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THESIS

INFLUENCE OF EXTRUSION ON PHYSICOCHEMICAL PROPERTIES OF WAXY RICE FLOUR AND ITS APPLICATION

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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The main objective of this study was to investigate the potential of waxy rice flour (WRF) substitution for wheat flour (WF) in order to reduce the amount of imported wheat as well as add the value of the waxy rice by using frozen cake as a model for studying. This research was divided into 3 parts: firstly, the effect of WRF substitution for WF on the properties of freeze-thawed cake was studied; secondly, the physical modification of WRF with different water feeding material in extrusion process was applied to modify the properties of WRF; lastly, the extruded waxy rice flour (exWRF) was substituted for WF to improve the properties, especially moisture loss, of freeze-thawed cake. In the second and third parts, rice flour was also studied in order to compare with WRF. The result indicated that repeated freeze-thaw cycles led to an increase in firmness and amylopectin retrogradation, and a denser matrix surrounding the air pores of WF cake, compared to those of fresh-baked cake. Sensory evaluation showed an increase in firmness and a decrease in firmness acceptability of freeze-thawed cakes. However, freeze-thawed cake with 10% w/w WRF substitution had significantly less firmness, less dense matrix and more acceptability than freeze-thawed WF cake. Although, native WRF could delay an increase in firmness, the moisture content of freeze-thawed cake continuously decreased after repeated freezing and thawing. Therefore, extrusion process was applied to modify the properties of WRF for improving the water absorption ability. As expected, the extruded flour had higher water absorption index than native flour. The result also indicated that extrusion caused molecular degradation, as observed by lower glass transition temperature and higher water solubility index, disruption of crystallinity and gelatinization. After that, extruded WRF was used to substitute for WF in frozen cake. Freeze-thawed cake with exWRF substitution had less moisture loss, firmness and dense matrix, and consequently obtained higher acceptance score than freeze-thawed WF cake. Substitution of exWRF had more pronounce effect on improvement of freeze-thawed cake than native WRF. Thus, exWRF could be used for WF substitution to improve the quality of freeze-thawed cake.

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Ornpicha Jongsutjarittam

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INFLUENCE OF EXTRUSION ON PHYSICOCHEMICAL PROPERTIES OF WAXY RICE FLOUR AND ITS APPLICATION

INTRODUCTION

Thailand is the world's seventh largest rice producer and the world's largest exporter. Thirty-four million tons of paddy were produced and 10 million tons worth 196 billion baht were exported in 2010 (Office of Agricultural Economics, 2012). Therefore, Thailand contributes to about a third of the total world rice trade. Seventy percentage of rice production in Thailand is non-waxy rice and the rest is waxy (glutinous) rice. Although Thailand is in the top ten of rice producers, it has to import other crops especially Durum wheat as a raw material for human food such as bakery product and instant noodle, and also the binder of marine feeds. In 2010, Thailand imported wheat as much as 1.4 million tons worth 15 billion baht (Office of Agricultural Economics, 2012). If the huge amounts of imported wheat could be reduced by using alternative crops that grow in Thailand, the cost for wheat import would be reduced and the value of waxy rice would be increased. As mentioned above, waxy rice is the second largest rice produced in Thailand. Therefore, in this study we attempted to add the value of waxy rice by substitution for wheat usage.

Waxy rice is used as a main ingredient for many food, such as sushi, rice balls, rice wine and traditional Asian desserts. Waxy rice flour (WRF) has various functions for processed foods, such as stabilizer, tenderizing agent and coating agent (McKenzie, 1993). However, the physicochemical properties of native WRF may not be suitable for using in place of wheat flour (WF) in wheat flour-based products; furthermore, the native WRF may have some limitations to the physical conditions in food processing. Therefore, the modification process should be applied to the native flour in order to modify its characteristics and achieve the required properties that is suitable for production of desired food products. The physical modification of native flour could improve the processability, texture, and stability of product. Moreover the maximum overall performance of the process and product could be achieved (Manson, 2009).

Extrusion is a versatile process that have been applied in the food industry for more than 50 years (Harper, 1989) for producing many kinds of food products, such as half-product snacks, ready-to-eat cereals, pasta, and meat analogs. For starch-based materials, the combination of temperature, moisture and mechanical shear force in the extrusion process leads to the changes in the physicochemical properties, such as granular disruption, loss of crystalline structure and polymer degradation (Colonna *et al.*, 1989) and also functional properties of the extruded starch and flour.

One of the important characteristic of extruded starch or flour is pre-gelatinized property which could absorb water and provide viscosity at a room temperature (Wurzburg, 1995). Hence, it can be used as a functional ingredient in food products, such as pudding, gravy, sauce and cream. While, the application of extruded starch or flour in bakery product has not been widely studied. Only few studies were carried on that topic, such as bread prepared with extruded corn and barley (Filipović *et al.*, 2009; Gill *et al.*, 2002) and cookies formulated with rice and black bean extrudated flour (Bassinello *et al.*, 2011). However, the application of extruded flour on the fresh-baked and frozen cakes has not been studied yet.

Cake is a 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 (Gelinas *et al.*, 1999; Karaoğlu and Kotancilar, 2009) depending on the ingredients and formulas, the aeration and stability of batters, and the baking and thermal setting stages (Gómez *et al.*, 2007). Normally, the shelf-life of cake is between one and four weeks (Karaoğlu *et al.*, 2008) relying on the storage conditions 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 crumb to crust of the cake, increased firmness and loss of aroma and flavor of the cake (Guy, 1983; He and Hoseney, 1990). An extension of the cake shelf-life can be achieved by changing the formulation, the incorporation of some additives such as a hydrocolloid, modification of packaging conditions, and frozen storage (Gómez *et al.*, 2011).

Changing the formula of bakery products by addition or substitution of waxy flour, especially waxy wheat flour (WWF), for WF to retard staling has been studied. For cake, Hayakawa *et al.* (2004) reported that addition of WWF could improve the shelf-life of cake by maintaining moistness, softness and stickiness. They suggested that WWF could be used as an antistaling ingredient in cake. Furthermore, addition 30% WWF for WF for bread making could reduce its firmness (Bhattacharya *et al.*, 2002 and Qin *et al.*, 2009). Therefore, in this study WRF which is waxy flour produced in Thailand was interested to apply to cake product in order to retard staling.

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 also deteriorate final products (Cauvain, 1998). The freezing process affect the quality of both cake baked from frozen batter and frozen baked cake. The volume, palatability and desirability scores of cake baked from frozen batter and frozen baked cake were lower, but the compressibility values were higher than those of fresh baked cake (Owen and Duyne, 1950). The frozen baked cake had a better quality and larger volume, compared to cake baked from frozen batter. Freezing 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). In addition, it has been demonstrated that temperature fluctuations during storage and transportation cause changes in the structure of ice crystals and recrystallization (Phimolsiripol *et al.*, 2008) which could deteriorate the quality and shorten the shelf-life of a frozen product (Blond and 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.

In this research, the potential of WRF for WF substitution was investigated in order to reduce the amount of imported wheat as well as increase value of the waxy rice using frozen cake as a model for studying. The possibility of using native WRF substitution for WF in order to retard staling of freeze-thawed cake and the causes of the effects were examined. In addition, extrusion process was applied to modify the physicochemical and functional properties of the native WRF, and then the extruded WRF (exWRF) was utilized for improving the quality of fresh and frozen cakes. Moreover, rice flour (RF), which has relatively higher amount of amylopectin, was also modified by extruder and then substituted for WF in freeze-thawed cake in order to compare with WRF.

Hypotheses

Part 1: Influence of WRF substitution for WF on characteristics of batter and freeze-thawed cake.

(1) Freezing and thawing have a negative effect on cake, such as an increase in its moisture loss and firmness, resulting in lower acceptability by consumer.

(2) WRF substitution for WF can retard the starch retrogradation in freeze-thawed cake leading to relatively lower firmness of freeze-thawed WRF cake than that of WF cake.

Part 2: The effect of moisture content on physicochemical properties of exWRF.

(1) Extrusion process changes physicochemical properties of WRF and RF. Due to the different amylose and amylopectin content between WRF and RF, exWRF and extruded non-waxy rice flour (exRF) have different physicochemical properties.

(2) Changing of water feed rate in extrusion process has an effect on the physicochemical properties of extruded product.

Part 3: Application of exWRF for quality improvement of freeze-thawed cake.

(1) Substitution of extruded flour for WF can reduce the baking loss and moisture loss in freeze-thawed cake because of high water absorption ability of the extruded flour.

(2) Extruded rice flour can be used as an ingredient to substitute for WF.

OBJECTIVES

The main objective of this study was to investigate the potential of WRF for WF substitution in order to reduce the amount of imported wheat as well as add the value of the waxy rice. The frozen cake was used as a model for studying.

This study was divided into 3 parts with the sub-objectives as follows;

Part 1: Influence of WRF substitution for WF on characteristics of batter and freeze-thawed cake.

This part aimed to study the effect of freezing and thawing on the quality of freeze-thawed cake. After that, the influence of WRF substitution for WF on the batter's properties (viscosity, density, and microstructure) and fresh-baked and freeze-thawed cake's characteristics (moisture content, firmness, microstructure, and enthalpy of melting retrograded amylopectin) were studied. Moreover, sensory evaluation of both intensity and acceptability tests of cake firmness was also evaluated.

Part 2: The effect of moisture content on physicochemical properties of exWRF.

In this part, extrusion process was used to modify the properties of WRF by studying the effect of different moisture contents of the feeding material for extrusion on the physicochemical properties including disruption of the granular structure, loss of starch crystallinity, water absorption and water solubility index and pasting and gelatinization properties of WRF. Moreover, the effect of the moisture content in the extrusion process on the glass transition temperature value of exWRF was also investigated. In addition, exRF was also studied in order to compare with the waxy varieties.

Part 3: Application of exWRF for quality improvement of freeze-thawed cake.

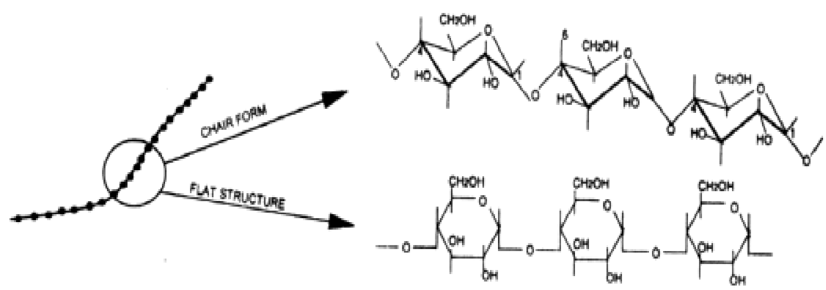
For the last part, we aimed to investigate the possibility of exWRF substitution for WF to retard the moisture loss and an increase in firmness of freeze-thawed cakes. The application of exWRF substitution for WF in freeze-thawed cakes was studied. Similar to part 1, the properties of batter and fresh-baked and freeze-thawed cakes were studied. Moreover, substitution with exRF for WF was also examined for comparison to exWRF.

LITERATURE REVIEW

1. Starch

Starch is a reserved polysaccharide in plants and it is a major component in cereal grains (wheat, rice, rye and corn), root vegetables (cassava and potato), and other plants (chestnut and almond). Starch is composed of two types of glucose polymers, i.e. amylose and amylopectin (Figure 1).

(a)



(b)

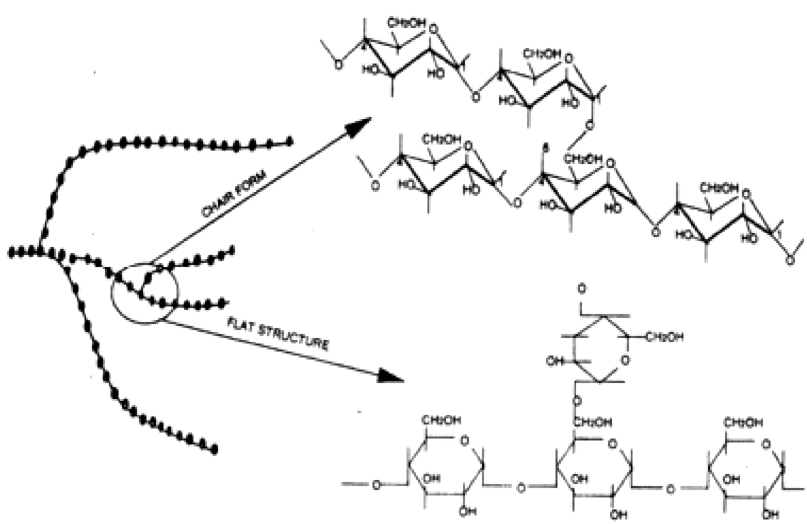


Figure 1 Chemical structure of (a) amylose and (b) amylopectin molecules

Source: Murphy *et al.* (2000)

Amylose is a linear molecule linked by α-(1,4)-linked D-glucopyranosyl residual with 9-20 branch points per molecule by α-(1,6) linkage (Biliaderis, 1998; Hizukuri *et al.*, 1981). The molecular weight (Mw) of amylose ranges between 2.0×10^5 and 1.2×10^6 (Hizukuri *et al.*, 1989). The conformation of amylose in aqueous is highly disordered coil with short-range helical structure. For amylopectin, it is a much larger molecule, compared with amylose, which Mw of 10^7-10^9 linked by α-(1,4) linkage with highly branched by α-(1,6) linkage (Biliaderis, 1998). Amylopectin consists of several chains differing in chain length (CL) (Figure 2) (Hizukuri, 1986). The unbranched A-chains are joined to the molecule through reducing end group; B-chains (B1-B4) are linked to the molecule in the same way as A-chains carrying at least one A-chain; and a C-chain has the reducing end group of the molecule. A- and B1-chains are the shortest ones with 14-18 CL; B2-B4 chains are longer chains with 45-55 CL. The molar ratio of each fraction depends on the source of starch (Hizukuri, 1985, 1986).

