with substitution level of Sinlek brown rice flour. The decreased water solubility may be due to chemical composition having less soluble components. The trends of WSI and WAI in this study closely follow those observed for the substitution with black glutinous rice flour of wheat flour (Itthivadhanapong & Sangnark, 2016). The water absorption capacity of a flour depends on its chemical composition, especially protein and carbohydrate contents as these are hydrophilic (Falade & Christopher, 2015). Furthermore, amylopectin and amylose contents affect water absorption based on their structure, because water can be absorbed by amy-

Table 1. Properties of Sinlek brown rice flour substituted for wheat flour at different levels.

Flour mix (Sinlek:Wheat)	Amylose content (%)	Color coordinates				WSI (%)	WAI
		L	*a*	*b*	ΔE		
0:100	20.24 ± 1.29[a]	74.88 ± 0.22[a]	-0.12 ± 0.23[c]	4.76 ± 0.38[c]	-	5.24 ± 0.01[a]	1.83 ± 0.01[e]
25:75	18.11 ± 0.73[b]	74.26 ± 0.48[b]	0.44 ± 0.27[b]	4.74 ± 0.35[c]	0.99 ± 0.28[d]	4.82 ± 0.07[b]	1.92 ± 0.05[d]
50:50	15.15 ± 0.24[c]	73.00 ± 0.41[c]	0.76 ± 0.27[ab]	5.50 ± 0.37[b]	2.25 ± 0.35[c]	4.65 ± 0.12[b]	2.12 ± 0.01[c]
75:25	14.96 ± 0.90[c]	71.40 ± 0.39[d]	0.92 ± 0.33[a]	5.78 ± 0.41[b]	3.80 ± 0.35[b]	3.99 ± 0.02[c]	2.39 ± 0.03[b]
100:0	11.97 ± 0.11[d]	65.98 ± 0.67[e]	1.04 ± 0.27[a]	7.44 ± 0.11[a]	9.37 ± 0.66[a]	3.00 ± 0.17[d]	2.66 ± 0.01[a]

Notes: Mean ± SD in the same column with different superscripts are significantly different ($p \leq 0.05$).
WSI = Water solubility index
WAI = water absorption index

lopectin more abundantly than by amylose (Itthivadhanapong & Sangnark, 2016). Thus, high protein and low amylose content of Sinlek brown rice flour probably increased its water absorption. The changes in WSI and WAI by the substitution of Sinlek brown rice flour affected the texture of the eventual bakery product in grittiness and juiciness.

The pasting properties with Sinlek brown rice flour substitution for wheat flour at different levels are shown in Figure 1 and Table 2. The peak viscosity and breakdown decreased with Sinlek brown rice flour content, similarly as with black glutinous rice flour substitution (Itthivadhanapong & Sangnark, 2016). In contrast, final viscosity and setback tended to increase with Sinlek content. However, the pasting temperature slightly increased with Sinlek content, but there was no statistically significant difference among the ratios 0:100, 25:75, and 100:0 (p > 0.05). Thus, these results show that Sinlek brown rice flour had a high stability during heating and shear stress. This is probably because of amylose content and impurities (protein, lipid and non-starch polysaccharides) in Sinlek brown rice flour. Amylose content in flour mixes decreased with Sinlek brown rice flour content. The changes in pasting properties with substitution level of Sinlek brown rice flour probably resulted in increased temperature and time for baking and firmer products after baking. However, not only flour but also other ingredients such as fat (butter), sugar and egg can improve the texture in terms of softness and juiciness of brownies (Hui, 2006). The viscosity profile of Sinlek brown rice flour was close to moderate-swelling starches (Type B), which is normal to cereal starches. Viscosity patterns of starches can be classified into four types: high swelling starch (Type A), moderate-swelling starches (Type B), restricted swelling starch (Type C), and highly restricted starch (Type D) (Rafig, Jan, Singh, & Saxena, 2015).

