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Influence of waxy rice flour substitution for wheat flour on characteristics of batter and freeze-thawed cake

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ABSTRACT

This study aimed to improve the freeze-thawed cake properties by 10–20% waxy rice flour (WRF) substitution for wheat flour (WF). Viscosity of WRF-substituted batters was lower; consequently, trapped air was less uniformly distributed than WF batter. After five freeze-thaw cycles, firmness and enthalpy of melting retrograded amylopectin of WF- and WRF-substituted cakes increased and the matrix surrounding the air pores from SEM images was denser than in fresh-baked cakes. Sensory evaluation showed an increase in firmness and a decrease in firmness acceptability of freeze-thawed cakes. However, freeze-thawed cake with WRF substitution had significantly less firmness, less dense matrix and more acceptability than WF cake. This could have been due to a low amylose content of WRF and the spread of ruptured waxy rice starch granules around swollen wheat starch granules as observed by CLSM. Thus, WRF could be used for WF substitution to improve the firmness in freeze-thawed cake.

1. Introduction

Cake is a sweet baked dessert that is consumed worldwide by all ages in large quantities. Good cake quality can be described as high volume, low crumb firmness, uniform crumb structure, staling tolerance, and long shelf-life (Gélinas, Roy, & Guillet, 1999; Karaoğlu & Kotancilar, 2009) depending on the ingredients and formulas, the aeration and stability of batters, and the baking and thermal setting stages (Gómez, Ronda, Caballero, Blanco, & Rosell, 2007). Normally, the shelf-life of cake is between one and four weeks (Karaoğlu, Kotancilar, & Gerçekaslan, 2008) and then the quality is mainly downgraded due to the staling process and also microbial spoilage. Staling results from changes in the physical and chemical properties of the product; importantly starch retrogradation, moisture migration from crumbs to drier parts of the cake, increased firmness of the cake, and loss of aroma and flavor (Guy, 1983; He & Hosene, 1990). An extension of the cake shelf-life period could be achieved by changing the formulation, the incorporation of some additives such as a hydrocolloid, modification of packaging conditions, and frozen storage (Gómez, Ruiz, & Oliete, 2011).

Freezing with subsequent frozen storage is one of the efficient methods applied for slowing down the staling process and inhibiting microbial growth; thus, the shelf-life of bakery products can be extended. However, this process could deteriorate final products (Cauvain, 1998). The freezing process led to an increase in batter density and viscosity which reduced cake height and volume and increased hardness of cake crumb (Gómez et al., 2011). After reheating in an oven, frozen cake had significantly lower values of volume, palatability, and desirability than fresh baked cake (Owen & Duyne, 1949). While, frozen baked cakes after thawing had a better quality and larger volume, compared to those baked from frozen batters (Skarha & Duyne, 1955). It has been aptly demonstrated that temperature fluctuations during storage and transportation cause changes in the structure of ice crystals and recrystallization (Phimolsiripol, Siripatrawan, Tulyathan, & Cleland, 2008) which could deteriorate the quality and shorten the shelf-life of a frozen product (Blond & Meste, 2004). Moreover, the refreezing-thawing process caused by temperature fluctuations may produce defects in the texture of frozen products making them unacceptable to the consumers. However, there has been no research focus on the effect of freeze-thaw cycles on the quality of frozen cakes.

The effect of alternative flour composition on staling retardation has been studied by the addition or substitution of substances for wheat flour in bakery products. Generally, the addition or substitution of waxy wheat flour lowered the loaf volume, but maintained the moistness and softness of bread (Bhattacharya, Erazo-Castrejón, Doehlert, & McMullen, 2002; Hayakawa, Tanaka, Nakamura, Endo, & Hoshino, 2004) and cake (Hayakawa et al., 2004) after storage at room or refrigerated temperature for several days. However, an excessive addition or substitution (>30% waxy wheat flour of the total wheat flour) resulted in less acceptable properties of the products, such as stickiness, lumpiness and less crispy texture (Hayakawa et al., 2004). Furthermore, Seyhun, Sumnu, and Sahin (2005) found that 5% substitution of maize, waxy maize, potato, and pregelatinized potato starches for cake flour reduced the moisture loss during baking leading to less firmness, compared to wheat cake

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during 5-day storage at 20 °C. However, the quality improvement of freeze-thawed cake by using WRF has not been studied yet.

WRF contains little or no amylose (<3%) according to Tester and Morrison (1990). A paste of WRF had a higher viscosity and lower retrogradation rate than that of non-waxy rice flour (Luh, 1999). Charoenrein and Preechathammawong (2012) found that WF reduced the syneresis and formation of the spongy structure in freeze-thawed rice starch gel resulting in the retardation of textural changes. Therefore, WRF could be used for preserving the quality of frozen starch-based food.

In this study, we investigated the possibility of WRF substitution for WF to retard staling of freeze-thawed cakes as well as studying the causes of the effects. Confocal laser scanning microscopy (CLSM), which is a powerful technique used for observing the characteristics and location of starch granules in a paste or gel (Charoenrein & Preechathammawong, 2012; Hongsprabhas, Israkarn, & Rattanawattanaprakit, 2007), was used to observe the microstructure of swollen starch granules in paste containing WF and WRF. Furthermore, differential scanning calorimetry (DSC) was used to measure the thermal properties of fresh cake (Ji, Zhu, Qian, & Zhou, 2007) and freeze-thawed cake. To date, the application of these two techniques to study the properties of freeze-thawed cakes has not been reported in the literature.

The objectives of this study were to determine the influence of WRF substitution for WF on the viscosity, density, and microstructure of batter, and the moisture content, firmness, microstructure, and enthalpy of melting retrograded amylopectin of fresh and freeze-thawed cake. Moreover, sensory evaluation of both intensity and preference tests of cake firmness was also evaluated.