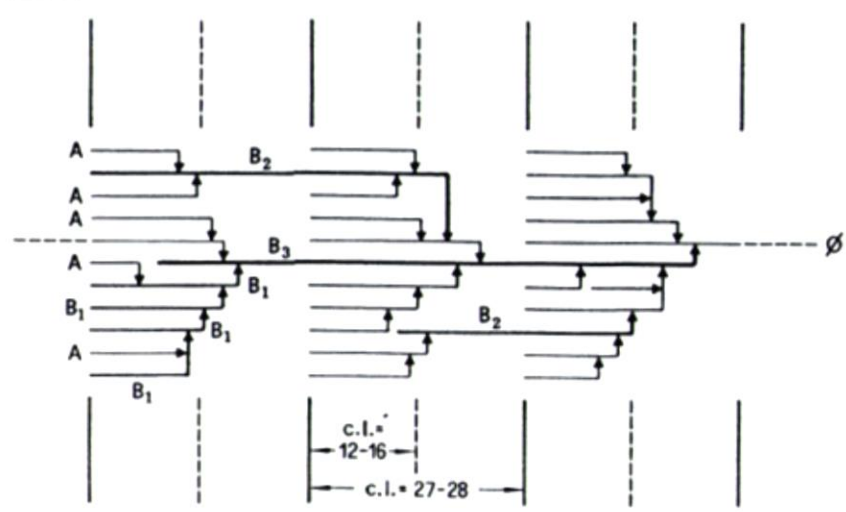


Figure 2 Amylopectin model consisting of A-, B-, and C- chains

Source: Hizukuri (1986)

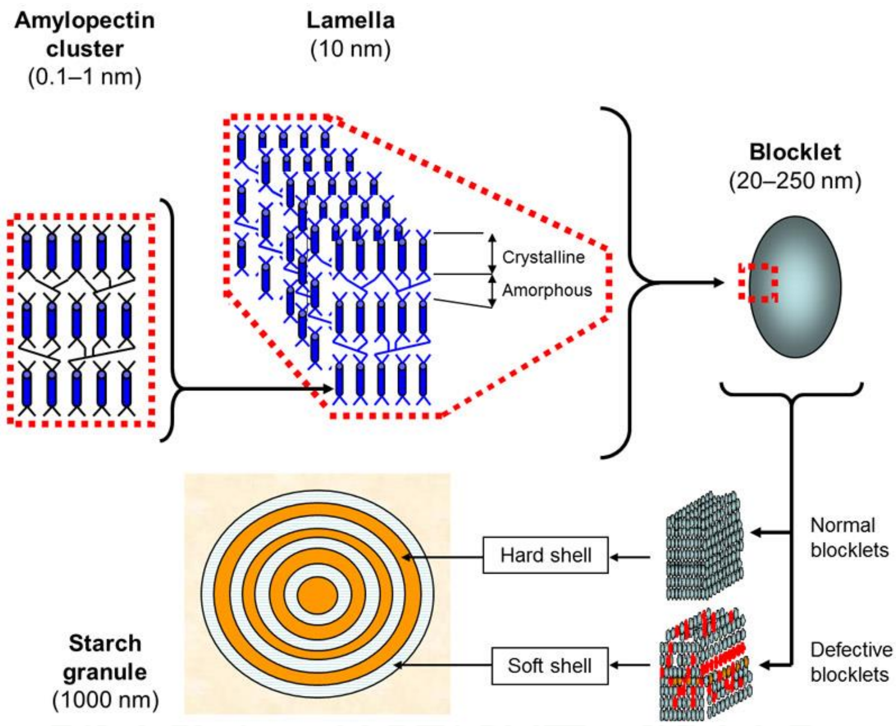


Figure 3 Organization of the starch granule

Source: Taiz and Zeiger (2011)

Figure 3 shows the levels of the organization of the starch granule. Amylopectin molecules are tightly packed together to form the clusters of double helices (0.1-1 nm). The association of amylopectin double helices interspersed with amorphous regions creates crystalline lamella (~10 nm). The blockles (20-250 nm) is the ordered aggregation of several crystalline-amorphous lamellae into an asymmetric structure an axial ratio of 3:1, called “normal blocklets”. While the “defective blocklets” is the blocklets that the regular formation is disturbed by amylose and other materials, such as water and lipids. The ordered aggregation of normal and defective blocklets form the concentric rings of hard (crystalline) and soft (semi-crystalline) shells in the starch granule (Taiz and Zeiger, 2011). Thus, the amylopectin molecules play a major role in the architecture of the blocklets while other components regulate the strength and flexibility of the starch granules (Tang *et al.*, 2006). The starch granules vary in size (1-100μm) and shape (spetical, elliptical, angular, lenticular, etc.)

according to the botanical source (Huber *et al.*, 2006) which can use as the diagnostic characteristics for identification and characterization of starch.

The ratio of amylose to amylopectin is different among the starches depending on the variety, source, and harvest season, but levels are generally in the range of 25-28% and 72-75% for amylose and amylopectin, respectively (Van *et al.*, 2006). The example of unusual ratio are waxy wheat starch and waxy rice starch which consist of 0-2% amylose. On the contrary, high-amylose wheat starch has amylose content up to 30-37% (Keeratipibul *et al.*, 2008).

Starch is a basic in gradient in many food products, such as bread, pudding and sauce. Moreover, it is a multifunctional ingredient in food products, most commonly, as a bulking agent, binder, fat replacer, texture modifier and raw material for other starch related products. Starch-based food plays a major row in the human diet due to their bulking quality and ability to contribute to satiety (Niba, 2006).

2. Starch gelatinization and retrogradation

2.1 Starch gelatinization

Native starch granules are insoluble in water at the room temperature due to the regions of complex molecular order (double-helical association of starch polymers). The presence of sufficient heat, moisture and shear leads to disruption and reorganization of the ordered structure of starch granules; consequently, they irreversibly lose the granular order, which is accompanied with an increase in granular hydration, swelling and leaching of amylose molecules and other soluble components (Figure 4). This process is called starch gelatinization. Gelatinization results in the formation of viscous paste, which composes of granule remnants dispersed in a continuous phase of solubilized starch (Huber *et al.*, 2006; Niba, 2006). The viscosity of starch is greatly influenced by the ratios of amylose to amylopectin (Niba, 2006).

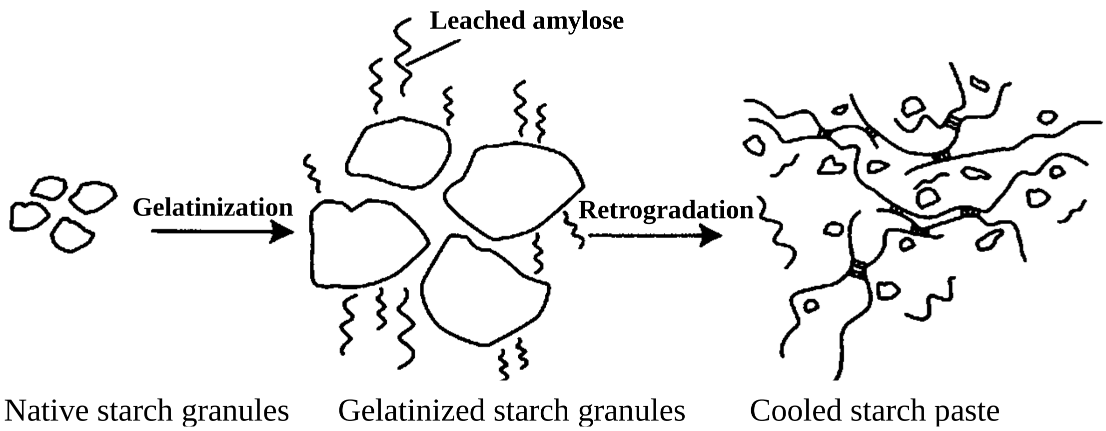


Figure 4 The structural changes associated with gelatinization and retrogradation

Source: Huber *et al.* (2006)

2.2 Starch retrogradation

As a paste cooled, the leached linear amylose molecules aggregate, form double helical structure and then form a continuous three-dimensional gel network contributed to the thickening characteristics of starch, which is called retrogradation (Figure 4). The dispersed phase of starch gel network consists of amylopectin-rich regions and granule remnants which disperse in the water continuous phase. The branched molecules of amylopectin limit their intermolecular association and they flavor initial water solubility, thus amylopectin molecules slowly aggregate in time (Huber *et al.*, 2006).

Retrogradation of starch in food product influences the product quality. The extent of retrogradation in particular during cold storage also affects the stability of starch-containing product. Moreover, freeze-thaw cycles lead to the extensive retrogradation (Niba, 2006) which could deteriorate the quality of frozen food.

3. Waxy rice and non-waxy rice

Among the cereals, rice and wheat are equal important for the food sources for humankind (Shinde *et al.*, 2014). According to an FAO report 20% of energy supply through diet in the world is provided by rice, while the corresponding figures for wheat and maize are 19% and 5%, respectively (Agriculture and Consumer Protection, 2014). Furthermore, starch from wheat and rice is predominant in food industry application.

Rice (*Oryza sativa* L.) is one of the staple foods consumed worldwide, especially in Asia. The composition of rice granules varies widely depending on the varieties. Based on the amylose content, rice can be divided into non-waxy rice and waxy rice. Non-waxy rice has 15-27% amylose, while waxy varieties contain almost no amylose content (Guy, 1994).

Glutinous rice (*Oryza sativa* var. glutinosa), or commonly known as waxy or sticky rice, is characterized by its appearance and very low amylose content. The appearance of the milled glutinous grain is opaque which is opposed to the translucent appearance of non-waxy rice. However, after cooking, the waxy rice turns into translucent while the non-waxy rice becomes turbid (Keeratipibul *et al.*, 2008; Mutters and Thomson, 2009). For cooking, unlike boiling of non-waxy rice, before steam cooking, the waxy rice requires overnight soaking in water because it has lower water uptake than non-waxy rice. The texture of cooked waxy rice is sticky and adherent (Keeratipibul *et al.*, 2008). Because of the different eating quality, waxy rice is used as a main ingredient for sushi, rice balls, rice wine and traditional Asian desserts, such as Japanese Moji, Thai Khaolam, and Korean Chalddeok. Moreover, waxy rice flour can be added to many processed foods as stabilizer in sauces, gravies and pudding, as tenderizing agent in frozen food and as coating flour for fried food (McKenzie, 1993).

Since it has a very high amount of amylopectin, the physicochemical properties of this starch determinately depend on the amylopectin structure, including degree of polymerization, degree of branching, chain length and chain-length distribution (Wang and Wang, 2002). Furthermore, the amylose and amylopectin contents influence the swelling behavior, pasting properties, as well as thermal characteristic of waxy rice starch (Tester and Morrison, 1990).

The waxy rice varieties had significantly lower pasting temperatures and viscosities, especially breakdown and setback viscosities, than those of non-waxy rice varieties (Kang *et al.,* 2006; Vandeputte *et al.*, 2003). Ibáñez *et al.* (2007) also found that waxy rice flour had a lower pasting temperature and setback viscosity; however, the viscosities of peak, hot paste and breakdown were higher than those of non-waxy rice flour. High breakdown viscosity described the ease of maximally swollen starch granules to be broken by heating. The waxy rice granules have this property; as a result the paste is sticky (Kang *et al.*, 2006). The inconsistent results of pasting properties are due to the differences in rice cultivars which affect the amylose and amylopectin contents, and the method for measurement.

The thermal properties of waxy rice differ from those of non-waxy rice. The research of Kang *et al.*, 2006 demonstrated that waxy rice had slightly higher gelatinization transition temperature and gelatinization enthalpy than non-waxy rice, which could be a result from the differences in amylose content, structure of amylose and amylopectin, and amylose-lipid complex. Waxy rice had more amylopectin content which was more crystalline, leading to more resistance to gelatinization, as compared to non-waxy rice. Moreover, chain-length distribution of amylopectin may also affect the transition temperature of rice starch. The retrogradation transition temperature and melting enthalpy of retrograded waxy starch after storing for 7 days at 4°C ranged from 40.48 to 56.19°C and from 0.50 to 0.13 J/g, respectively. The retrogradation enthalpy differed among the cultivars as a consequence of amylose to amylopectin ratio and amylopectin chain length. The gelatinized waxy rice starches did not retrograde during 7-day storage at 5°C (Wang and Wang, 2002). After that the retrogradation occurred and increased continuously. Each cultivar had different degree of retrogradation which was controlled by a combined effect of the A and B1 chain levels, average chain length (CL), and exterior chain lengths (ECL). A higher proportion of B1 chain and a longer CL of amylopectin provided greater retrogradation, while a higher proportion of A chain and a shorter ECL resulted in smaller retrogradation (Jane *et al.*, 1999; Shi and Seib, 1992).

4. Wheat

According to the US classification, wheat (*Triticale aestivum* L.) is divided into Durum, Hard Red Spring, Hard Red Winter, Soft Red Winter, Hard White, and Soft White. Wheat can be used as a raw material for productions of food, alcoholic beverage, chemical and pharmaceutical products, as well as cattle feed; however, wheat is mostly used for flour production. Wheat flour is used as a main ingredient for bakery products and pasta. It is also good for being a thickening agent for soups, sauces and gravies.

Starch is the most abundant component of wheat flour (70–75%) and it consists mainly of two glucose polymers, which are 25-28% of amylose and 72-75% of amylopectin (Van *et al.*, 2006). Wheat flour contains ~7-14% protein content and ~80-85% of total wheat protein are gluten protein which is a major storage protein in wheat (Manley *et al*., 2011) and possesses the viscoelastic properties (Brahim *et al*., 1999). Therefore, the properties of wheat flour vary not only as a result of the amylose and amylopectin content, but also the amount of gluten.

Jarosla and Les (2008) studied the pasting behaviors of wheat starch and flour from thirty-eight varieties. The peak viscosity of starch pastes ranged from 172 to 259 RVA Units (RVU) and the peak viscosity range of flour pastes was 159 to 272 RVU. While, Ming *et al.* (1997) reported that the peak viscosity of wheat starch from seven cultivars varied from 190 to 323 RVU. Lower apparent and total amylose contents caused the higher peak and breakdown but lower final viscosity, and setback. The peak viscosity was increased of 22 and 25 RVU with 1% reduction in apparent or total amylose content, respectively. Chen *et al*. (2010) studied the effect of gluten on pasting properties of wheat starch. They reported that it was a significant downtrends of peak time, peak viscosity, trough viscosity, final viscosity and setback with increasing the addition of gluten. They suggested that it could be because (1) self-adsorption of gluten influences on the transportation of available water and starch concentration in the paste, and (2) thermal characteristics of gluten affects the transmission of gelatinization energy. However, breakdown was not affected by the amount of the gluten.

The new wheat types, waxy wheat and high amylose wheat, were produced to have the specific structures and unique physicochemical and functional properties for enhancing or improving the quality of food products. Waxy wheat starch, similar to waxy rice starch, swells rapidly and spent shorter time to reach peak viscosity than normal wheat starch. The viscosities at setback and breakdown of waxy wheat were lower than those of normal wheat during 7-day 4°C storage (Hayakawa *et al.*, 1997).

5. Starch modification

Different types of starch have different properties and they are applied to the food product for nutritional, functional and sensory purposes. However, the native starch has some limitations in its resistance to the physical conditions in food processing. Therefore, starch need to be modified to enhance their physical properties to be more resistant to the processing conditions and provide better food quality, such as reduction of starch gel syneresis, textural improvement, and resistance to freeze-thawed stability (Alexander, 1992).

Starch modification, which resulted in a structural and physicochemical changes, can be obtained through chemical, physical, enzymatic applications (or combination of these methods) and genetic modification. Around 75% of food starch added as an ingredient is chemically and/or physically modified. The type of modification is listed in Table 1.

Table 1 Type of starch modification and modified product

Type of modification		Modified products
Chemical modification	Cross-linking	Distarch phosphate
	Substitution	-Starch ester: acetylated starch, starch phosphate, octenylsuccinate-treated starch -Starch ether: hydroxypropylated starch, carboxymethylated starch, cationized starch
	Conversion	-Acid converted starch -Oxidized starch -Bleached starch -Pyroconversion (dextrinization): dextrin, British gum
Physical modification	Pregelatinization	Pregelatinized starch
	Heat treatment	-Heat-moisture treated starch -Annealed starch
	Radio treatment	Radio treated starch
Enzymatic modification		-Maltodextrin -Cyclodextrin -Amylose
Genetic modification		-Amylose free starch -High amylose starch

Source: modified from Belitz and Grosch (1999) ; Wurzburg (1995)

6. Extrusion and starch modified by extrusion

6.1 Extrusion

Extrusion is a versatile process that is a highly adaptable, cost effective and energy efficient technology having been applied in the food industry for more than 50 years (Harper, 1989) for producing many kinds of food products, such as half-product snacks, ready-to-eat cereals, pasta, and meat analogs. The extrusion process involves raw materials feeding into the extruder, and then they are mixed, cooked and kneaded before passing through a narrow opening die. The extruded products may expand which is caused by sudden evaporation of water, and/or they may be further processed, such as shaping, coating with seasoning agent, and drying.