3.3 Brownie properties

Physical properties of five formulations of brownies containing different amounts of Sinlek brown rice flour are shown in Table 3. The color parameters of brownies slightly differed in the L values, but the a values did not differ significantly (p > 0.05). However, the b values of brownies significantly decreased with level of Sinlek brown rice flour ($p \leq 0.05$), which is similar to the yellowness of Sinlek brown rice cookie (Somboonpanyakul, Hudthagosol, Meekhruerod, Lasukhang, & Chuaduangpuy, 2012). Sinlek brown rice flour decreased yellowness of the brownies due to the brown pigments in this flour. The yellow color parameter (b) represented the carotenoid level that was low in brown rice (Lam berts & Delcour, 2008). The total color difference (ΔE) from the formulation of brownie at the ratio of 0:100 (100% wheat flour) as reference slightly increased with substitution level of Sinlek brown rice flour, but the 50:50 flour mix did not significantly differ from the others (p > 0.05). This indicates that the flour mix ratio did not strongly affect the color intensity of brownies. The brownie from Sinlek brown rice flour had similar darkness to the brownie from wheat flour (control) because of the amount of cocoa powder in formulation and the non-enzymatic browning reactions (including Maillard reaction and caramelization) that were similar in all the brownies. Browning in bakery products depends on the ingredients, particularly amino acids, proteins, sugar and leaving agents; and on operating conditions such as temperature and water activity (Purlis, 2010). According to texture analysis, the substitution of Sinlek brown rice flour at the ratios 0:100, 25:75, or 50:50 did not significantly influence the texture of brownies, but the ratios 75:25 and 100:0 gave significantly decreased springiness, cohesiveness and chewiness of the brownies ($p \leq 0.05$). Regarding hardness, the substitution of Sinlek brown rice flour at the ratio 75:25 did not significantly differ from the ratio of 0:100, but the ratio 100:0 gave lower hardness of the brownie ($p \leq 0.05$) than the others. Wheat flour contains gluten protein, which is a mixture of glutenins and gliadins. Glutenins contribute to the elastic characteristic of gluten, while gliadins contribute to extensibility. Gluten structure is formed when water is added to flour and is mixed. Glutens are the main structure builders in the product, and they hold the air and give the product a chewy and elastic texture (Shewry, Halford, Belton, & Tatham, 2002). Therefore, the substitution of rice flour for wheat flour significantly decreased chewiness and elastic texture. The increase in Sinlek brown rice flour substitution level in brownie reduced hardness indicating that the products became softer due to the low amylose content in Sinlek brown rice flour (Lu *et al.*, 2013). Moreover, it can be observed that hardness, chewiness and adhesiveness decreased with Sinlek content. These results are similar to a previous study about the substitution of different rice flours in chiffon cakes (Ferng, Liou, Yeh, & Chen, 2016). In addition, the particle size distribution of rice flour also affected the textural properties. In the study of Kim & Shin (2014), the hardness and springiness of cupcakes decreased as the particle size decreased.

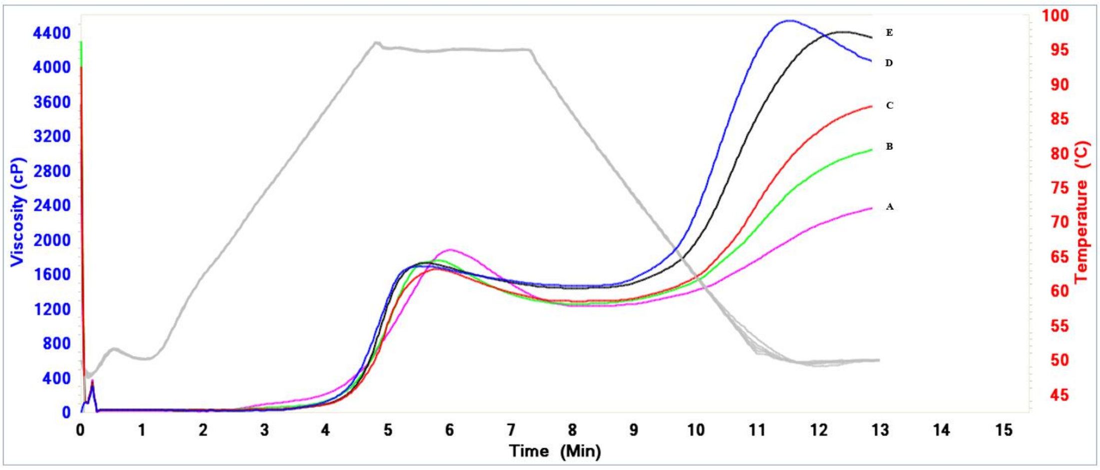

Figure 1. Pasting viscosities of flour mixes of Sinlek brown rice flour and wheat flour at the ratios 0:100 (A), 25:75 (B), 50:50 (C), 75:25 (D) and 100:0 (E) % (w/w).

Table 2. Pasting properties of Sinlek brown rice flour substituted for wheat flour at different levels.