2. Materials and methods

2.1. Materials

Ingredients for cake making were cake-specific wheat flour (WF) (United Flour Milled Public Co., Ltd, Samutprakarn, Thailand), waxy rice flour (WRF) (Thai Wah Food Public Co., Ltd, Bangkok, Thailand), sugar, fresh whole eggs, unsalted butter, pasteurized milk, emulsifier (SP®), and salt. The moisture contents of the WF and WRF were 12.20% and 12.14%, respectively.

2.2. Pasting profile of wheat flour and waxy rice flour

Suspensions of 8% (w/w) flour (WF, WRF, and 10%, 15%, and 20% (w/w) WRF substitution for WF) were prepared in distilled water and analyzed by a Rapid Visco Analyzer (RVA, model RVA3D, Newport Scientific Instrument & Engineering, NSW, Australia). The slurry was held at 50 °C for 1 min, heated to 95 °C with a constant rate of 60 rpm and then held at 95 °C for 2.50 min. After that, it was cooled to 50 °C and held at 50 °C for 2 min. The data were reported as the average of duplicate measurements.

2.3. Microstructure of wheat flour and waxy rice flour before and after freezing

Suspensions of 1% (w/w) flour were prepared in distilled water and allowed to stand at room temperature (27 °C) for 10 min. The sample was heated in a water bath at 75 °C for 30 min and was cooled down at room temperature for 1 min before staining with Rhodamine B solution (0.2%, w/w). After incubation for 10 min, each sample was loaded onto a slide and a cover slide was applied. Each sample was observed by CLSM (Axio Imager MI, Carl Zeiss Pte Ltd, Germany) with the LSM 5 PASCAL program. An HeNe laser was used to excite the Rhodamine B at 543 nm.

2.4. Batter and cake preparation

The formula for cake preparation was 200 g WF, 200 g sugar, 360 g fresh whole eggs, 70 g water, 40 g butter, 30 g milk, 4 g emulsifier (SP®), and 3 g salt. The batter was prepared by mixing using a high speed blender (HW-BDC2L, House worth, China). At first all ingredients, except the WF, were mixed at a maximum speed for 30 s. Then, 200 g WF was added and mixed together at minimum speed for 10 s. Then, the mixture was filled into a Cream Profi Whip 1730 (ISI GmbH, Vienna, Austria) and shaken well. Next, 150 g of batter was placed into a butter coated pan (diameter 125 mm and depth 60 mm) and baked in an electric oven CP-N (Chung Pu Paking Machinery, Taiwan) at 190 ± 2 °C for 30 min. After baking, cake was removed from the pan and cooled upside down at 25 ± 2 °C for 1 h. Then, it was kept in a sealed nylon bag.

In order to study the effects of WRF substitution for WF, the basic control cake formulation was used, but the amount of WF was substituted by WRF at 10%, 15%, and 20% (w/w).

The WF and WRF flour were blended together before mixing with other ingredients.

2.5. Freezing and thawing

Cake in a sealed nylon bag was frozen in a cryogenic cabinet freezer (Minibatch 1000L; Bangkok Industrial Gas Co., Bangkok, Thailand) with −40 °C atmospheric temperature until the central temperature of the cake reached −25 °C. The frozen cakes were stored in a chest freezer (Sanyo refrigerator model SF-C1497, Sanyo Electric Co., Ltd, Osaka, Japan) at −18 °C for 20 h and thawed at room temperature (25 ± 2 °C) for 4 h until the central temperature of the cake reached 25 °C. This process was continued for 4-more cycles. After each cycle, the samples were taken for further analysis.

2.6. Batter analysis

Immediately after mixing, the density of each batter sample was determined in triplicate as the ratio of the batter weight to the water weight filled in a standard container.

The viscosity of the batter was measured five times by using an HBDVII+ viscometer (Brookfield Eng Labs Inc., MA, USA) with spindle-21 resolved at 100 rpm (adapted from a method of Gómez et al. (2011)).

The microstructure of the batter was observed under a light microscope DME (Leica, NY, USA) coupled with a color video camera SSC-DC318P (Sony, Japan) at 40× magnification. Each sample was prepared by placing a drop of freshly mixed batter on a microscope slide and applying a cover slip.

2.7. Cake analysis

Cakes were weighted and photographed after 1 h after removal from the pan. All quality measurements were performed within 4 h after baking.

Baking loss after baking was measured in triplicate and calculated as follows:

$$\%\,\text{Baking loss} = \frac{(\text{Weight of batter before baking} - \text{Weight of cake after baking})}{\text{Weight of batter before baking}} \times 100$$

Moisture content was determined in triplicate according to AACC Approved Methods 44–15A (AACC, 1995).

2.8. Texture analysis

For texture determination, the crust was removed and the central crumb section was sliced into four 25 mm × 25 mm × 20 mm samples for measurement. The firmness of each sample was determined using a texture analyzer (TA-XT plus, Stable Micro System, Surrey, UK) equipped with a 100 mm probe (P100) according to the texture profile analysis (TPA) procedure (five replicates per treatment). The conditions of TPA were: pre-test speed, 1 mm s^{-1}; test speed, 2 mm s^{-1}; post-test speed, 1 mm s^{-1}; distance, 8 mm; trigger type, auto 20 g, and time, 2 s. The firmness was defined as the peak force during the first compression cycle.

2.9. Sensory analysis

The cake samples were prepared using the same method and the same size as for texture analysis. Then, they were evaluated for the intensity and acceptability of crumb firmness. The intensity test was evaluated by 10 trained panelists using a 9-point scale (1 = less firmness and 9 = most firmness). The preference test was conducted by 30 untrained panelists using a hedonic 9-point scale (9 = like extremely and 1 = dislike extremely).