Extruder can be categorized as single-screw or twin-screw extruder. The operation diagrams of extruders are displayed in Figure 5. In a single-screw extruder, the feeding section is the first section where raw materials are collected and pushed into the transition section. Within this section, the materials are initially compressed, and then they begin to be cooked and changed in structure. The last section is a metering section, where high shear, heat buildup and pressure buildup occur, leading the product out of the die. For twin-screw extruder, the operation is slightly more complex than the single-screw one since a wide variety of operations can be achieved within a single machine by adjusting the screw configurations. Twin-screws can be operated as four categories which are counter-rotating and intermeshing, counter-rotating and non-intermeshing, co-rotating and intermeshing and co-rotating and non-intermeshing. The variables that influence the extrusion process are feed rate, screw geometry (pitch, diameter, flight, degree of intermeshing, and type of screw mixing), screw speed, and die characteristics (Heldman, 1997). These parameters have an effect on the processability and product quality.

(a)

(b)

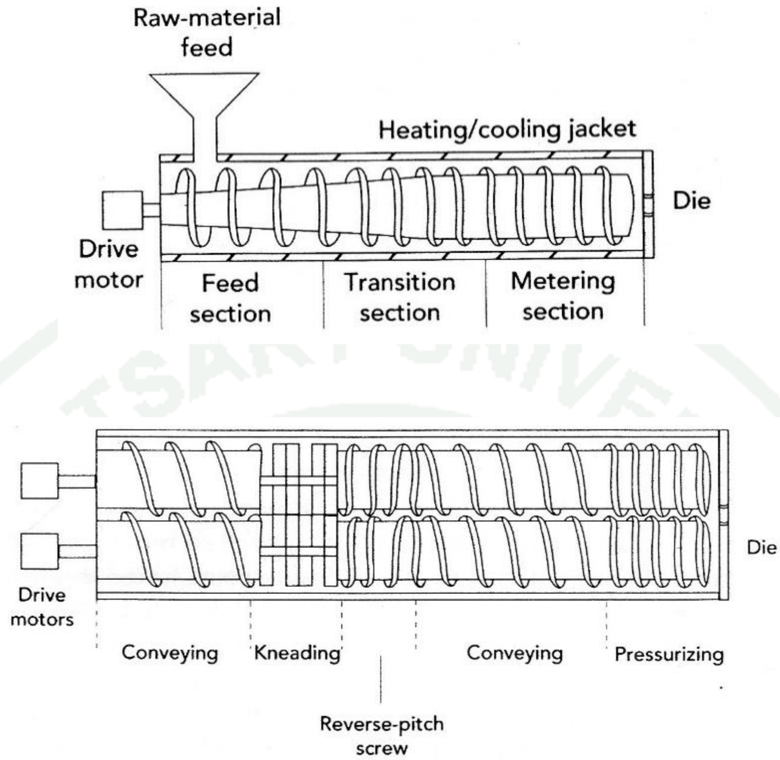


Figure 5 Operation diagrams of (a) single-screw extruder and (b) twin-screw extruder

Source: Heldman (1997)

6.2 Starch and flour Modified by extrusion

Extrusion have been applied to modify the properties of various starch and flour, in corn starch (Blanche and Sun, 2004; El-Dash *et al.*, 1983) waxy maize starch (Willett *et al*., 1997), high-amylose maize starch (Lopez *et al*., 2007), banana starch (González *et al.*, 2007), rice flour (Guha and Ali, 2002; Hagenimana *et al.*, 2006 Sompong *et al.*, 2011) and barley four (Gill *et al.*, 2002). For starch-based materials, the combination of temperature, moisture and mechanical shear force in the extrusion process causes structural transformations, such as granular disruption, loss of crystalline structure and polymer degradation (Colonna *et al*., 1989). As a result, the physicochemical and functional properties of the extruded product differ from the native starch.

Unlike the simple heating method that has no molecular degradation, extrusion involves the degradation of starch molecules into smaller oligosaccharide units. Under controlled moisture, temperature and shear force in the extruder, starch gelatinization occurs. Typically, gelatinization leads to an increase in viscosity because starch molecules unfold and are easier to entangle. However, the shear force in the extruder breaks the strands into smaller molecules, lowering the viscosity. Once these molecules exit the die, they are cooled and form new networks and strands which affect texture, color and nutritional quality of the final products (Heldman, 1997). The model for starch modification by the combination of heat, moisture and shear force in extrusion and simple heating method is presented in Figure 6.

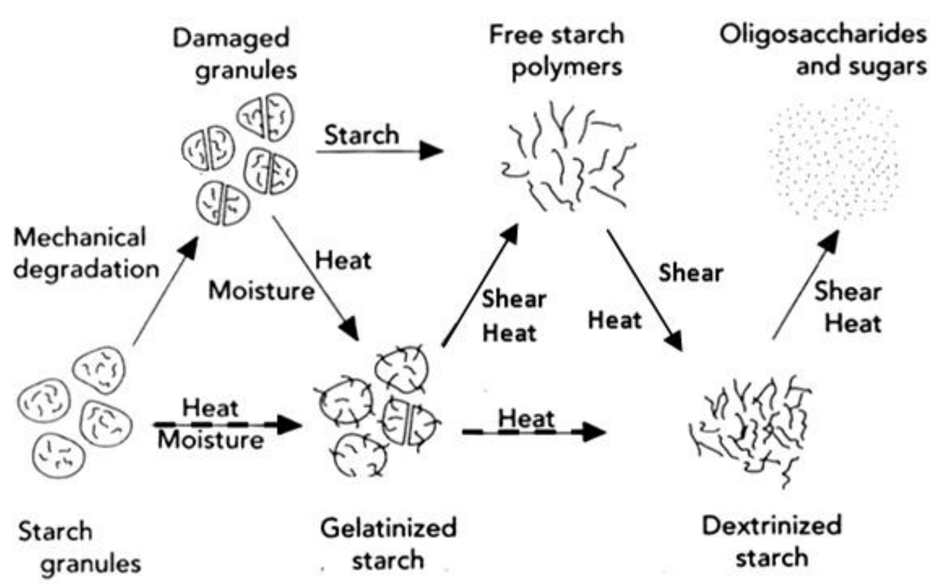


Figure 6 Model for starch modification by the combination of heat, moisture, and shear force in extrusion (→) and simple heating method (···►)

Source: Heldman (1997)

The high shearing of extrusion led to the high degradation in corn starch molecules (Blanche and Sun, 2004). Moreover, the combination of severity of extrusion conditions consisting of high shear rate, high screw speed, and low moisture content resulted in the greater degradation of starch molecules.

The water soluble index (WSI), which is the measurement for degree of starch degradation, of extruded banana starch was decreased with temperature and moisture content increasing but it was not affected by screw speed (González *et al.*, 2007). The water absorption index (WAI), which is an indicator for swelling ability of starch, of extruded banana starch was increased when screw speed, temperature, and moisture content of the extrusion process were high.

Guha and Ali (2002) investigated the molecular degradation resulting from extrusion process of three rice starch varieties which were different in the amount of amylose and amylopectin by gel permeation chromatography. They reported that during co-rotating twin-screw extrusion rice starch was degraded from high molecular weight fraction, amylopectin, into low molecular weight one, amylose. The highest molecular degradation was found in waxy rice while high amylose rice had the lowest degradation which was in line with the result from Sompong *et al.* (2011). They reported that the rice varieties with low amylose content led to higher starch degradation during extrusion than those with higher amylose contents. As a result, the WSI values of lower amylose varieties increased, while extruded rice with higher amylose content led to an increase in WAI value.

Hagenimana *et al.* (2006) studied the effect of screw speed, barrel temperature and feeding moisture content on characteristics of extruded rice flour by response surface methodology. They reported that an increase in extrusion temperature decreased WAI which was due to an increase in starch degradation. The highest WAI were obtained at 19-22% moisture content. For WSI, lower moisture content, higher barrel temperature and higher screw speed resulted in the highest WSI value. Moreover, the combination with harsh conditions caused an increase in degraded starch granules leading to higher water soluble products.

Willett *et al*. (1997) reported that waxy maize starch was degraded after passing through the first and second extrusions. The initial co-rotating twin-screw extrusion reduced the molecular weight of native starch from 336×10^6, which was amylopectin, to 40×10^6 and it was further degraded during the second extrusion measured by multi-angle laser light scattering.

Kaletunc and Breslauer (1993) studied the influence of twin-screw extruder on the starch fragmentation by monitoring the glass transition temperature (T_g) of extruded corn flour. They found that the extrusion-induced fragmentation into smaller molecule which was the primary cause of change in T_g of extruded flour. Furthermore, the extent of fragmentation by increasing process severity caused lower T_g value in extruded flour.

Not only has the effect on starch molecular degradation, extrusion also had an effect on the morphology of starch granules which could be observed by scanning electron microscope (Figure 7). The surface of pre-gelatinized maize starch was irregular stone-like shape with lots of holes (Yan and Zhengbiao, 2010). Lopez *et al.* (2007) found that after “mild” and “extreme” extrusion process high-amylose maize starches lost their granular structures and the extruded starch were irregular shape consisting mostly of amorphous particles. González *et al.* (2007) stated that the native starch granules of banana were round and oval shape, whereas the cylindrical structures were found after extrusion.

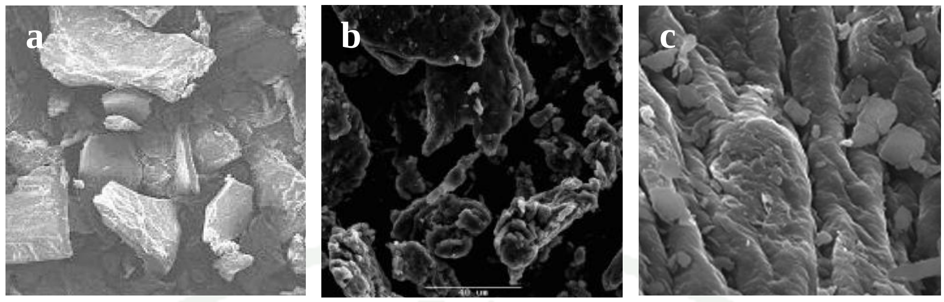


Figure 7 Scanning electron micrographs of the extruded starch of (a) maize (300×), (b) high-amylose maize (700×), and (c) banana (1200×).

Source: González *et al.* (2007); Lopez *et al.* (2007); Yan and Zhengbiao (2010)

No gelatinization peak was observed in extruded corn starch indicating that the starch granules were completely gelatinized or starch molecules might be depolymerized as a result of extrusion (Blanche and Sun, 2004). While its endotherm showed the formation of amylose-lipid complex at 110°C and the melting enthalpy of complexes increased with treatment severity; higher shear, higher screw speed and lower moisture content. They mentioned that low moisture content and high temperature strongly contributed to starch degradation and favored the formation of amylose-lipid complex. Hasjim and Jane (2009) also found the formation of amylose-lipid complex in extruded maize starch.

For crystallinity structure, after passing through the extrusion process, the crystallinity of extruded waxy maize starch was eliminated showing that the waxy maize starch was converted into a homogenous thermoplastic melt (Willett *et al*., 1997). The similar result was found in banana starch that the crystallinity pattern of extruded sample showed an amorphous peak which was due to high disorganization of the starch molecules after extrusion process (González *et al.*, 2007). Hasjim and Jane (2009) reported that extrusion caused the formation of a V-type diffraction pattern in extruded maize starch which reflected the formation of amylose-lipid complex.

Moreover, extrusion has an influence on the pasting properties of the extruded starch and flour. The viscosity of a paste depends on the degree of gelatinization and molecular break down (El-Dash *et al.,* 1983). Hagenimana *et al.* (2006) found that the extruded rice flour had much lower viscosity values than unprocessed flour. All extruded flour displayed lower peak viscosity than unprocessed flour which indicated that ungelatinized starch polymers were still present. It was agree with the result of extruded wheat flour (Barres *et al.*, 1990). Blanche and Sun (2004) reported that the hot paste and final viscosity was higher in the extruded corn starch prepared at higher-moisture content and lower-screw speed which could be assumed that lower starch transformation degree occurred at these conditions, compared to the extrusion process with lower-moisture content and higher-screw speed.

In addition after extrusion, the properties of native starch or flour for process ability, food texture and food stability could be improved; moreover, the maximum overall performance of the process and product could be achieved (Manson, 2009). One of the important characteristic of extruded starch or flour is pre-gelatinized property which could absorb water and provide viscosity at a room temperature (Wurzburg, 1995). Therefore, it can be used as an ingredient in food products, such as pudding, gravy, sauce and cream. The highly water absorption ability of extruded starch or flour could also prevent the moisture loss of the frozen food products.

7. Cake, Staling and Staling Retardation of Bakery Products

7.1 Cake

Cake is a macroporous material, produced by heating the liquid foam, cake batter, to set as a stable solid foam. Then it requires a cooling period to allow the gelatinized starch to gel and firm up the cake. Cake is a complex system where air bubbles are dispersed in a continuous liquid phase with dry ingredient dispersing or suspending. The structure of cake is developed by mixing, which involves starch gelatinization, protein denaturation, CO_2 production from leavening agent, and interactions between other ingredients, and baking stage (Meza *et al*., 2011; Sahin, 2008). The main ingredients of cake batter are wheat flour, sugar, egg, liquid, fat or fat replacer, and sometimes, emulsifier, salt and leavening agent. The change in batter properties, such as composition and structure, may have the effect on the performance during baking and the quality of cake after baking and during storage. For example, reduction of wheat gluten resulted in lowering batter viscosity which led to a reduction in cake volume (Wilderjans *et al*., 2008).

Good quality cake can be described as high volume, low crumb firmness and uniform structure, staling tolerance, and long shelf-life (Gelinas *et al*., 1999) depending on the ingredients and formulas, the aeration and stability of batters, and the baking and thermal setting stages (Gómez *et al*., 2007). Normally, the shelf-life of cake is between one and four weeks depending on the storage conditions, and then the quality is mainly defected from staling process resulting from changing of physical and chemical properties of the product, such as starch retrogradation and moisture loss leading to an increase in cake's firmness and loss of aroma and flavor (He and Hoseney, 1990) and microbial spoilage. One important aim of the cake industry is an extension of cake shelf-life period which can be done by changing formulation, incorporation of some additives such as hydrocolloid, modification of packaging condition, and frozen storage (Gómez *et al*., 2011).

7.2 Staling of bakery products

Staling of the bakery products relates to the physical and chemical changes leading to the loss of freshness and quality of the baked products. An increase in the crumb firmness, which is the most important change resulting from staling, is caused by moisture loss from crumb to the atmosphere and starch retrogradation during storage (Cauvain, 1998).

The staling process is involved in the change of starch crystallinity. During baking, starch is transformed from an ordered to disordered state via gelatinization. After leaving out from the oven, the disordered starch re-orders again, referred to retrogradation (Cauvain, 1998). It has been proved that amylopectin crystallization takes the responsibility for staling. During storage, starch gel, which consists of swollen starch granules with amylose gel, slowly increased in the stiffness, while the stiffness of amylose gel was stable. The reason that amylopectin retrogradation caused the starch gel stiffness was described by amylopectin retrogradation within starch granules stiffening the granules; consequently, the amylose gel matrix was reinforced, leading to the stiffness of starch gel. The stiffness and crystallinity of starch gel were reduced to original point by heating at 95ºC but those in amylose gel were not affected which showed that staling was thermo-reversible behavior. This thermo-reversible crystallization was found in the amylose-free stored swollen granule, indicating amylopectin crystallization (Morris, 1990). Therefore, the staling of starch gel is due to crystallization of amylopectin and its associates with the firmness of the stale bakery products.