Flour mix (Sinlek:Wheat)	Pasting temp. (ºC)	Peak viscosity (RVU)	Trough (RVU)	Final viscosity (RVU)	Breakdown (RVU)	Setback (RVU)
0:100	85.88 ± 0.45^{b}	1886.00 ± 25.51^{a}	1232.67 ± 18.01^{c}	2376.00 ± 14.23^{e}	653.33 ± 7.51^{a}	1143.33 ± 11.59^{e}
25:75	86.48 ± 0.81^{b}	1767.67 ± 31.53^{b}	1261.00 ± 32.19^{bc}	3059.67 ± 30.01^{d}	506.67 ± 2.52^{b}	1798.67 ± 62.07^{d}
50:50	88.37 ± 0.46^{a}	1667.33 ± 9.71^{c}	1305.00 ± 19.00^{b}	3627.33 ± 63.41^{c}	362.33 ± 20.60^{c}	2322.33 ± 64.67^{c}
75:25	87.88 ± 0.51^{a}	1751.33 ± 15.89^{b}	1473.00 ± 28.62^{a}	4322.33 ± 14.01^{a}	278.33 ± 12.74^{d}	2849.33 ± 22.01^{a}
100:0	86.23 ± 0.79^{b}	1710.67 ± 57.06^{bc}	1492.00 ± 55.38^{a}	3986.67 ± 24.19^{b}	218.67 ± 9.02^{e}	2494.67 ± 63.52^{b}

Notes: Mean ± SD in the same column with different superscripts are significantly different ($p \leq 0.05$).

Table 3. Physical properties of brownies with different contents of Sinlek brown rice flour.

Flour mix (Sinlek: Wheat)	Color				Texture			
	L	a^{ns}	*b*	ΔE	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
0:100	19.93 ± 1.59^{c}	2.87 ± 1.00	6.20 ± 2.52^{a}	-	615.76 ± 77.64^{ab}	0.97 ± 0.03^{a}	0.60 ± 0.03^{a}	358.92 ± 60.55^{a}
25:75	21.67 ± 0.76^{bc}	2.43 ± 0.55	4.87 ± 1.03^{ab}	2.45 ± 0.60^{b}	645.48 ± 122.57^{a}	0.94 ± 0.02^{a}	0.55 ± 0.02^{a}	332.67 ± 66.86^{a}
50:50	24.20 ± 1.11^{a}	3.27 ± 1.07	4.60 ± 0.95^{ab}	4.36 ± 1.40^{ab}	628.03 ± 101.39^{a}	0.93 ± 0.02^{a}	0.57 ± 0.03^{a}	331.23 ± 43.94^{a}
75:25	23.73 ± 0.74^{ab}	2.50 ± 2.00	3.23 ± 0.40^{bc}	5.15 ± 0.25^{a}	521.98 ± 74.52^{b}	0.77 ± 0.08^{b}	0.40 ± 0.06^{b}	164.30 ± 58.45^{b}
100:0	22.27 ± 1.96^{ac}	2.07 ± 0.40	1.60 ± 1.73^{c}	5.53 ± 1.40^{a}	311.64 ± 30.76^{c}	0.71 ± 0.05^{c}	0.38 ± 0.02^{b}	83.17 ± 9.68^{c}

Notes: Mean ± SD in the same column with different superscripts are significantly different ($p \leq 0.05$).
Mean ± SD in the same column with ns are not significantly different ($p > 0.05$).

Sensory characteristics of brownies in terms of color, softness, juiciness, flavor and overall acceptance are shown in Table 4. The brownies with Sinlek brown rice flour and wheat flour at the ratio 75:25 gave the highest sensory scores in all attributes, especially in softness and overall acceptance ($p \leq 0.05$). Brownies with Sinlek brown rice flour and wheat flour at the ratio of 0:100 (control), 25:75 or 100:0 had significantly lower softness than that with 75:25 ratio ($p \leq 0.05$). The hardness of brownies decreased with the Sinlek content (Table 3). Brownies with Sinlek brown rice flour and wheat flour at the ratio 75:25, or at 100:0, gave higher color scores ($p \leq 0.05$) than the others. Sinlek brown rice flour significantly affected the consumer acceptability of the color of the brownies. In juiciness and flavor, the brownie at the ratio 75:25 gave the highest scores, but without statistically significant difference to the ratio 50:50. However, these results indicate that Sinlek brown rice flour can be used to substitute for wheat flour in brownies at the ratio 75:25. The control brownies with 100% wheat flour gave the lowest overall acceptance score; this is probably due to the baking conditions. In this study, temperature and time for preparation of brownies were fixed controlled variables, but these may have affected the product characteristics.