2.10. Determination of the microstructure of fresh and freeze-thawed cakes by scanning electron microscopy (SEM)

The fresh-baked and frozen cakes with and without WRF substitutions were cut into samples (3 mm × 3 mm × 3 mm). For fixing, the samples were incubated in 2% glutaraldehyde in 0.5 M phosphate buffer solution for 24 h at room temperature. Samples were rinsed three times with a phosphate buffer solution. Next, they were dehydrated in 30%, 40%, 50%, 60%, 70%, 80%, and 90% ethanol for 12 h at each concentration followed by three times with absolute ethanol for 24 h. After that, the samples were dried using a critical point dryer. They were mounted on a stub coated with gold and observed using a microscope (JSM-5600LV, JEOL Ltd., England). The magnification and accelerating voltage are showed on each SEM image.

2.11. Retrogradation properties of freeze-thawed cakes

The retrogradation properties of freeze-thawed cakes were analyzed using DSC (Pyris-1, Perkin Elmer, CT, USA) with the modified method of Yu, Ma, and Sun (2010). Fresh and thawed cakes were cut into samples (2 mm × 2 mm) and soaked in 50%, 70%, 90%, and 99% ethanol for 12 h at each concentration. After that, the samples were separated from the solution using a Büchner funnel and dried at 37 °C in a hot air oven (ULE500, Memmert, Frankfurt, Germany). The dried cake sample was blended using a blender (Moulinex-Y46, Paris, France) and passed through a 100-mesh sieve screen. The duplicate samples (6 mg dry basis) were mixed with distilled water (1:2, w/w) in a hermetically sealed stainless steel pan and allowed to stand at room temperature for 24 h. The samples were held isothermally at 25 °C for 1 min before heating to 140 °C at a rate of 10 °C/min.

2.12. Experimental design and statistical analysis

Each experiment was repeated twice. Batter and cake characteristics and result from sensory evaluation were analyzed using analysis of variance followed by Duncan's test ($p \leq 0.05$) using the SPSS software program (version 17.0, SPSS Inc, IL, USA).

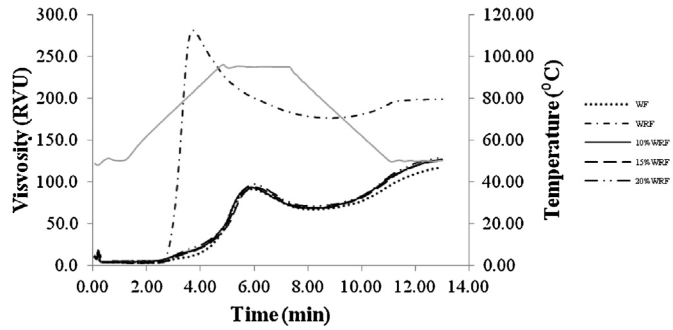

Fig. 1. Pasting profile of wheat flour, waxy rice flour and waxy rice flour substitutions for wheat flour (WF, wheat flour; WRF, waxy rice flour; 10%, 15%, and 20% WRF are 10%, 15%, and 20% of waxy rice flour substitution, respectively).

3. Results and discussion

3.1. Pasting profile of wheat flour and waxy rice flour

The pasting properties of WF, WRF and WF blended with WRF at 8%, w/w was shown in Fig. 1. WRF had the highest peak viscosity while WF had the lowest peak viscosity. The difference in paste viscosity depended on the flour composition and starch gelatinization. The higher peak viscosity during heating indicated the higher ability of the starch granules to swell before rupture (Leach, 1965). Therefore, the starch granules of wheat were more resistant to swelling than those of waxy rice. Although the gelatinization temperatures of wheat and waxy rice starches were quite similar, around 65–70 °C depending on the variety (Chaiwanichsiri, Ohnishi, Suzuki, Takai, & Miyawaki, 2001; Tester & Morrison, 1990). For WF, the pasting temperature was higher than for WRF (Chaiwanichsiri et al., 2001). The crystallinity of wheat starch was 35.5% (Morrison, Tester, & Gidley, 1993) which was higher than those of waxy rice starch 18.5–23.8% (Lu, Wung, Zhao, & Lu, 2009). Moreover, WRF had lower amylose content, which associates with the strength of the micellar network in starch granules, than WF leading to higher swelling power of WRF than WF (Wootton & Tumaalii, 1984). As a result, penetration of water into wheat starch granules was more difficult than into waxy rice starch granules, consequently, lower pasting temperature of WRF than WF. It is possible that wheat granules were not broken at the peak time, and subsequently, the viscosity of the wheat paste dropped because the RVA was running in the cooling mode. The higher level of WRF substitution for WF tended to increase the peak viscosity, breakdown, and final viscosity, which was in agreement with Charoenrein and Preechathammawong (2012).

3.2. Microstructure of wheat flour and waxy rice flour before and after heating

In order to investigate the distribution of swollen starch granules of wheat and waxy rice, unheated and heated suspensions (1%, w/w) of WF, WRF, and 10% WRF were stained with Rhodamine B and observed using CLSM. The unheated wheat starch granules were disk-shaped and spherical (Fig. 2a) whereas the waxy rice granules were polygonal (Fig. 2b). They were clearly distinguishable from each other in a suspension containing both WR and WRF (Fig. 2c). After heating at 75 °C for 30 min, the deformation of starch granules was observed. The wheat granules were swollen (Fig. 2d) while the waxy rice granules were deformed and dispersed with a greater circumference (Fig. 2e). In a suspension containing 10% WRF substituted for WF (Fig. 2f), the spread of rupture of swollen waxy rice starch granules around swollen wheat starch granules was clearly seen leading to separation of the WF granules. As a result of this, we