The rate of starch retrogradation is associated with storage temperature, specific volume and moisture content of the baked products (Cauvain, 1998). The maximum staling rate of each kind of baked goods is different depending on their ingredients. To illustrate, cake has its maximum staling rate at 25ºC, compared to 4ºC of bread due to the higher proportion of soluble materials, such as sugar, in cake than that in the bread dough (Cauvain, 1998).

7.3 Staling retardation of bakery products

Many researchers have tried to delay the staling process of bakery goods, especially bread, by incorporating additive ingredients, such as α-amylase, maltodextrins, lipids, emulsifiers, and shortening. Most of them effectively work on retarding bread retrogradation by two mechanisms which are an increase in dough water content, and formation of a complex with starch molecule (Bhattacharya *et al.*, 2002). Moreover, the effect of alternative flour on staling retardation has been studied by addition or substitution of other types of flour for wheat flour in bakery products, and many of the researches have been focusing on waxy wheat flour (WWF).

7.3.1 Addition with other flour

The fresh bread added 0-15% waxy wheat flour were not significantly different in sensory score, but the highest score was in bread added 7% WWF (Qin *et al.*, 2009). The weight loss of bread was decreased when the proportion of WWF increased. Addition of 22% WWF effectively reduced bread firmness (texture analyzer and regression equation) and weight loss after 6-day preservation at room temperature.

Hayakawa *et al.*, 2004 studied the influence of WWF blended with wheat flour on the quality of bread and cake after one-day storage at room temperature or at 4°C in refrigerator. The loaf volume of bread was lower, bread crumb changed from dense to open bubble structure as the ratio of WWF increased. Moreover, addition of WWF to WF affected eating qualities of bread (crispiness, hardness and chewiness or palatability) evaluated by 10 skilled panelists. An excessive addition (>20% WWF) resulted in less acceptable properties of the products (sticky, lumpy, or less crispy textures). However, the suitable level of WWF addition should be ≤30% or ≤10% for more preference. Similar to bread, cake volume was decreased with an increase in the levels of WWF. Incorporation of <20% WWF could improve the shelf-life characteristics of bread and cake by maintaining moistness, softness and stickiness. They suggested that WWF could be used as an antistaling ingredient in cake.

Addition 30% of WWF for WF for bread making caused smaller loaf volume, compared to bread made of WF only, but from the overall appearance of loaf added with 10-20% WWF was not different from the control (Bhattacharya *et al.*, 2002). The addition of 20-30% of WWF delayed an increase in firmness of bread during 5-day storage at room temperature. The total moisture contents over time of bread crumb blended with WWF were not significantly different from those of bread crumb made without WWF blending which implied that retardation of firming process was not moisture related.

7.3.2 Substitution of other flour

Partially substituted tapioca flour blend for WF (100:0, 75:25, and 50:50) was studied in cake (Chaiya and Pongsawatmanit, 2011). An increase in tapioca flour content decreased batter density leading to the rising of the specific volume of blended cake. Furthermore, the hardness was reduced with 50% tapioca flour substitution.

Whole WWF substitution for WF resulted in an increase in water absorption of bread dough, compared to bread dough made from WF (Hung *et al.*, 2007). Bread baked from whole WWF was significantly lower in specific volume and big gas cell distribution than that from WF. Bread with whole WWF had lower firmness than WF bread during storage for 3 days at 22ºC. Dough prepared from partial substitution of 30% or 50% whole WWF for WF absorbed a high amount of water, leading to high moisture content in bread crumb which provided lower firmness after 1-day storage. After 3-day storage, the firmness rapidly increased because of water evaporation and bread crumb retrogradation. The substitution by 10% whole WWF in bread generated higher firmness value as compared to that of the control because it had a higher amount of dietary fiber then WF but the water absorption was not changed. The weak point of whole WWF was the generation of the dark-brown color which was due to high amounts of phenolic compounds and bitter taste to the bread crumb.

The effect of cake flour replaced with 5% various starch types (maize, waxy maize, amylomaize, potato and pregelatinized potato starches) on the staling process of microwave-baked cake was investigated (Seyhun *et al*., 2005). They reported that all starch types, with the exception of amylomaize, reduced the weight or moisture loss during baking because those starches provided higher water binding capacities, compared to cake flour. Moreover, these starches led to less firmness of cake during 5-day storage at 20ºC, compared to control.

The bread crumb substituted of WWF for commercial hard-type wheat flour was softer with increasing degree of substitution (20-40%) during 7-day storage (Morita *et al*., 2002). In addition, the firmness of bread crumb substituted by WWF after refreshing by oven heating at 110°C for 15 min was lower than that of bread crumb made from WF. The moisture content of bread crumb substituted with WWF seemed to be higher than that of the control, which could be due to the higher water absorption of WWF. They also suggested that reduction of amylose content in bread by substitution of WWF led to retardation of staling.

In addition, due to the higher water absorption ability of extruded starch, it could improve the property of bakery products. However, there has been a few research about applying extruded starch or flour to the bakery products. For example, the substitution of WF with native barley flour reduced the loaf volume of bead (Figure 8a); however, substitution with 15% extruded barley flour (extrusion conditions: moisture 50%, screw speed 50 rpm and temperature 130°C) had higher acceptable properties on loaf volume (Figure 8b), firmness and color than bread substituted with native barley flour (Gill *et al.*, 2002). Substitution of extruded corn (extrusion conditions: moisture 12.3% and temperature 90°C) at 10-30% for WF decreased bread volume around 15 to 40 %, but 10-20% substitution along with bread improver could improve bread volume and retarded its staling (Filipović *et al*., 2009).

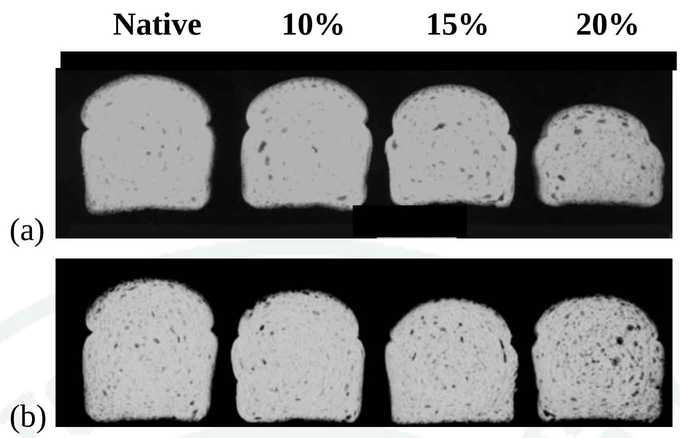


Figure 8 Cross-section view of bread with native (a) and extruded (b) barley flour substitution for wheat flour at 10, 15 and 20% w/w

Source: Gill *et al.* (2002)

8. Freezing Technology and Frozen Cake

Freezing is one of the most popular preservation techniques using during storage and transportation of a wide variety of food, such as ready-to-eat food, meat and meat products, bakery products, and desserts.

8.1 Freezing technology

The purpose of the freezing process is to decrease the temperature of the product to a temperature range leading to the formation of ice crystals within the product structure. The temperature of the product is reduced as much as it economically feasible in the attempt to reduce the quality deterioration rates of the product followed by storage at low temperature (most commonly -18°C) (Rahman, 2012). As a result, the storage life of a perishable food is extended (Heldman, 1997).

In freezing process, the sensible and latent heat of fusion of water within the product are removed by a surrounding low temperature medium (Figure 9), consequently, water (liquid) is transformed into ice (solid) and aqueous phase with water content about 20g per 100g sample. Heat is removed from the product's surface by convention and by conduction within the product leading to the formation of the frozen layer at the surface and the freezing part move from the outside towards the thermal center (Rahman, 2012). A typical freezing process consists of three stages (Figure 10) (1) pre-cooling section for reducing the temperature of the product to its freezing point, (2) phase transition stage of water to ice by removing the latent heat of crystallization which is an important step for determination of the process efficiency and frozen product quality, and (3) tempering stage where the product temperature falls from freezing point toward the freezing medium to the storage temperature (Kiani and Sun, 2011; Rahman, 2012).

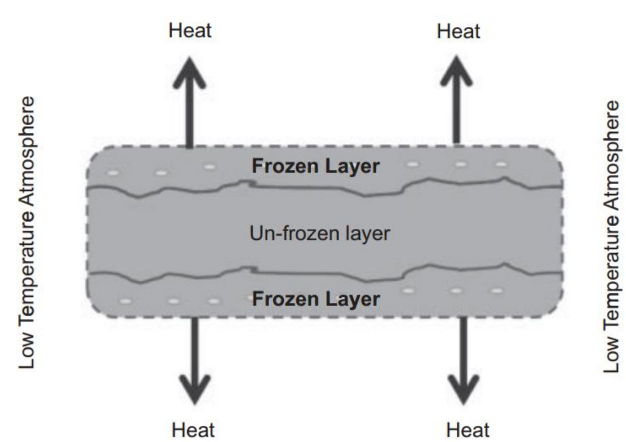


Figure 9 Diagram of freezing process.

Source: Rahman (2012)

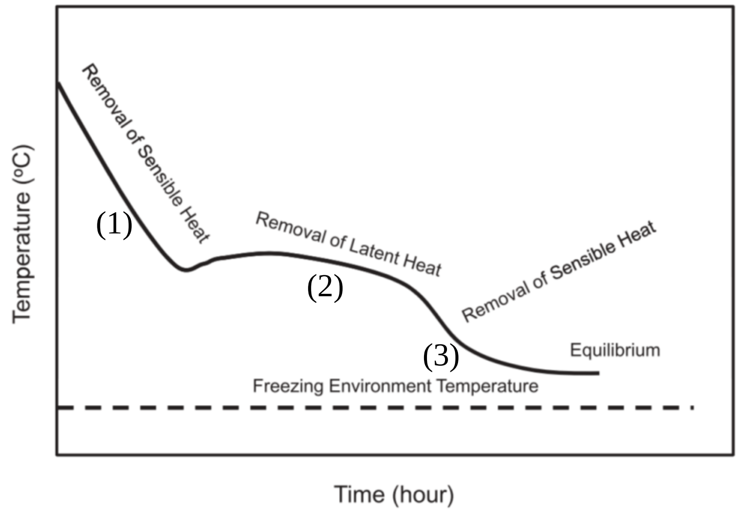


Figure 10 Temperature-time curve for freezing process consisting of (1) pre-cooling (2) phase transition and (3) tempering stages.

Source: modified from Rahman (2012)

It has been demonstrated that temperature fluctuations during storage and transportation cause changes in the structure of ice crystals and recrystallization (Phimolsiripol *et al*., 2008) which could deteriorate the quality and shorten the shelf-life of frozen product (Blond and Meste, 2004). Moreover, the refreezing-thawing process caused by temperature fluctuations may produce defects in the texture of frozen product making them to be unacceptable by consumer.

8.2 Frozen cake

Freezing has been claimed as an ideal method for preservation of nearly all baked products, such as bread, cake, pie, pastry and pizza. The freezing technique has been apply for the baked goods to reduce costs of production and distribution (Pruthi, 1999) and also increase the shelf-life of baked products. However, this process could deteriorate final products (Cauvain, 1998).

For frozen cake, the quality of frozen cake, especially cake crumb, is affected by the interactions between its ingredients during freezing process and storage. The deterioration of frozen cake could be a result from the staling process due to starch retrogradation; drying because of crumb’s moisture migration and redistribution; and crumb weakening caused by structural disruption from ice crystal formation and growth during storage (Cauvain, 1998).

Few researchers focused on the effect of freezing on the quality of frozen cake. For example, the effect of freezing on the quality of shortened cakes by comparison between fresh baked cakes, cakes baked from frozen batter and reheated frozen cakes was studied by Owen and Duyne (1950). The result showed that freezing decreased the cake volume, palatability and desirability scores, but increased the compressibility values compared to fresh baked cakes. The volume of frozen cakes was lower with longer freezing storage (up to 16 weeks). The frozen baked cakes reheated in an oven, had a better quality and larger volume than those baked from frozen batter. The similar result also reported for spice cakes (Skarha and Duyne, 1995). In addition, they stated that the volume, compressibility and palatability of frozen baked cakes thawed at room temperature for 5 hours did not differ significantly from those thawed in an oven at 300°F (148.9°C) for 30 minutes.

Recently, The effect of batter freezing on quality of layer and sponge cakes was studied (Gómez *et al*., 2011). The freezing process led to an increase in batter density indicating a lower volume of retained gas in batters that had been frozen. The air bubbles were more irregularly distributed and were larger after freezing (Figure 11) which could be a result of the breakdown of interfacial films that stabilize the interfaces between fat/water and fat/air in the layer cake, or due to weakening of proteins that stabilize the emulsion by the formation of ice crystal or an increase in solute concentration in the sponge cake. This irregular distribution could lead to coalescence phenomena and loss of air due to movement of the bubbles to the batter surface. As a result, the volume and height of cakes made from frozen batter were lower, but the crumb hardness were higher than those made from non-frozen batter.

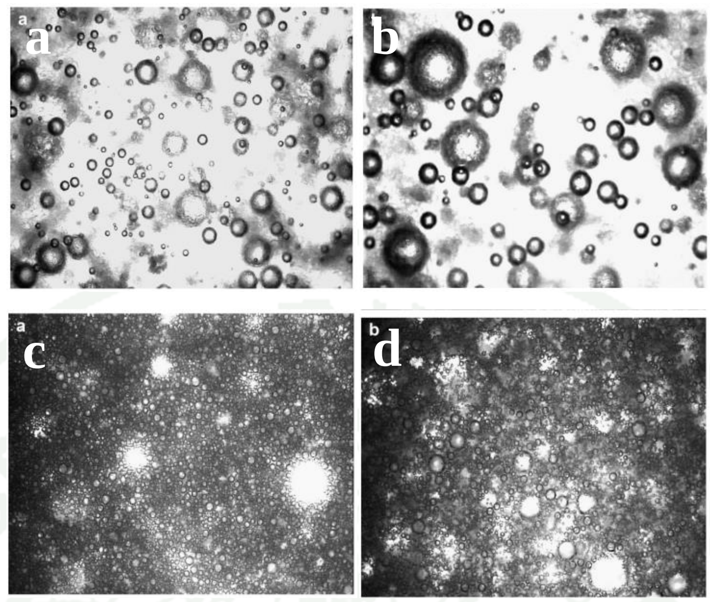


Figure 11 Internal structure (40×) of the batter of layer cake (a and b) and sponge cake (c and d) without previously frozen (a and c) and after freezing (b and d).

Source: Gómez *et al.* (2011)

The results of this research will provide fundamental understanding of the influence of freezing on the change in quality of frozen cake, especially after repeated freezing and thawing process. Moreover, the way to improve the quality of freeze-thawed cake by native and extruded WRF substitution for WF will be suggested.