3.4 Proximate analysis of brownies

Chemical compositions of the control brownie and that with substitution of Sinlek brown rice flour for wheat

Table 4. Sensory evaluation scores of brownies with different contents of Sinlek brown rice flour.

Flour mix (Sinlek:Wheat)	Color	Softness	Juiciness	Flavor	Overall acceptance
0:100	6.72 ± 1.51^b	5.58 ± 2.01^c	5.25 ± 1.77^c	6.19 ± 1.44^b	5.79 ± 1.91^d
25:75	7.02 ± 1.48^b	5.97 ± 1.69^{bc}	5.85 ± 1.68^{bc}	6.58 ± 1.09^b	6.25 ± 1.60^{cd}
50:50	7.02 ± 1.44^b	6.49 ± 1.79^b	6.15 ± 1.84^{ab}	6.86 ± 1.41^{ab}	6.78 ± 1.68^{bc}
75:25	8.05 ± 0.89^a	7.35 ± 1.46^a	6.85 ± 1.56^a	7.40 ± 1.09^a	7.70 ± 0.86^a
100:0	7.70 ± 1.38^a	5.70 ± 1.68^c	6.15 ± 1.90^b	6.65 ± 1.34^b	6.95 ± 1.09^b

Notes: Mean ± SD in the same column with different superscripts are significantly different ($p \leq 0.05$).

flour at the ratio 75:25 are shown in Table 5. Brownies with Sinlek brown rice had higher contents of protein and fat but lower content of moisture than the control ($p \leq 0.05$). There was no difference in carbohydrate contents of these brownies ($p > 0.05$). The carbohydrate content in brownies was similar to fat content because the similar amounts of carbohydrate (110% sugar and 100% flour) and fat (190% butter), which were the major ingredients in the brownie formulation. In addition, Sinlek brown rice brownie contained more iron (16.22 ± 0.11 mg/kg) than the wheat flour brownie (8.84 ± 0.23 mg/kg). In recent years, the application of flour mixes with flours, starches or other ingredients, in products as substitutes for wheat flour has been well studied, because consumers need healthy foods with high nutritional value and good appearance. In addition, some food products avoid wheat flour in order to be gluten-free products for consumers with gluten-intolerance. Rice is a locally grown raw material in Asian countries, and it can be processed into gluten-free flours to replace wheat flour. The substitution of brown rice flour for wheat flour, totally or partially, could be used in bakery products such as bread, biscuits/cookies, and pastry products (Noorfarahzilah *et al.*, 2014). Therefore, the substitution of Sinlek brown rice flour in brownies is an alternative choice to improve their nutritional quality, especially to enrich iron content in food.

Table 5. The chemical composition of wheat flour brownie (control) and 75% Sinlek brown rice flour brownie.

Chemical composition	Wheat flour brownie (0:100)	Sinlek brown rice flour brownie (75:25)
Moisture (%)	$14.73^a \pm 0.33$	$12.46^b \pm 0.40$
Protein (%)	$10.35^b \pm 0.21$	$10.77^a \pm 0.09$
Fat (%)	$33.79^b \pm 0.84$	$37.18^a \pm 1.11$
Carbohydrate^{ns} (%)	37.55 ± 0.90	36.57 ± 1.57
Iron (mg/kg)	$8.84^b \pm 0.23$	$16.22^a \pm 0.11$

Notes: Mean ± SD in the same row with different superscripts are significantly different ($p \leq 0.05$).
Mean ± SD in the same row with ns are not significantly different ($p > 0.05$).

4. Conclusions

From this study, it can be concluded that the dry-milled Sinlek brown rice flour is an excellent source of protein and iron, but is low in amylose content. The substitution of Sinlek brown rice flour for wheat flour increased water absorption and resistance to heating and shear stress during pasting. In brownies, Sinlek brown rice flour can be used as a substitute for wheat flour at up to 75%, producing higher scores in all sensory attributes and high nutritional quality, especially in iron content. This study indicates that Sinlek brown rice flour could be considered a nutritional ingredient in bakery products for the development of value-added products. However, the functional properties of flours, and physical and chemical modifications of starches, along with development of other food products, should be studied for further applications in various food industries in the future.

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