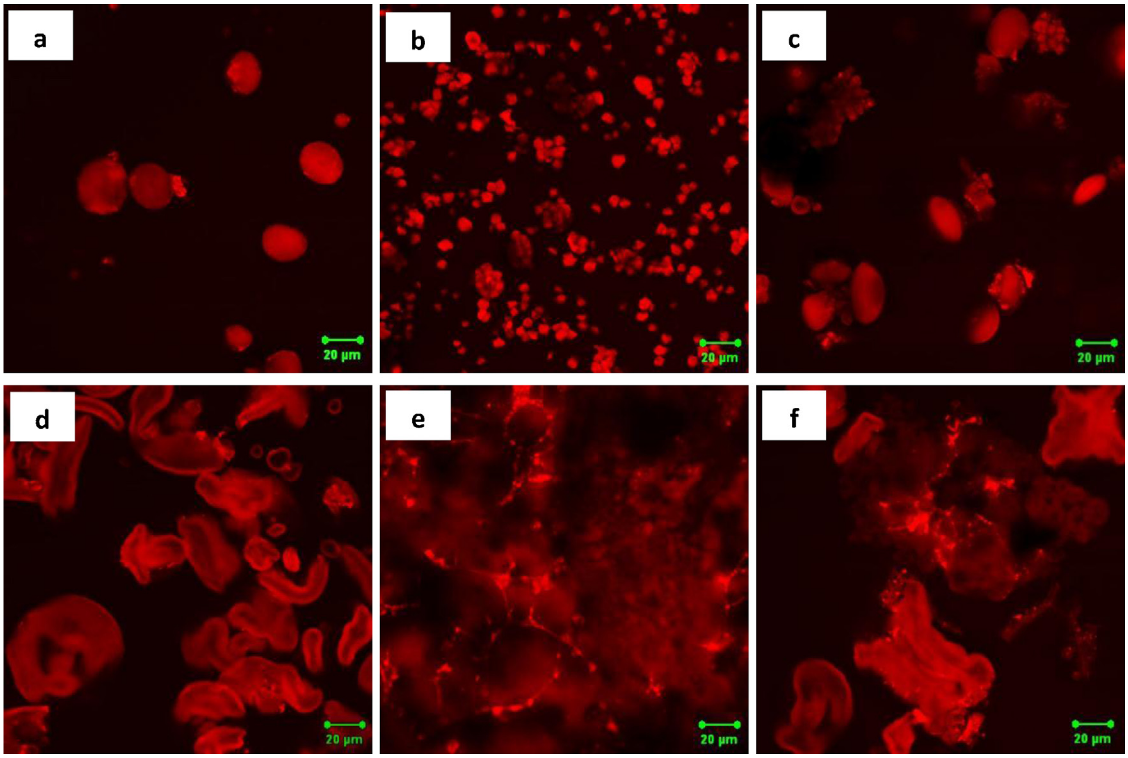

Fig. 2. Confocal laser scanning micrographs of unheated (a–c) and heated (d–f) flour solutions (8%, w/w). Starch granules of wheat (a and d), waxy rice (b and e) and 10% waxy rice flour substitution (c and f) were stained with Rhodamine B (appear as red).

hypothesize that WRF substitution could reduce the starch retrogradation of WF and substitution of WRF in WF cake could improve the firmness in freeze-thawed cake.

3.3. Batter characteristics

3.3.1. Viscosity and density of batter

The viscosity of batter was found to decrease with increasing WRF substitution (Table 1). However, there was no significant ($p > 0.05$) difference in viscosity between batter substitution with 10%, 15%, or 20% WRF. This could have been due to the fact that substitution of WRF for WF decreased the wheat gluten, which was the main cause of viscosity development in the batter during mixing (Loewe, 1993) which was in line with the results of Wilderjans, Pareyt, Goesaert, Brijs, & Delcour (2008) who reported that the batter viscosity decreased with lower gluten content. A higher batter viscosity prevented the loss of air and increased batter stability. Batter density is related to the amount of incorporated air in the batter; thus, batter with a higher viscosity should have a lower density. However, there was no significant ($p > 0.05$) difference in density between the control batter and those samples with WRF substitution. This might have been because the viscosity of each batter did not differ enough to making an appreciable difference in density.

3.3.2. Microstructure of batter

The microstructure of the cake batter samples with different levels of WRF substitution for WF was evaluated under a light microscope. Fig. 3 shows that there is more uniform distribution of air bubbles in the batter prepared with WF, while the batter substituted with WRF shows a larger variation in bubble size distribution which could have been a result of the lower viscosity of the batter substituted with WRF. Low viscosity of batter could lead to the coalescence of bubbles into a larger bubble (Irene, Geana, Gros, & Trystram, 2006); thus, larger bubbles were exhibited in batter substituted with WRF.

3.4. Cake properties

For the properties of the unfrozen cake samples, any baking loss (Table 1) between the control and the fresh-baked cake substituted with WRF was not significant ($p > 0.05$). Fresh-baked cake samples made from 15% and 20% WRF substitution had a dense gummy layer at the bottom (Fig. 3c and d) and their heights were significantly ($p \leq 0.05$) less than those of WF cake (Table 1). This could have been the result of the diluted gluten content which serves as a binder in the cake (Donelson & Wilson, 1960). Another reason could have been due to the lower resistance to swelling of the waxy rice granules than those of wheat as mentioned in previous discussion of the

Table 1
Characteristics of cake batter and fresh-baked cake.