MATERIALS AND METHODS

Materials

1. Cake-specific wheat flour (WF) (United Flour Milled Public Co., Ltd., Thailand)
2. Emulsifier (SP®) (Caltech Corp., Ltd., Thailand)
3. Fresh whole egg (Supermarket)
4. Fresh whole egg (Chicken farm, Faculty of Agriculture, Kasetsart University, Thailand)
5. Pasteurized milk (Charoen Pokphand Meiji Co., Ltd., Thailand)
6. Rice flour (RF) (Cho Heng Rice Vermicelli Factory Co., Ltd., Thailand)
7. Salt (Thai Refined Salt Co., Ltd., Thailand).
8. Sugar (Mitr Phol Sugar Corp., Ltd., Thailand)
9. Unsalted butter (Kim Chua Group Co., Ltd., Thailand)
10. Waxy rice flour (WRF) (Thai Wah Food Public Co., Ltd., Thailand
11. Waxy rice flour (WRF) (Cho Heng Rice Vermicelli Factory Co., Ltd., Thailand)

Chemical substances

1. Magnesium nitrate ($Mg(NO_3)_2$, Lobachemie, India)
2. Rhodamine B ($C_{28}H_{31}ClN_2O_3$, Sigma, India)
3. Glutaraldehyde (Merck, Germany)
4. Potassium dihydrogen orthophosphate (KH_2PO_4, Ajax Finechem Phy Ltd., New Zealand)
5. di-Potassium hydrogen orthophosphate (K_2HPO_4, Ajax Finechem Phy Ltd., New Zealand)
6. Potassium sulphate (K_2SO_4, Carlo Erba Reagenti, Italy)
7. Ethanol (C_2H_5OH, Merck, Germany)

Instruments

1. Blender for dry material (Turbora TRK-01, Napat Inter Ltd., Thailand)
2. Blender for wet material (HW-BDC2L, House worth, China)
3. Centrifuge (Z206A, Hermle, Germany)
4. Chest freezer (SF-C1497, Sanyo, Japan)
5. Confocal laser scanning microscopy (CLSM, Axio Imager MI, Germany)
6. Cream whipper (Profi Whip-1730, ISI GmbH, Austria)
7. Critical point dryer (CPD, K850, Energy Beam Sciences, USA)
8. Cryogenic cabinet freezer (Minibatch 1000 L, Bangkok Industrial Gas, Thailand)
9. Differential scanning calorimeter (DSC, Pyris-1, Perkin Elmer, USA)
10. Electric oven (CP-N, Chung Pu Paking Machinery, Taiwan)
11. Extruder (EV25-A120, Clextral, France)
12. Hot air oven (ULE-500, Memmert, Germany)
13. Light microscope (Leica DME, Leica Microsystems, USA)
14. Rapid visco analyzer (RVA, RVA-3D, Newport Scientific Instrument & Engineering, Australia)
15. Scanning electron microscope (SEM, JSM-5600LV, JEOL, Japan)
16. Spectrophotometer (GENESYSTM10, Thermo Electron Corporation, USA)
17. Texture analyzer (TA-XT plus, Stable Micro Systems, UK)
18. Viscometer (HBD-VII+, Brookfield, USA)
19. Vortex mixer (Vortex-Genie2, Scientific Industries Inc., USA)
20. Water bath (Schutzart DIN40050-IP20, Memmert, Germany)
21. X-ray diffractometry (XRD, JDX-3530, JEOL, Japan)

Methods

Part 1: Influence of waxy rice flour (WRF) substitution for wheat flour (WF) on characteristics of batter and freeze-thawed cake.

In the first part, the potential of WRF for WF replacement was studied using frozen cake as a model. Firstly, the influence of freezing and thawing process on the quality of cake was examined. Then, the effect WRF substitution for WF on the properties of batter and freeze-thawed cake were studied. The moisture contents of the WF and WRF (Thai Wah Food Public Co., Ltd., Thailand) were 12.20 and 12.14%, respectively. The amylose contents were 32.33±0.12% for WF and 8.43±0.06% for WRF (Appendix Figure 1).

1.1 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. The pasting profiles of flour suspensions were analyzed by a RVA (AACC, 200). 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.

1.2 Microstructure of wheat flour and waxy rice flour before and after heating

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 a CLSM with the LSM 5 PASCAL program. An HeNe laser was used to excite the Rhodamine B at 543nm.

1.3 Batter and cake preparation

The formula for cake preparation was 200g WF, 200 g sugar, 360 g fresh whole eggs, 70 g water, 40 g butter, 30 g milk, 4 g emulsifier, and 3 g salt. The batter was prepared by mixing using a blender. At first all ingredients, except the WF, were mixed at a maximum speed for 30 s. Then, WF was added and mixed together at minimum speed for 10 s. Then, the mixture was filled into a cream whipper and shaken well. Next, 150g of batter was placed into a butter coated aluminium pan (diameter 125 mm and depth 60 mm) and baked in an electric oven 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 plastic bag (NY/LLDPE, 70 μm).

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.

1.4 Freezing and thawing

Cake in a sealed plastic bag was frozen in a cryogenic cabinet freezer with -40ºC atmospheric temperature until the central temperature of the cake reached -25°C. The freezing profile of cake was showed in Appendix Figure 2. Then the frozen cakes were stored in chest freezer (SF-C1497, Sanyo, Japan) at -18°C for 20 h and thawed at room temperature (25 ± 2ºC) 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.

1.5 Batter analysis

1.5.1 Density

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.

1.5.2 Viscosity

The viscosity of the batter was measured five times using a viscometer with spindle-21 resolved at 100 rpm (adapted from a method of Gómez *et al.*, 2011).

1.5.3 Microstructure

A drop of freshly mixed batter was placed on a microscope slide and a cover slip was applied. The microstructure of the batter was observed under a light microscope at 40× magnification.

1.6 Cake analysis

The weight of cakes were measured after 1-hour removal from the pan. Then, the middle of cake were cut with the thickness of 0.5 cm for taking a cross-section view picture. All quality measurements were performed within 4 h after baking.

1.6.1 Baking loss

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

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

1.6.2 Moisture content

Moisture content was determined in triplicate according to AACC Approved Methods 44-15A (AACC, 2000) .

1.6.3 Texture analysis

For texture determination, the crust was removed and the central crumb section was sliced into four (25×25×20) mm samples for measurement. The firmness of each sample was determined using a texture analyzer 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 20g, and time, 2s. The firmness was defined as the peak force during the first compression cycle.

1.6.4 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 = least firmness and 9 = most firmness). The acceptance test was conducted by 30 untrained panelists using a hedonic 9-point scale (9 = like extremely and 1 = dislike extremely). The sensory evaluation forms were showed in Appendix 1 and Appendix 2.

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

The fresh-baked and freeze-thawed cakes with and without WRF substitutions were cut into samples (3×3×3 mm). The samples were fixed in 2% glutaraldehyde in 0.5M 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 CPD. They were mounted on a stub coated with gold and observed using a SEM. The magnification and accelerating voltage are showed on each SEM image.

1.6.6 Retrogradation properties of freeze-thawed cakes

The retrogradation properties of freeze-thawed cakes were analyzed by DSC using nitrogen as the purge gas with the modified method of Yu *et al.* (2010). Fresh and thawed cakes were cut into samples (2×2×2 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. The dried cake sample was blended 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.

1.7 Experimental design and statistical analysis

Each experiment was repeated twice. Batter and cake characteristics 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).

Part 2: The effect of moisture content on physicochemical properties of extruded WRF (exWRF).

The result from part one indicated that WRF could retard an increase in firmness of frozen cake; however, it could not reduce the baking loss and moisture loss during repeated freezing and thawing. Thus, in this part, the extrusion process was used to modify the properties of WRF and RF was also studied in order to compare with WRF. The effect of different moisture contents in the feeding material on the physicochemical properties of modified flour was studied. In addition, the change in T_g value related to the molecular degradation resulted from the extrusion was studied. Since we could not buy a large amount of WRF from Thai Wah Food Public Co., Ltd. during Part 2 experiment, the WRF and RF were purchased from Cho Heng Rice Vermicelli Factory Co., Ltd. instead of Thai Wah Food Public Co., Ltd. The moisture content of WRF and RF were 12.55 and 11.88%, respectively.

2.1 Extrusion process

Extrusion was performed in a co-rotating twin screw extruder. The extruder barrel was segmented into 6 zones with the controlled temperature profile as follows: 30-30-45-45-60-75°C. The feed rate of flour and the screw speed were set at a constant 5 kg/h and 350 rpm, respectively. The water feed rate was varied from 0.5 to 1.0 l/h which resulted in different moisture contents of the feeding material as shown in Table 2. A single circular die with 3.2 mm diameter was used. The extruded products were cut into about 1 cm lengths before drying in a hot air oven at 40°C for 24 h. The dried extruded products were ground using a blender (for dry material) and passed through a 100-mesh sieve screen.

Table 2 Moisture content of waxy rice flour (WRF) and rice flour (RF) with different water feed rates in extrusion process.

Flour feed rate (l/h)	Water feed rate (l/h)	Calculated moisture content (%)	
		WRF	RF
5.0	0 (Native)	12.55	11.88
5.0	0.5	20.50	19.89
5.0	0.6	21.92	21.32
5.0	0.7	23.29	22.70
5.0	0.8	24.61	24.03
5.0	0.9	25.89	25.32
5.0	1.0	27.13	26.57

2.2 Characteristics of native and extruded WRF and RF

2.2.1 Microstructure

The native and extruded WRF and RF were dried in hot air oven at 40 °C for 3 d. After that, they were fixed on a stub and coated with gold. The samples were observed using a SEM with the magnifications and accelerating voltages are showed on each SEM image.

2.2.2 Relative crystallinity

The flour samples were equilibrated for 3 d in a desiccator containing saturated K_2SO_4 solution in order to prepare flour samples containing 20% H_2O (wet basis) according to the method of Phothiset and Charoenrein (2007). The crystallinity of native and extruded WRF and RF was determined by X-ray diffractometry (JDX-3530, JEOL, Japan) with Cu-K_α radiation (wavelength = 1.5406Å) and a Ni filter. The scanning diffraction angle (2θ) ranged from 5 to 40° and the X-ray generator was operated at 45 kV and 45 mA. The relative crystallinity (%) of the samples was calculated according to the method of Cheetham and Tao (1998).

2.2.3 Water absorption index (WAI) and water solubility index (WSI)

The WAI and WSI of native and extruded samples were measured according to the method of Anderson *et al.* (1969). The samples (2 g dry basis) were dispersed in water at 30 °C for 30 min with mixing every 5 min by vortex. Then, the supernatant was separated using a centrifuge at 3000×g for 15 min and placed into an aluminum pan and dried in the hot air oven at 90°C for 6 h. The WAI and WSI were determined as follows:

$$WAI\ (g/g) = \frac{Weight\ of\ wet\ sediment}{Dry\ weight\ of\ sample}$$

$$WSI\ (\%) = \frac{Weight\ of\ dry\ solid\ in\ supernatant}{Dry\ weight\ of\ sample} \times 100$$

2.2.4 Glass transition temperature

The native and extruded flour samples (6 mg on a dry basis) were weighed and placed in an aluminum pan and equilibrated for 24 h to a relative humidity of 52.9% at 25°C using saturated $Mg(NO_3)_2$. After that, the pan was immediately sealed and accurately weighed before analysis with a differential scanning calorimeter (DSC; Pyris-1, Perkin Elmer, USA) using nitrogen as the purge gas. The pans were cooled from 25°C to 5°C, held at this temperature for 1 min and then heated to 170°C at a rate of 10 °C/min. Then, they were cooled to 5°C and re-heated to 170°C at the same rate. No shift in the heat capacity could be observed in the second scan; therefore, the T_g values were determined from the onset of the heat capacity change over the glass transition. The example of thermogram was showed in Appendix Figure 3. All measurements were performed in triplicate.

2.2.5 Pasting properties

Suspensions of native and extruded flour were prepared at 8% (w/w) in distilled water. The pasting properties of each suspension were measured using a RVA (AACC, 2000). The method in part 1.1 was applied to analyze the pasting profile of the samples. All measurements were performed in triplicate.

2.2.6 Gelatinization properties

The native and extruded flour samples were weighed to provide 6 mg (dry basis) into a stainless steel pan, and then distilled water was added to obtain 70% moisture content. After that, the pan was hermetically sealed and kept at the room temperature (25 ± 2°C) for 24 h. The gelatinization properties of the samples were analyzed using the DSC modified from a method of Thirathumthavorn and Charoenrein (2005). The samples were placed and held isothermally at 25°C for 1 min in the DSC before heating from 25°C to 130°C at a rate of 10°C/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) were calculated. The data were reported as the average of triplicate measurements.

2.3 Experimental design and statistical analysis

The properties of the native and extruded WRF and RF were analyzed using analysis of variance followed by Duncan's test ($p \leq 0.05$) using the SPSS program (SPSS 17.0, SPSS Inc., USA). Each experiment was repeated twice.

Part 3: Application of extruded waxy rice flour (exWRF) for quality improvement of freeze-thawed cakes.

After WRF and RF (Cho Heng Rice Vermicelli Factory Co., Ltd., Thailand) was modified in the second part, the extruded WRF and RF were applied to substitute for WF at 10% (w/w) in order improve the quality of freeze-thawed cakes. The modified flour by the extrusion with the water feed rate at 0.8 l/h, which produced extruded WRF and RF with the highest WAI, was selected for continuing study in this part. The application of substitution of extruded WRF and extruded RF for WF was studied on the characteristics of fresh and freeze-thawed cakes. In this part, fresh eggs from chicken farm was used instead of eggs from supermarket.

3.1 Batter and cake preparation

The batter of cake was prepared according to the method in part 1.3. For studying the effects of extruded WRF and RF substitutions for WF, the basic control cake formulation was used, but the amount of WF was substituted by 10% (w/w) of extruded flour. The WF and substituting flour were blended together before mixing with other ingredients.

3.2 Freezing and thawing

Cake in a sealed a plastic bag was subjected to 5 freeze-thawed cycles according to the method in part 1.4. After that, the samples were taken for further analysis.

3.3 Batter analysis

The properties of batter substituted with extruded WRF and RF for WF; density, viscosity and microstructure, were observed according to the method in 1.5.

3.4 Cake analysis

Cakes were weighted and photographed after 1 hour after removal from the pan. All quality measurements of freeze-thawed cakes, which were baking loss, moisture content, texture, sensory analysis, microstructure and retrogradation properties, were performed within 4 h after baking according to the method in 1.6.

3.5 Experimental design and statistical analysis

Each experiment was repeated twice. Batter and cake characteristics 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).

Place

The Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University (Bangkhen campus), Thailand.

Duration

The study was performed from April 2012 to May 2014.

RESULTS AND DISCUSSION

Part 1: Influence of waxy rice flour (WRF) substitution for wheat flour (WF) on characteristics of batter and freeze-thawed cake.

In this study, we studied the adverse effect of freezing and thawing on the quality of frozen WF cake and then 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. WRF was used to substitute for WF at 10, 15, and 20% w/w. The properties of blended flour, batter and characteristics fresh and frozen cakes prepared with substituting flour were evaluated.

1.1 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 Figure 12. 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 (Tester and Morrison, 1990; Chaiwanichsiri *et al*., 2001). For WF, the pasting temperature was higher than for WRF (Chaiwanichsiri *et al*., 2001). The crystallinity of wheat starch was 35.5% (Morrison *et al.*, 1994) which was higher than those of waxy rice starch 18.5-23.8% (Lu *et al.*, 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 and 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).