% Substitution of WRF for WF	Batter		Cake	
	Viscosity (Pa s)	Density (g/cm^3)	Baking loss (%)	Height (mm)
0	6.07 ± 0.48^b	0.58 ± 0.01^a	17.70 ± 0.03^a	35.5 ± 0.03^b
10	4.70 ± 0.26^a	0.55 ± 0.01^a	17.22 ± 0.57^a	34.8 ± 0.21^b
15	4.82 ± 0.42^a	0.55 ± 0.02^a	17.93 ± 0.25^a	28.5 ± 0.31^a
20	4.13 ± 0.30^a	0.54 ± 0.02^a	17.31 ± 0.20^a	24.6 ± 0.19^a

Mean values in each column with different superscript letters are significantly ($p \leq 0.05$) different.

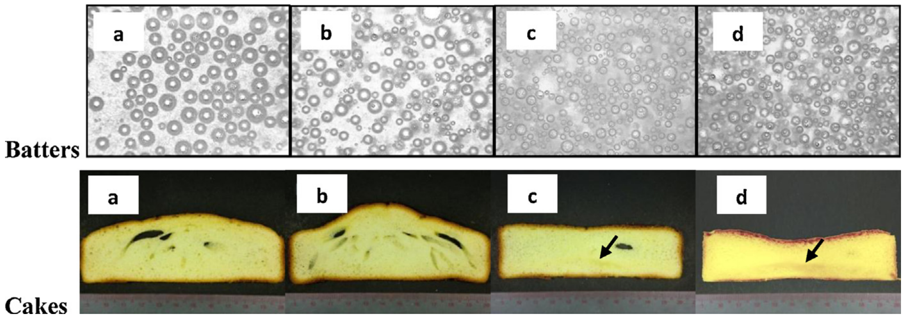

Fig. 3. Light micrographs (40×) of aerated cake batter and images of cakes prepared with wheat flour (WF) (a) and 10% (b), 15% (c) and 20% (d) waxy rice flour (WRF) substitution. Arrows indicate the dense gummy layer of the cake crumbs.

pasting profile; hence batter with 15% and 20% WRF substitution might require more water for swelling and gelatinization, and the subsequent development of a fully expanded structure. However, an identical amount of water was added to all samples; therefore, batter with 15% and 20% WRF substitution might have less water available for use in producing fully extended cake. In addition, the collapse of waxy rice granules during heating (Fig. 2e and f) could be a result in lower height of cake substituted with with15% and 20% WRF. Furthermore, the cake samples with 20% WRF substitution collapsed upon cooling which was consistent with the results of Miller, Trimbo, and Sandstedt (1967) and Wilderjans et al. (2008) that cake with low gluten exhibited a gummy layer and collapse during cooling. Miller et al. (1967) reported that a gummy layer developed first at the bottom followed by the formation of a band of air cells along the top of the batter which resulted in a weak structure and collapse. However, cake with 10% WRF substitution did not collapse after cooling (Fig. 3b) and its height was not different ($p > 0.5$) from WF cake (Table 1) which showed that it contained sufficient of WF gluten.

3.5. Effect of WRF substitution on freeze-thawed cake

In the study on the effect of the freezing and thawing process, cake substituted with 20% WRF was eliminated from consideration because of its obvious collapsed structure.

3.5.1. Moisture content of cake

The moisture content of freeze-thawed cake decreased significantly ($p \leq 0.05$) after the third freeze-thaw cycle (Table 2). This was caused by the loss of moisture during storage (Cauvain, 1998) and thawing. Furthermore, the freezing resulted in ice formation; as a result of this the concentration of unfrozen phase was increased which led to physical stress on the food matrix. Consequently, moisture readily separates from the matrix upon thawing of frozen food causing textural change (Reid, 1999; Rahman, 1999). However, no significant ($p > 0.05$) difference was observed in the moisture content of the control and WRF-substituted cake samples either in the fresh-baked or freeze-thawed cake.

3.5.2. Firmness of cake

The firmness of fresh-baked and freeze-thawed cake samples was shown in Table 2. The firmness of the WF cake samples increased significantly ($p \leq 0.05$) after the first freeze-thaw cycle, while those with 10% and 15% WRF substitution for WF increased significantly ($p \leq 0.05$) after the third cycle. The increase in the firmness values of the freeze-thawed cake was due to moisture migration during frozen storage from the inner to the outer part and then by loss to the atmosphere, and due to starch retrogradation (Kerr, 2004; Cauvain, 1998). Amylose was mainly responsible for the retrogradation associated with freezing and thawing (Charoenrein & Preechathammawong, 2012); therefore, cake containing WRF, which had 0–3% amylose content (Tester & Morrison, 1990), had a lower amount of amylose than wheat cake leading to a lower rate of retrogradation. As a consequence, the firmness of cake with different levels of WRF substitution was lower than that without WRF. Waxy rice granules were swollen to a higher degree and earlier than those of wheat (Fig. 1); moreover, the spread of ruptured waxy rice starch granules covering wheat granules (Fig. 2f) could prevent swollen wheat granules to pack tightly together and consequently reduction in the firmness of the cake. Charoenrein and Preechathammawong (2012) suggested that when adding waxy rice flour to rice starch the spread of highly swollen waxy rice granules reduced the degree of retrogradation of the freeze-thawed rice starch gel. After the fifth freeze-thaw

Table 2
Characteristics of fresh and freeze-thawed cakes.

Parameter	WRF substitution (%, w/w)	Number of freeze-thaw cycle			
		0	1	3	5
Moisture content (%)	0	43.85 ± 0.05^{cA}	43.46 ± 0.06^{bcA}	42.82 ± 0.41^{bA}	41.40 ± 0.57^{aA}
	10	43.46 ± 0.22^{cB}	43.48 ± 0.06^{cA}	42.42 ± 0.04^{bA}	41.40 ± 0.32^{aA}
	15	43.40 ± 0.11^{cB}	43.10 ± 0.42^{bcA}	42.38 ± 0.06^{bA}	41.57 ± 0.44^{aA}
Firmness value by texture analyzer (g)	0	281.95 ± 14.15^{aA}	430.68 ± 29.24^{bC}	687.41 ± 78.85^{cB}	$745.31.03 \pm 24.82^{cC}$
	10	282.79 ± 27.01^{aA}	324.88 ± 24.57^{aA}	478.60 ± 10.94^{bA}	493.03 ± 77.63^{bA}
	15	294.66 ± 30.27^{aA}	366.44 ± 15.93^{abB}	427.32 ± 54.38^{bA}	608.86 ± 105.85^{bB}
Firmness score by intensity test	0	5.54 ± 1.59^{aA}	5.06 ± 1.26^{aA}	n.a.	6.63 ± 1.1^{bB}
	10	4.06 ± 1.36^{aA}	4.60 ± 1.18^{aA}	n.a.	5.34 ± 0.77^{aA}
Firmness score by preference test	0	6.30 ± 1.56^{bA}	5.92 ± 1.31^{bA}	n.a.	4.64 ± 1.75^{aA}
	10	6.97 ± 1.38^{bA}	6.66 ± 1.23^{bB}	n.a.	5.77 ± 1.61^{aB}

n.a. = not available.
Mean values in each row with different lower case superscript letters (a–c) are significantly ($p \leq 0.05$) different.
Mean values in each column of each attribute with different upper case superscript letters (A–C) are significantly ($p \leq 0.05$) different.

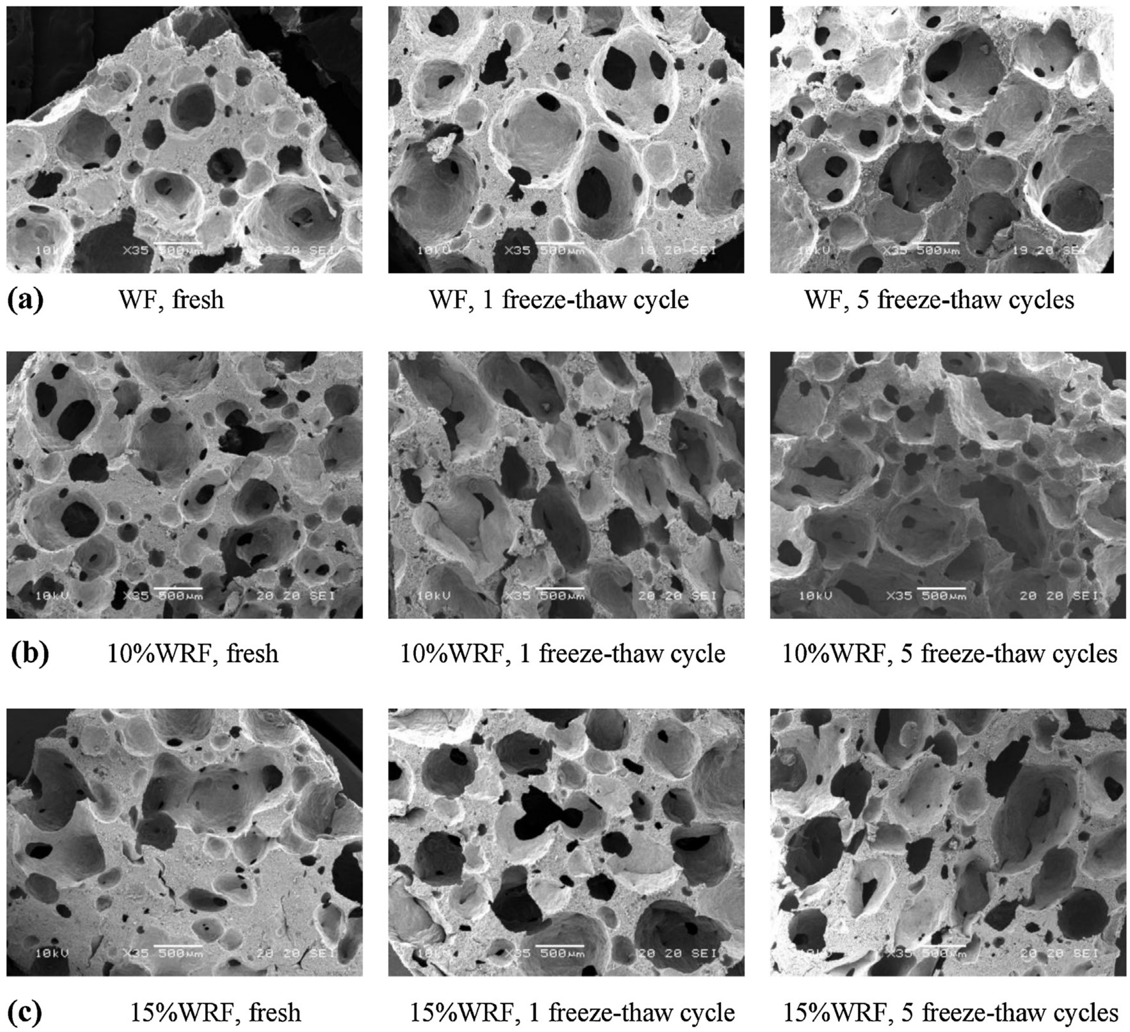

Fig. 4. Scanning electron microscope images (35×) of fresh and freeze-thawed cakes prepared with wheat flour (WF) (a), 10% (b) and 15% (c) waxy rice flour (WRF) substitution.

cycle, the firmness of cake samples substituted with 0%, 10%, and 15% WRF was raised by 62.17%, 42.64%, and 51.60%, respectively (Table 2). The cake samples with 15% WRF substitution had some dense gummy layer; therefore, they had higher firmness values than those with 10% WRF substitution. These results showed that the substitution of WRF for WF at levels of 10% and 15% improved the firmness of freeze-thawed cake.