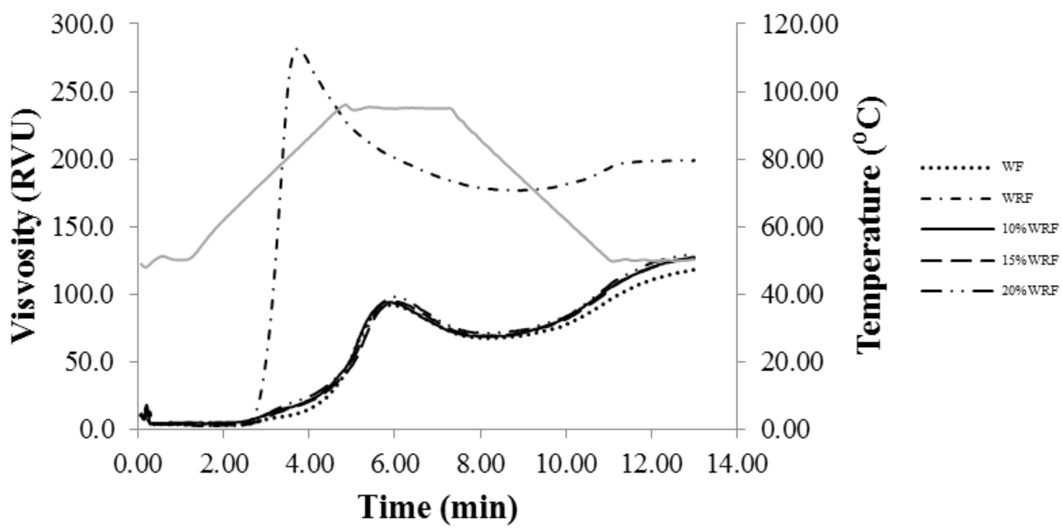


Figure 12 Pasting profile of wheat flour, waxy rice flour and waxy rice flour substitutions for wheat flour (WF, wheat flour; WRF, waxy rice flour; 10%, 15%, and 20% WRF are 10%, 15%, and 20% w/w of waxy rice flour substitution, respectively).

1.2 Microstructure of wheat flour and waxy rice flour before and after heating

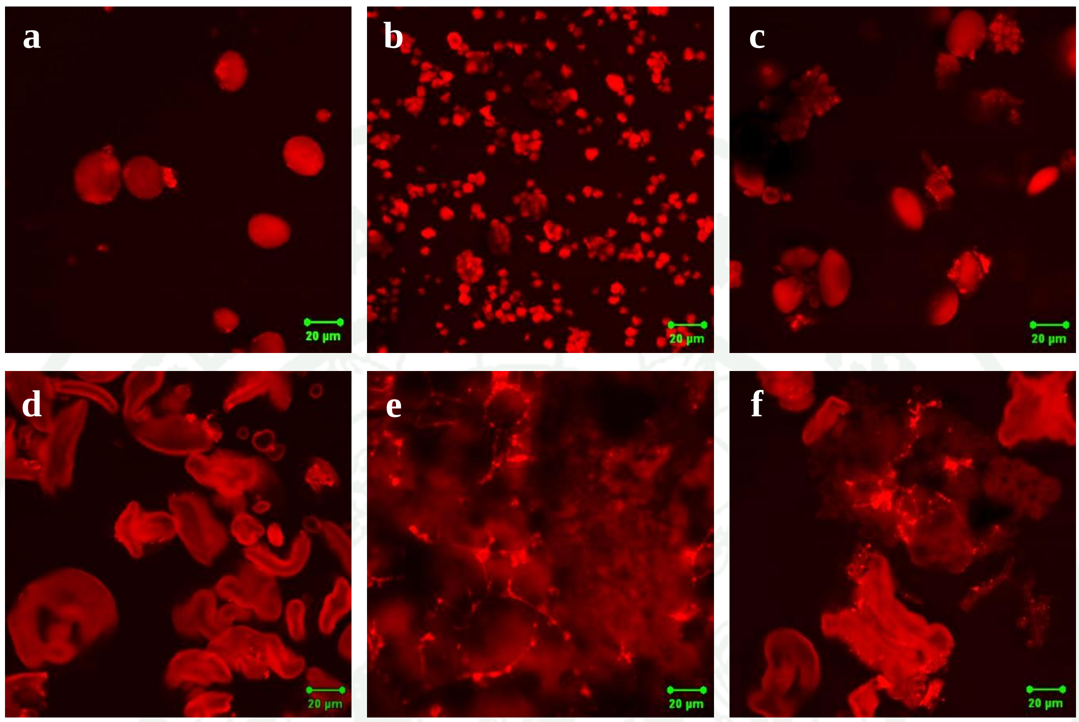


Figure 13 Confocal laser scanning micrographs of unheated (a, b and c) and heated (d, e and f) flour solutions (8% w/w). Starch granules of wheat (a and d), waxy rice (b and e) and 10% w/w waxy rice flour substitution (c and f) were stained with Rhodamine B (appear as red). Bars=20µm.

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 substitution of WF were stained with Rhodamine B and observed using CLSM. The unheated wheat starch granules were disk-shaped and spherical (Figure 13a) whereas the waxy rice granules were polygonal (Figure 13b). They were clearly distinguishable from each other in a suspension containing both WF and WRF (Figure 13c). After heating at 75°C for 30 min, the deformation of starch granules was observed. The wheat granules were swollen (Figure 13d) while the waxy rice granules were deformed and dispersed with a greater circumference (Figure 13e). In a suspension containing 10% WRF substituted for WF (Figure 13f), 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 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.

1.3 Batter characteristics

1.3.1 Viscosity and density of batter

The viscosity of batter was found to decrease with increasing the level of WRF substitution (Table 3). 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 (Loewe, 1993) during mixing. This finding was in line with the results of Wilderjans *et al*. (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 make an appreciable difference in density.

Table 3 Characteristics of cake batter and fresh-baked cake

Substitution of WRF for WF (% w/w)	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.

1.3.2 Microstructure of batter

The microstructure of the cake batter samples with different levels of WRF substitution for WF was evaluated under a light microscope. Figure 14 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 larger bubbles (Allais *et al.*, 2006); thus, larger bubbles were exhibited in batter substituted with WRF.

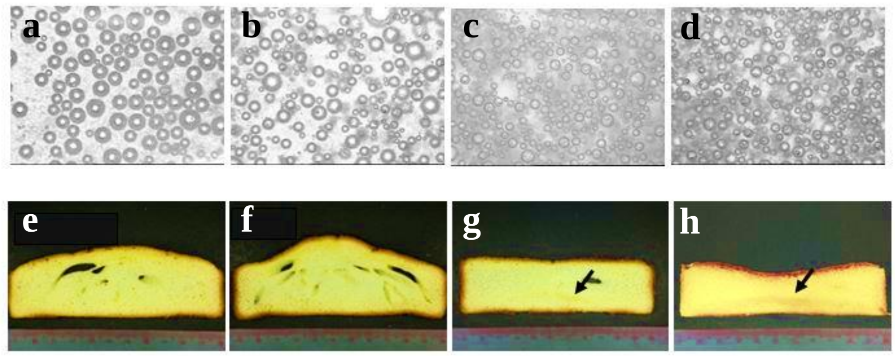


Figure 14 Light micrographs (40×) of aerated cake batter (a-d) and images of cakes (e-h) prepared with wheat flour (WF) (a,e) and 10% (b,f), 15% (c,g) and 20% (d,h) w/w waxy rice flour (WRF) substitutions. Arrows indicate the dense gummy layer of the cake crumbs.

1.4 Cake properties

For the properties of the unfrozen cake samples, any baking loss (Table 3) 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 (Figures 14g and 14h) and their heights were significantly ($p\leq0.05$) less than those of WF cake (Table3). This could have been the result of the diluted gluten content which serves as a binder in the cake (Donelson and 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 pasting profile; hence batter with 15% and 20% WRF substitutions 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 (Figures 13e and 13f) could be a result in lower height of cake substituted with 15% and 20% WRF. Furthermore, the cake samples with 20% WRF substitution collapsed upon cooling which was consistent with the results of Miller *et al.* (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 (Figure 14b) and its height was not different ($p>0.5$) from WF cake (Table 3) which showed that it contained sufficient of WF gluten.

1.5 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.

1.5.1 Moisture content of cake

The moisture content of freeze-thawed cake decreased significantly ($p \leq 0.05$) after the third freeze-thaw cycle (Table 4). 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 (Rahman and Perera, 1999; Reid, 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.

Table 4 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^{cA}	43.48±0.06^{cA}	43.42±0.04^{bA}	41.40±0.32^{aA}
	15	43.40±0.11^{cA}	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±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.d.	6.63±1.10^{bB}
	10	4.06±1.36^{aA}	4.60±1.18^{aA}	n.d.	5.34±0.77^{aA}
Firmness score by acceptance test	0	6.30±1.56^{bA}	5.92±1.31^{bA}	n.d.	4.64±1.75^{aA}
	10	6.97±1.38^{bA}	6.66±1.23^{bB}	n.d.	5.77±1.61^{aB}

n.d. = not determined

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.

1.5.2 Firmness of cake

The firmness of fresh-baked and freeze-thawed cake samples by texture analyzer were shown in Table 4. 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 (Cauvain, 1998; Kerr, 2004). Amylose was mainly responsible for the retrogradation associated with freezing and thawing (Charoenrein and Preechathammawong, 2012); therefore, cake containing WRF 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 (Figure 12); moreover, the spread of ruptured waxy rice starch granules covering wheat granules (Figure 13f) could prevent swollen wheat granules to pack tightly together and consequently reduction in the firmness of the cake. Charoenrein, S. and N. 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 cycle, the firmness of cake samples substituted with 0%, 10% and 15% WRF was raised by 211.63%, 74.34% and 106.63%, respectively (Table 4). 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, compared to the control.

1.5.3 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 4) 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 acceptance test, the fresh-baked cake had a significantly ($p \leq 0.05$) higher acceptance score than the sample passing through the fifth freeze-thaw cycle (Table 4). This result corresponded with work by Owen and Duyne (1950) 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 acceptance score than the control.

The results of the sensory evaluation on firmness acceptance (Table 4) 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 as shown in Appendix Figure 4). The fifth freeze-thaw cycle led to more firmness in the cake and lower acceptance by the panelists, compared to unfrozen cake. Substitution of 10% WRF provided freeze-thawed cake with lower firmness and a higher acceptance than the non-substituted samples.

1.6 Microstructure of fresh and freeze-thawed cakes evaluated by SEM

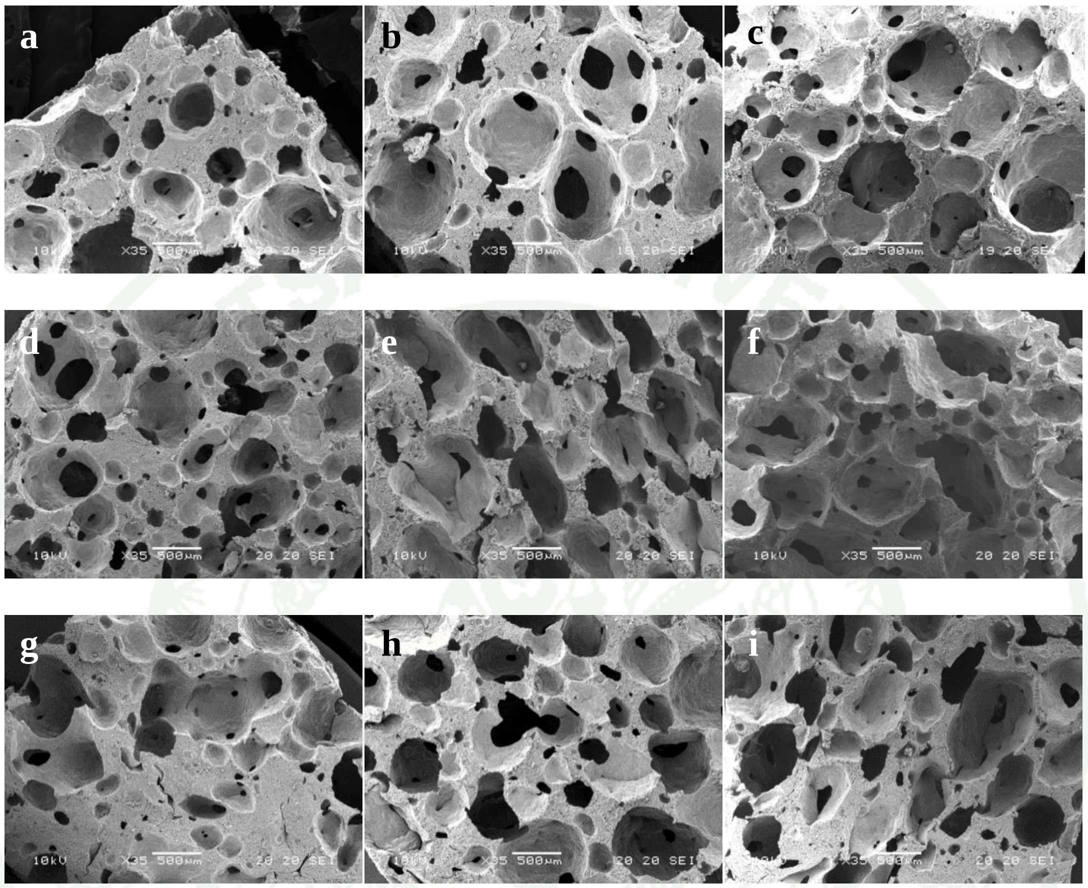


Figure 15 Scanning electron microscope images (35×) of fresh cake (a, d and g) and cakes with the first (b, e and h) and fifth (c, f and i) freeze-thaw cycle prepared with wheat flour (WF) (a-c), 10% (d-f) and 15% (g-i) waxy rice flour (WRF) substitution. Bars = 500µm.

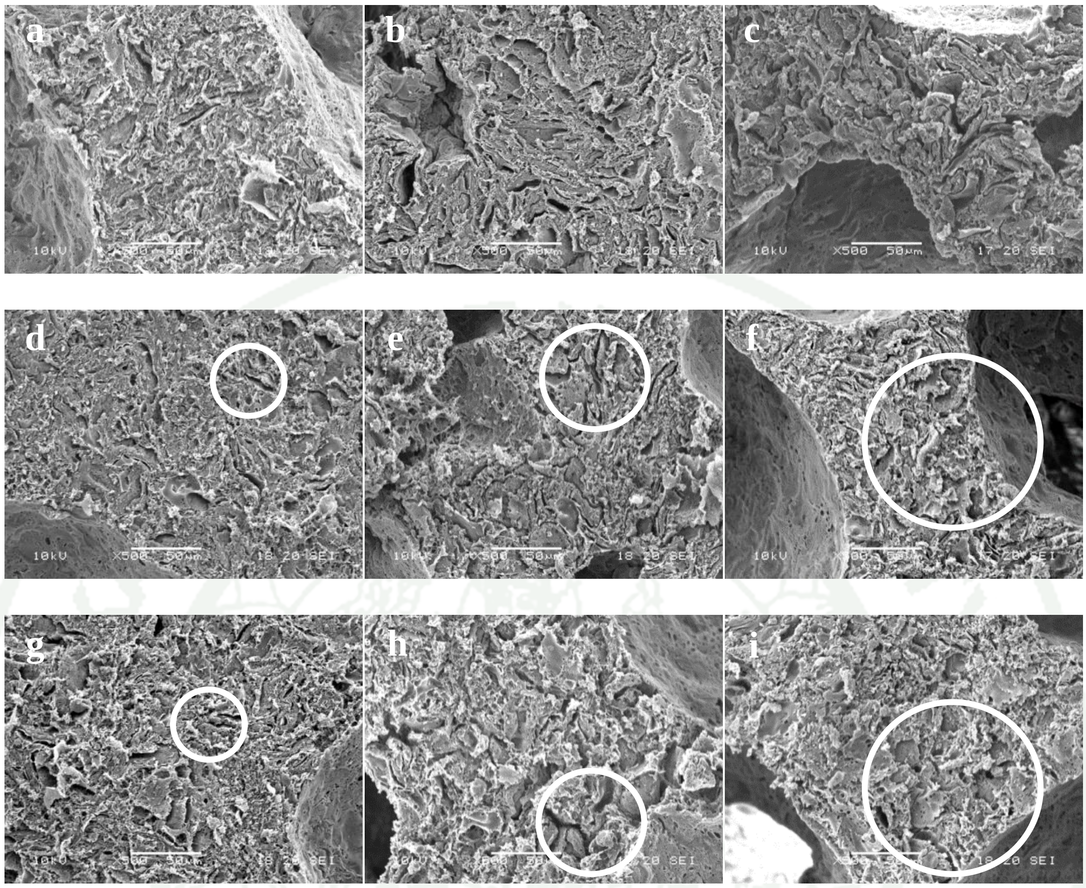


Figure 16 Scanning electron microscope images (500×) of fresh cake (a, d and g) and cakes with the first (b, e and h) and fifth (c, f and i) freeze-thaw cycle prepared with wheat flour (WF) (a-c), 10% (d-f) and 15% (g-i) waxy rice flour (WRF) substitution. The circles indicates channel-like structure. Bars = 50µm.