3.5.3. Sensory evaluation of fresh and freeze-thawed cakes

Based on the best expansion and the lowest increase in the firmness of the cake samples, sensory evaluation was performed by comparing the control with cake substituted 10% WRF. The result of the intensity test (Table 2) showed that the firmness of the control significantly ($p \leq 0.05$) increased after passing through the fifth freeze-thaw cycle, whereas that of cake substituted with 10% WRF showed no significant ($p > 0.05$) increase. The firmness of freeze-thawed cake with 10% WRF substitution was lower than that of the control. In preference test, the fresh-baked cake had a significantly ($p \leq 0.05$) higher acceptability score than the other samples passing through the fifth freeze-thaw cycle (Table 2). This result corresponded with work by Owen and Duyne (1949) who reported that defrosted cake had a significantly lower desirability score than fresh-baked cake. However, freeze-thawed cake with 10% WRF substitution for WF had a significantly ($p \leq 0.05$) higher firmness score than the control.

The results of the sensory evaluation on firmness (Table 2) correlated with the firmness value evaluated by the texture analyzer ($r^2 = 0.9979$ for WF cake and $r^2 = 0.9963$ for 10%WRF cake). The fifth freeze-thaw cycle led to more firmness in the cake and lower acceptability by the panelists, compared to unfrozen cake. Substitution of 10% WRF provided freeze-thawed cake with lower firmness and a higher preference than the non-substituted samples.

3.6. Microstructure of fresh and freeze-thawed cakes evaluated by SEM

To study the effects of WRF substitution for WF on the firmness of fresh-baked and freeze-thawed cakes, the microstructure of cake samples was examined using SEM at 35× and 500× magnification (Figs. 4 and 5). These magnifications clearly showed the differences in microstructure between fresh-baked and freeze-thawed cakes prepared with WF or WRF substitution. The thickness of matrix surrounding the air pores represented the compactness of matrix. The matrix with high compactness was thin but dense. Repeating the freeze-thaw cycle caused a thinner and rougher matrix compared to fresh-baked cake (Fig. 4). A channel-like structure of the cake crumbs was noticeable in fresh-baked cake (Fig. 5), similar to the finding reported by Turabi, Sumnu, and Sahin (2010).

After the first freeze-thaw cycle, channels were clearly observed and the matrix seemed to be more compact than in fresh-baked cake. Furthermore, the matrix was distinctly thinner in cake

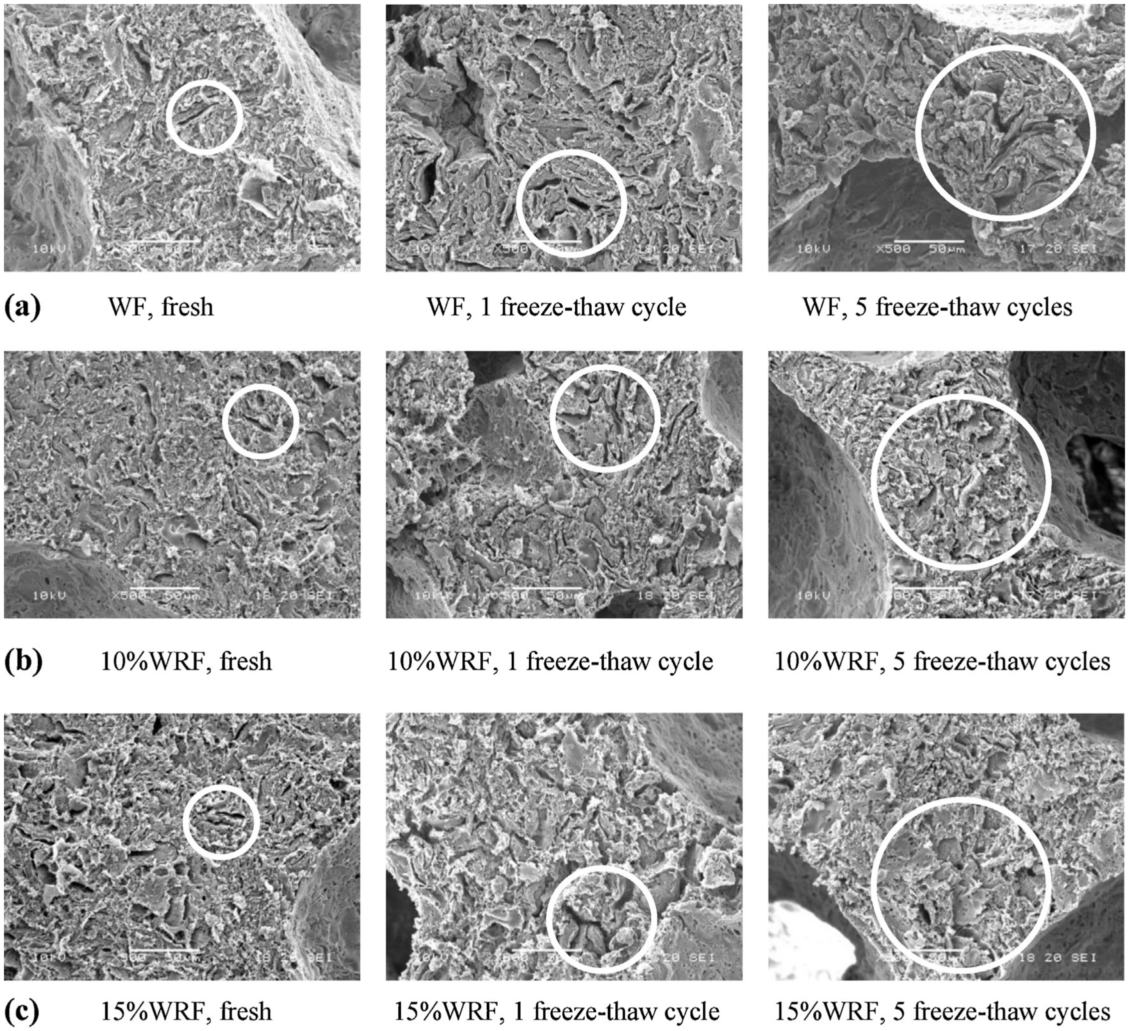

Fig. 5. Scanning electron microscope images (500×) of fresh and freeze-thawed cakes prepared with wheat flour (WF) (a), 10% (b) and 15% (c) waxy rice flour (WRF) substitution. Circles indicate the channel-like structure of the cake crumbs.

subjected to the fifth freeze-thaw cycle than fresh-baked cake, and the largest and deepest channels were well defined. The more freeze-thaw cycles the cakes were subjected to, the larger the ice crystals that formed and amlylose retrogradation increased (Charoenrein & Preechathammawong, 2012). Consequently, well defined channels and a tough matrix could be seen which led to an increase in cake firmness after repeated freeze-thawed cycles (Table 2). Although the microstructure of fresh-baked cake prepared with WF looked similar to those samples prepared with the different levels of WRF substitution (Fig. 5), freeze-thawed cakes with 10% WRF substitution had a less compact matrix compared to cakes with no WRF. This resulted from the lower amylose content and the higher swollen granules of WRF compared to WF, as mentioned earlier in the discussion on cake firmness. Hence, the firmness of freeze-thawed cakes with 10% WRF substitution was significantly ($p \leq 0.05$) lower than in WF (Table 2). With 15% WRF substitution, fresh-baked cake had less air pores (Fig. 4), which resulted from the unstable batter (discussed previously in the batter viscosity section) and a thicker matrix than either WF or cake substituted 10% WRF. As a consequence, the macrostructure of cake with 15% WRF substitution showed a non-fully expanded structure (Fig. 3c). This could have been the result of a significantly ($p \leq 0.05$) higher firmness value for the 15% compared to the 10% WRF substation after being subjected to the fifth freeze-thaw cycle (Table 2). These structural findings corresponded well with the firmness results from the texture analyzer.

3.7. Retrogradation properties of freeze-thawed cakes

An increase in the firmness of cake samples during unfrozen storage could have resulted from amylopectin retrogradation (Armero & Collar, 1998). Therefore, the enthalpy of melting retrograded amylopectin in fresh and freeze-thawed cake samples was measured using DSC. Table 3 shows a peak occurred at approximately 48.8 °C which is a characteristic of the melting of retrograded amylopectin that was detected in the cake samples subjected to five freeze-thaw cycles. The enthalpy of melting retrograded amylopectin (ΔH) of these cakes ranged between 1.02 and 1.08 J/g. However, it was not detected in fresh cakes and cakes subjected to only one freeze-thaw cycle. The results of DSC correlated to the firmness values evaluated by the texture analyzer, which showed that cake subjected to five freeze-thaw cycles was significantly ($p \leq 0.05$) firmer than fresh cakes and cakes after freeze-thaw cycle (Table 2). Furthermore, it was consistent with microstructure of cake after 5 freeze-thaw cycles that had the most compact structure (Fig. 5), which indicated that repeated freezing and thawing resulted in an increase in amylopectin retrogradation leading to a higher firmness value and consequently, a lower acceptability score. The ΔH value of cake subjected to five freeze-thaw cycles

Table 3
Retrogradation properties of flour prepared from fresh and freeze-thawed cakes.

Number of freeze-thaw cycles	%WRF substitution	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g dried flour)
Fresh	0	n.d.	n.d.	n.d.	n.d.
	10	n.d.	n.d.	n.d.	n.d.
	15	n.d.	n.d.	n.d.	n.d.
1	0	n.d.	n.d.	n.d.	n.d.
	10	n.d.	n.d.	n.d.	n.d.
	15	n.d.	n.d.	n.d.	n.d.
5	0	49.6 ± 0.0	55.1 ± 0.4	60.0 ± 0.6	1.08 ± 0.22
	10	48.2 ± 0.2	54.1 ± 0.0	59.0 ± 0.4	1.01 ± 0.19
	15	48.6 ± 0.6	54.4 ± 0.4	59.6 ± 0.6	1.02 ± 0.29

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, ΔH = enthalpy of melting retrograded flour.
n.d. = not determined.

showed no significant ($p > 0.05$) difference between cake substituted with 10% and 15% WRF which was not consistent with the firmness values evaluated by texture analyzer which showed that 10% and 15% WRF substitution could reduce the firmness of freeze-thawed cake. This result implied that an increase in the firmness of frozen cake was not a simple function of amylopectin retrogradation which was consistent with Ji et al. (2007). Furthermore, it could be possible that freezing and thawing had a stronger effect on amylopectin retrogradation than that of WRF substitution.

4. Conclusion

Repeated freezing and thawing of cake samples resulted in the greater firmness than in unfrozen cake which was due to the more compact structure, lower moisture content and higher starch retrogradation in the freeze-thaw samples. However, this study demonstrated that WRF could be used as a substitute for WF at level of 10% (w/w) in freeze-thawed cake. After passing through the fifth freeze-thaw cycle, the firmness of WRF-substituted cake was lower than that made from WF. WFR could retard the starch retrogradaion by spreading swollen waxy rice starch granules to cover wheat starch granules producing a subsequent lowering of the firmness of freeze-thawed cake which could have been a result of the lower amylose content and the highly swollen characteristics of WRF during baking. The higher amounts of WRF substitution (15% and 20%) for WF diluted the wheat gluten, so that consequently, the cake had a gummy layer and collapsed upon cooling.

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