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× magnifications (Figures 15 and 16). 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 (Figure 15). A channel-like structure of the cake crumbs was noticeable in fresh-baked cake (Figure 16), similar to the finding reported by Turabi *et al.* (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 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 amylose retrogradation increased (Charoenrein and 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 4). Although the microstructure of fresh-baked cake prepared with WF looked similar to those samples prepared with the different levels of WRF substitution (Figures 16a, 16d and 16g), 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 4). With 15% WRF substitution, fresh-baked cake had less air pores (Figure 15g), 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 (Figure 14c). 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 4). These structural findings corresponded well with the firmness results from the texture analyzer.

1.7 Retrogradation properties of freeze-thawed cakes

An increase in the firmness of cake samples during unfrozen storage could have resulted from amylopectin retrogradation (Armero and Collar, 1998). Therefore, the enthalpy of melting retrograded amylopectin in fresh and freeze-thawed cake samples was measured using DSC. Table 5 shows a peak occurred at approximately 54.5°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 the first freeze-thaw cycle (Table 4). Furthermore, it was consistent with microstructure of cake after five freeze-thaw cycles that had the most compact structure (Figures 16c, 16f and 16i), which indicated that repeated freezing and thawing resulted in an increase in amylopectin retrogradation leading to a higher firmness value and consequently, a lower acceptance score. The ΔH value of cake subjected to five freeze-thaw cycles showed no significant ($p > 0.05$) difference between WF cake and cakes 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 the report of 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.

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 retrogradation 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.

Table 5 Retrogradation properties of flour prepared from fresh and freeze-thawed cakes

Number of freeze-thaw cycles	WRF Substitution (% w/w)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{ret} (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_{ret} = enthalpy of melting retrograded flour

n.d. = not detectable (no peak)

The result from the first part showed that repeated freeze-thaw cycles resulted in a higher firmness of freeze-thawed cake which was due to the more compact structure, lower moisture content and higher starch retrogradation than those of fresh-baked cake. However, substitution of WRF for WF could retard the starch retrogradation by spreading swollen waxy rice starch granules to cover wheat starch granules (Figure 17) evident from the confocal laser scanning micrographs. As a result, the firmness of freeze-thawed cake substituted with WRF was subsequently lowered than freeze-thawed WF cake.

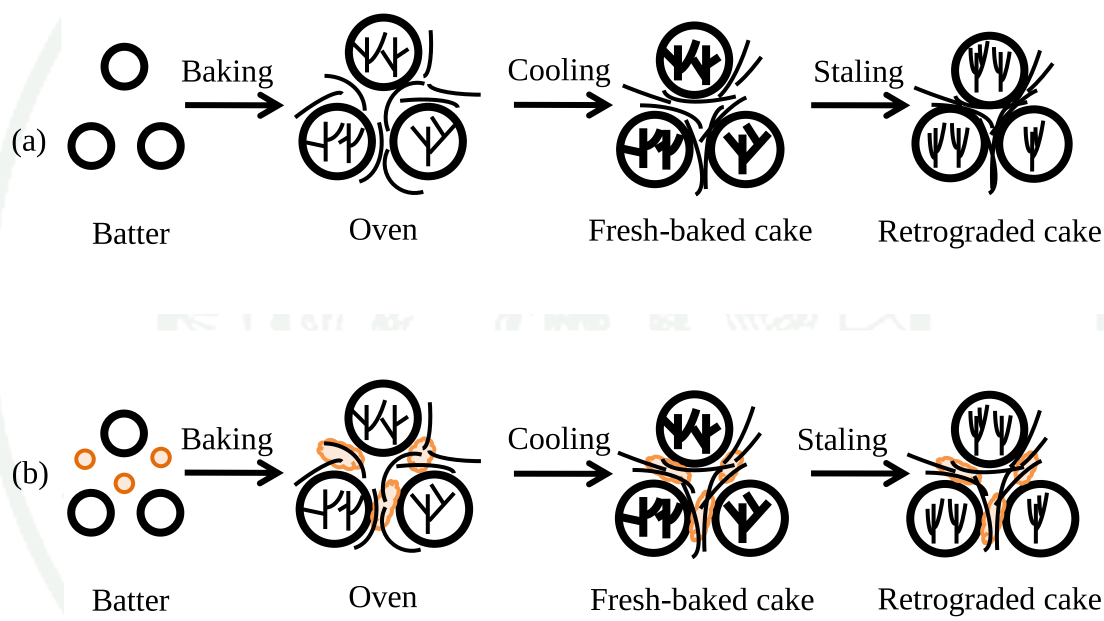


Figure 17 Staling of cakes prepared with wheat flour only (a) and with waxy rice flour substitution for wheat flour (b) (O = wheat granules and o = waxy rice granules)

Although WRF seems to be an effective agent to improve the firmness of freeze-thawed cake, it could not retard the baking loss and moisture loss during repeated freezing and thawing process. A reduction in moisture content in frozen bakery products could affect their quality and palatability resulting in lower consumer acceptance.

Part 2: The effect of moisture content on physicochemical properties of extruded waxy and non-waxy rice flour

In this part, extrusion was applied to produce the modified WRF in order to increase its water absorption ability which could have a potential to reduce the baking loss and moisture loss in frozen bakery products. RF was also modified for comparison with the properties of exWRF. Moreover, the effect of different moisture contents in the feeding material for extrusion on the physicochemical properties including the changes in granular structure, starch crystallinity, water solubility characteristics, glass transition temperature and pasting and gelatinization properties of exWRF and exRF was studied.

2.1 Characteristic of extruded products

The appearance of the extruded products of rice flour could be affected by the amylose and amylopectin content of the flour and the moisture of feeding materials which can be adjusted by the water feed rate in the extrusion process. Table 2 shows that the extrusion with the lowest water feed rate (0.5 l/h) resulted in the lowest moisture content in the feeding flours (20.50% for exWRF and 19.89% for exRF), and the exWRF products expanded and then collapsed immediately after emerging from the die (Figure 18a), whereas exRF did not show any obvious collapse (Figure 18). The expansion and collapse of products were reduced when a higher water feed rate was used (Figures 18b-f and 18h-l) which agreed with the results of Singh *et al.* (2007) who reported extrusion with a higher water feed level decreased the expansion of the extruded product. In the current study, the extrusion process with a lower water feed rate resulted in dry and hard extruded products with a lower moisture content (Table 6); however, sticky and soft extruded flour with a higher moisture content was produced using an extrusion process with a higher water feed rate. At the same water feed rate (as clearly seen at a water feed rate 0.7 l/h, Figures 18c and 18i), the expansion of extruded products was lower in the exRF than for the exWRF because of the higher amylose content of the RF compared to that of the WRF. The linear amylose chains were difficult to pull apart during expansion because they aligned themselves in the shear field of extrusion (Martínez *et al.*, 2012) leading to lower expansion of the extruded RF; however, amylopectin starches were not as hard as amylose starches which also favored expansion (Valle *et al.*, 1996).

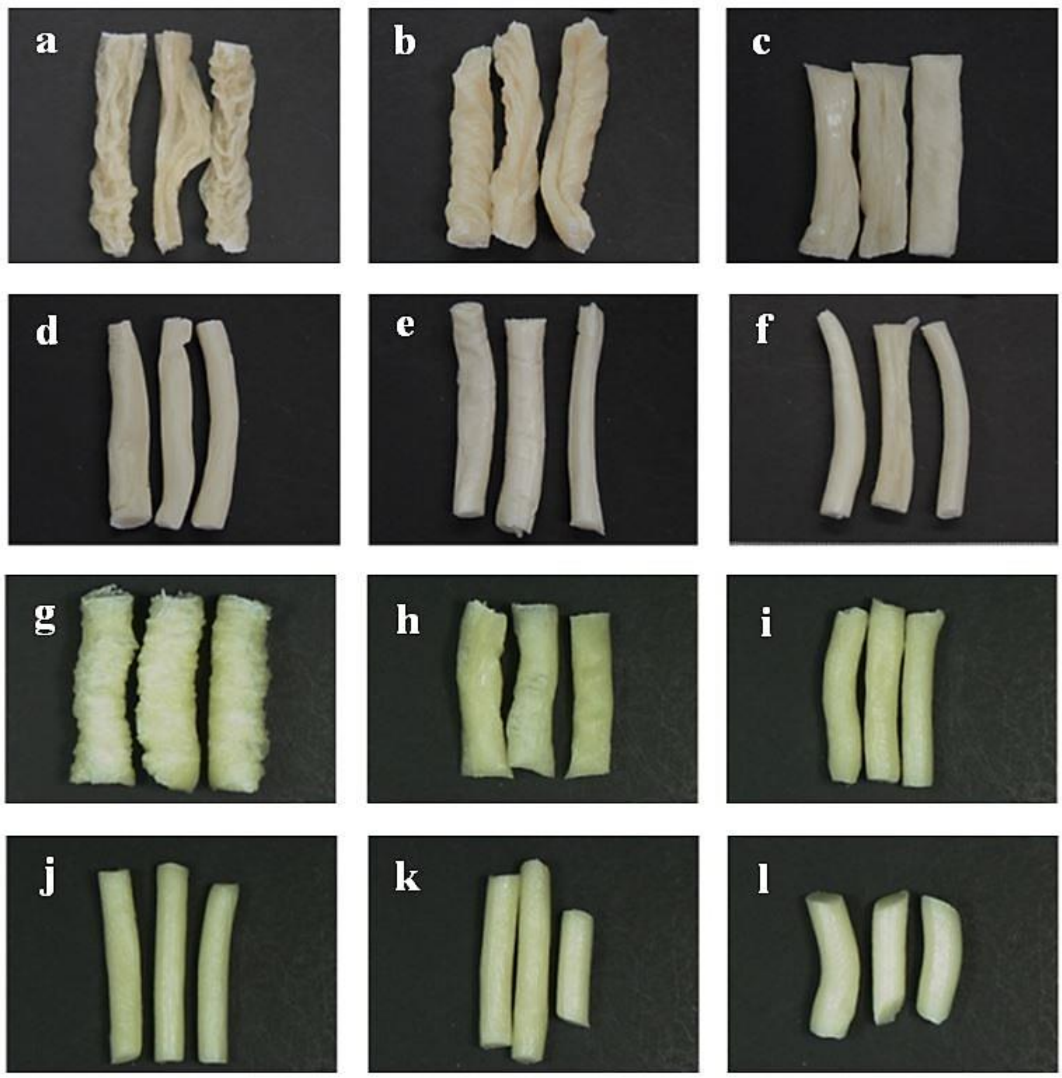


Figure 18 Products of waxy rice flour (a-f) and rice flour (g-l) modified by extrusion process with water feed rate at 0.5 l/h (a, g), 0.6 l/h (b, h), 0.7 l/h (c, l), 0.8 l/h (d, j), 0.9 l/h (e, k) and 1.0 l/h (f, l).

Table 6 Moisture content and characteristics of native and extruded waxy rice flour (WRF) and rice flour (RF).

Flour	Water feed rate (l/h)	Moisture content (%) of extruded product	WAI (g/g)	WSI (%)	Gelatinization properties			
					T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g dry flour)
WRF	Native	12.55 ± 0.59^a	2.16 ± 0.15^d	0.69 ± 0.02^a	62.3 ± 0.93^a	69.7 ± 1.62^a	77.1 ± 3.64^a	8.9 ± 3.67^b
	0.5	20.08 ± 0.05^b	0.55 ± 0.03^a	82.45 ± 0.65^g	n.d.	n.d.	n.d.	n.d.
	0.6	22.15 ± 0.84^c	1.00 ± 0.04^b	73.00 ± 1.96^f	66.1 ± 0.03^b	72.1 ± 0.47^b	76.8 ± 2.21^a	0.2 ± 0.12^a
	0.7	22.90 ± 0.58^c	1.85 ± 0.07^c	59.83 ± 0.62^e	65.8 ± 0.97^b	72.0 ± 0.35^b	77.3 ± 0.75^a	0.7 ± 0.14^a
	0.8	24.13 ± 0.25^{cd}	3.04 ± 0.06^f	39.04 ± 0.62^d	66.5 ± 0.13^b	72.5 ± 0.12^b	77.8 ± 0.03^a	1.1 ± 0.08^a
	0.9	25.37 ± 1.24^d	3.07 ± 0.08^f	25.94 ± 0.21^c	66.2 ± 0.73^b	72.0 ± 0.35^b	77.6 ± 0.20^a	1.6 ± 0.29^a
	1.0	29.37 ± 1.44^e	2.72 ± 0.01^e	12.25 ± 0.06^b	65.3 ± 0.07^b	71.6 ± 0.12^b	77.1 ± 0.36^a	2.5 ± 0.05^a
RF	Native	11.88 ± 0.68^a	2.05 ± 0.07^a	0.34 ± 0.01^a	76.1 ± 0.07^a	79.3 ± 0.10^a	83.3 ± 0.57^a	6.9 ± 0.10^b
	0.5	16.89 ± 0.57^b	6.01 ± 0.12^b	25.90 ± 0.23^g	n.d.	n.d.	n.d.	n.d.
	0.6	18.67 ± 0.31^c	8.78 ± 0.06^f	9.32 ± 0.10^f	n.d.	n.d.	n.d.	n.d.
	0.7	20.89 ± 0.45^d	8.17 ± 0.04^e	6.70 ± 0.05^e	n.d.	n.d.	n.d.	n.d.
	0.8	22.59 ± 0.04^e	7.47 ± 0.04^d	4.92 ± 0.02^d	78.2 ± 0.18^c	81.1 ± 0.47^b	84.0 ± 0.58^a	0.1 ± 0.02^a
	0.9	23.89 ± 0.37^f	6.87 ± 0.05^c	3.85 ± 0.03^c	77.2 ± 0.68^b	80.6 ± 0.35^b	84.0 ± 0.79^a	0.2 ± 0.10^a
	1.0	25.59 ± 0.49^g	6.03 ± 0.03^b	2.84 ± 0.02^b	75.4 ± 0.06^a	80.2 ± 0.23^b	84.8 ± 0.08^a	0.3 ± 0.02^a

Data are mean ± standard deviation

WAI = water absorption ability, WSI = water solubility inde

[a-g]: Means with different superscripts in the same column for each rice flour are significantly ($p \leq 0.05$) different

n.d. = not detectable (no peak)

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, ΔH_{gel} = enthalpy of gelatinization

2.2 Microstructure of native and extruded WRF and RF

SEM micrographs showed that the starch granules of the native WRF and RF were polygonal (Figures 19a, 19f, 20a and 20f) but the starch granules were bound together and appeared as large particles as a result of extrusion (Figures 19b-e and 20g-j). After the product emerged from the extrusion die, a sudden disequilibrium between the extruded product and the atmosphere caused steam generation within the product (Miller, 1994); consequently pores, on the exWRF particles could be observed (Figures 20b-e). However, no pores were visible on the exRF particles (Figures 20g-j). The higher peak and final viscosity of the exRF (Figure 23b) made it difficult for the RF extrudate to form pores. Moreover, the smoother surfaces of the exRF compared to those of the exWRF may have resulted from the presence of amylose which could leach out of the starch granules during the extrusion cooking and bind to the surface of the exRF particles (Gill *et al.*, 2002). At 3000× magnification (Figure 20), the formation of the coarse structure could be seen in the extruded flour of both the WRF and RF which may have been produced by an alignment of starch molecules during shear stress in the extrusion process (González *et al.*, 2007). Sample with a higher water feed rate in the extrusion process had smoother surfaces which represented less severe conditions during the extrusion process because the moisture acted as a plasticizer during extrusion and lowered the extent of shear degradation (Lai and Kokini, 1991) leading to less damage in the extruded flour compared to samples with coarser surfaces resulted from using a lower water feed rate.

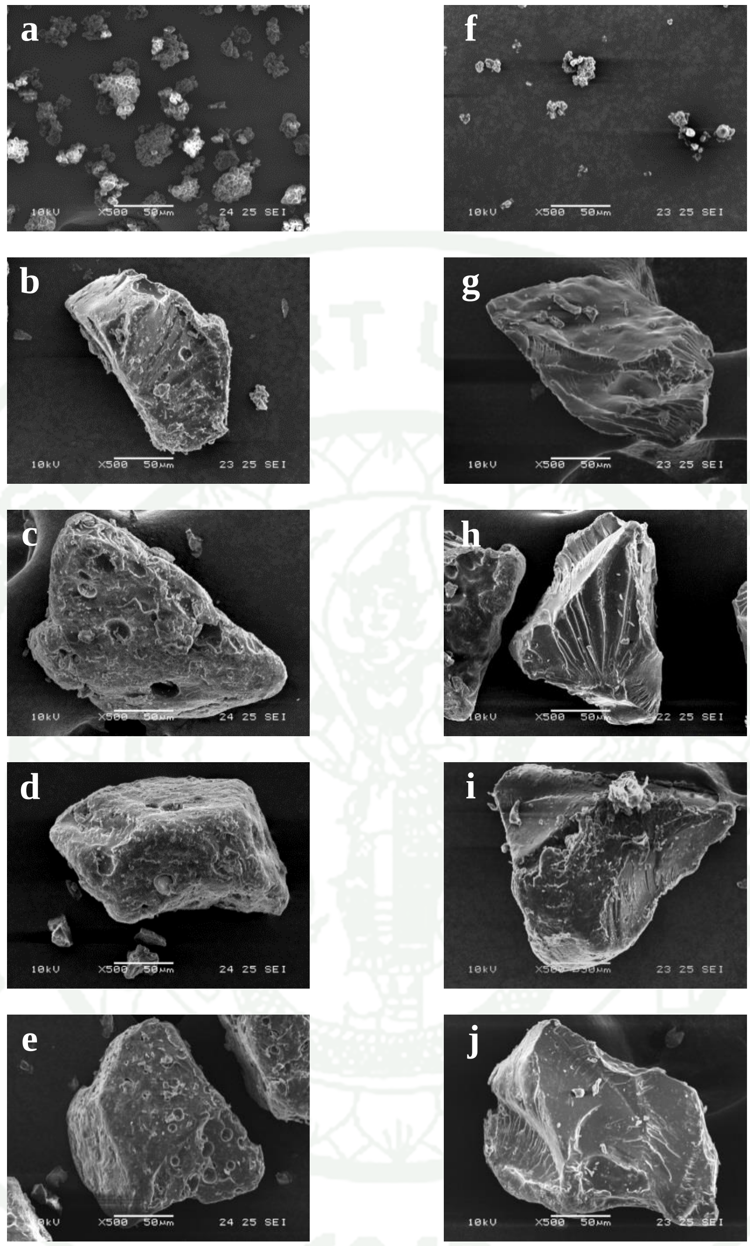


Figure 19 Scanning electron micrographs (500×) of native waxy rice flour (a) and rice flour (f). Waxy rice flour (b-e) and rice flour (g-j) were modified by extrusion process with water feed rate at 0.5 l/h (b, g), 0.6 l/h (c, h), 0.7 l/h (d, i), and 1.0 l/h (e, j). Bars = 50µm.

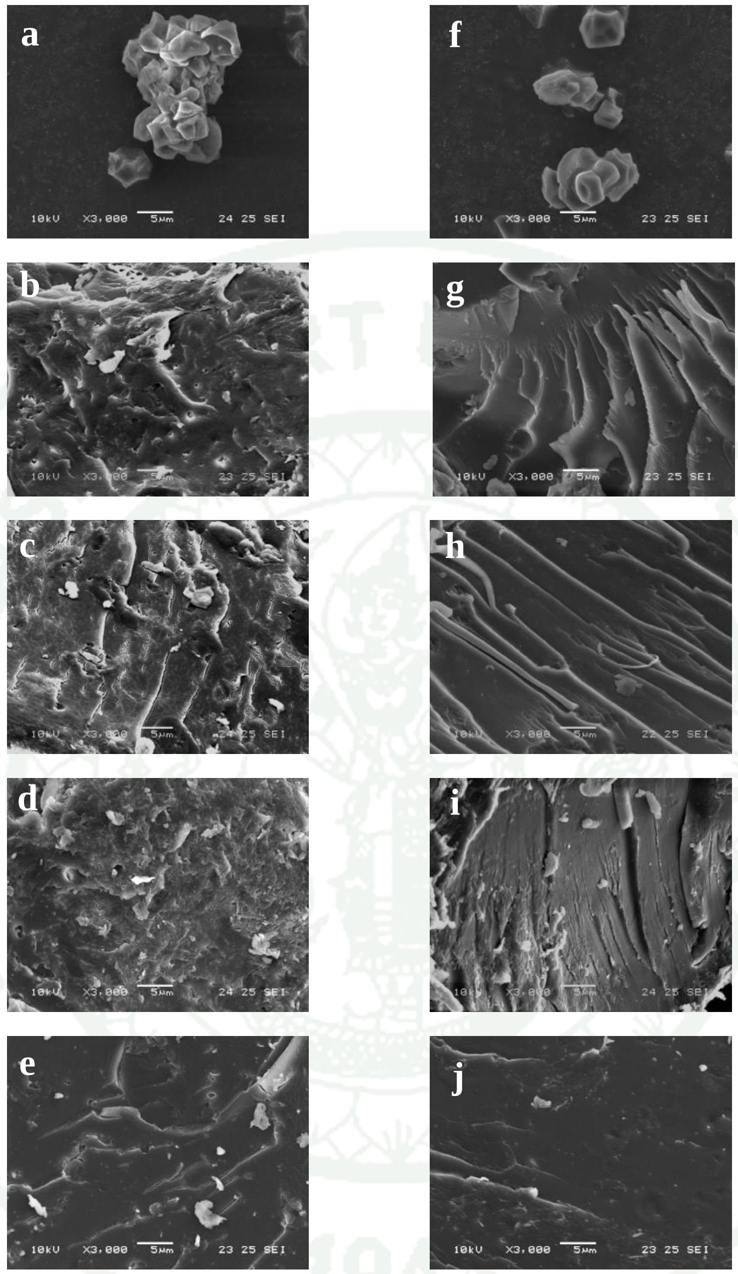


Figure 20 Scanning electron micrographs (3000×) of native waxy rice flour (a) and rice flour (f). Waxy rice flour (b-e) and rice flour (g-j) were modified by extrusion process with water feed rate at 0.5 l/h (b, g), 0.6 l/h (c, h), 0.7 l/h (d, i), and 1.0 l/h (e, j). Bars = 5µm.

2.3 Starch crystallinity of native and extruded WRF and RF

The X-ray diffractograms of the native WRF and RF showed an A-type pattern with 29.13% and 23.34% relative crystallinity, respectively (Figure 21). For the exWRF, the lower water feed rate applied, the lower the crystallinity of the exWRF. In Figure 21a, exWRF with the lowest water feed rate shows an amorphous peak which represents the complete destruction of starch crystallinity during extrusion by the mechanical disruption of the molecular bonds due to the intense shear fields within the extruder (Wen *et al.*, 1990) at a low moisture content. Similarly, the relative crystallinity of the exRF reduced with a lowering of the water feed rate from 1.0 l/h to 0.8l/h. However, the extrusion process with the water feed rate at 0.7 l/h resulted in a change in the X-ray diffraction pattern of the exRF from the A-type to the V-type pattern with additional peaks at 12.7° and 19.8° (2θ) (Figure 21b), which indicated the formation of complexes between starches and native lipids (Zobel, 1964). The peak heights for the V-type pattern and the percentage of crystallinity increased when the water feed rate lower than 0.7 l/h. This confirmed the formation of amylose-lipid complexes caused by the severity of the process. At low water feed rate, the increasing shear force of the process could increase the availability of amylose chains, together with, the high temperature during the extrusion process that could promote molecular movement (Blanche and Sun, 2004); therefore, amylose-lipid complexes were produced when using the lower water feed rate in extrusion. Additionally, the intense shear field of the extrusion could cause molecular bond disruption (Wen *et al.*, 1990), and the complexes between starches and lipids resulted from the ability of amylose to bind to lipids (Bhatnagar and Hanna, 1994); therefore, it could be possible that, during severe extrusion (moisture content lower than 22.70%), the double helix structure of amylose was disrupted and unfolded which could favor forming the complexes with native lipids. Our results were consistent with the results from Bhatnagar and Hanna (1994) who found that the formation of complexes increased with a higher amylose content in corn starch but no V-type pattern was observed in extruded waxy corn starch.

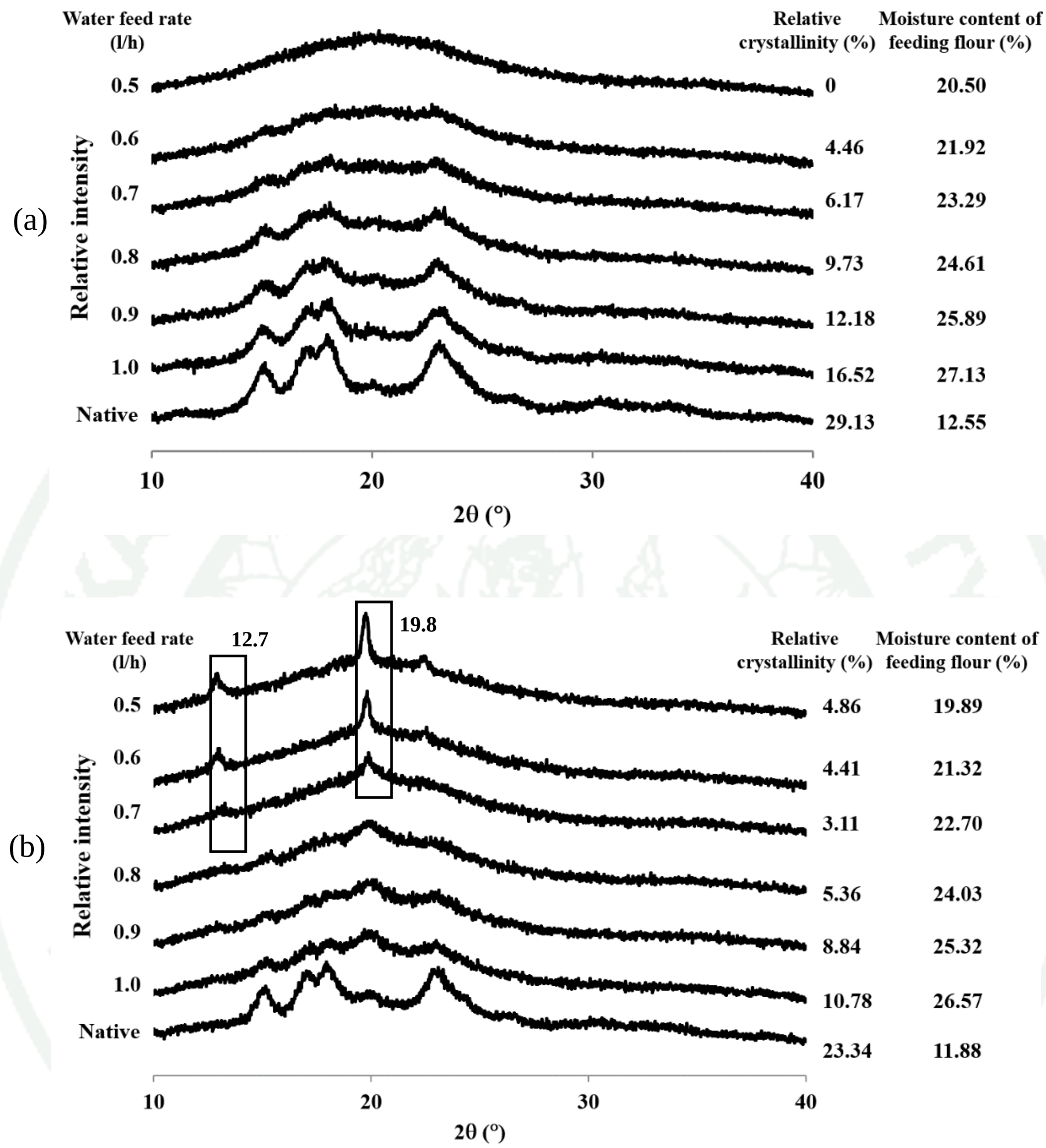


Figure 21 X-ray diffraction patterns and relative crystallinity of native and extruded waxy rice flour (a) and rice flour (b) by extrusion process with water feed rate at 0.5-1.0 l/h. The extruded rice flour shows additional peaks at 12.7° and 19.8°(2θ) indicating a change in crystallinity pattern from A-type to V-type.

2.4 Water absorption index (WAI) and water solubility index (WSI) of native and extruded WRF and RF

The WAI values of the exWRF and exRF were significantly higher than those of native flour samples, with the exception of the extrusion process using the WRF with a water feed rate lower than 07 l/h that had a lower WAI than the native WRF (Table 6). The WAI could be increased by the degree of starch damage which was caused by starch gelatinization and extrusion-induced gelatinization (Colonna *et al*., 1989). At room temperature, the gelatinized starch had a higher ability to absorb water than did the native starch granules. Thus, the WAI of the extruded flours was higher than that of the native flours. The WAI of the extruded flours increased with an increase in the water feed rate and reached a maximum when the water feed rate was 0.8 l/h and 0.6 l/h for WRF and RF, respectively. The WAI reduced with a higher water feed rate, due to the less severe conditions in the extrusion process leading to less damage of the extruded flours (Figures 19 and 20). However, the extrusion process at high temperature with a low amount of water could cause melting crystalline of amylopectin molecules (Colonna *et al*., 1989) or severe damage to the extruded flour which can be seen in the SEM images (Figures 19 and 20). As a result, the damaged particles lost their ability to absorb water; consequently, the WAI of the exWRF with water feed rate lower than 0.7 l/h was lower than that of the native WRF.

The WSI measures the degree of starch degradation in the extrusion process (Ding *et al*., 2005). Similarly to the WAI, the WSI values of the exWRF and exRF were significantly higher than those of native flour samples with maxima obtained when using the lowest water feed rate because of dextrinization in the extrusion system at high temperature but with low water or shear fragmentation of the starch during extrusion at low water feed rate which led to an increase in the number of water-soluble carbohydrate molecules (Colonna *et al*., 1989) resulting in the higher WSI. An increase in the water feed rate reduced the process severity; consequently, the WSI was lowered and continued to decrease and was close to the WSI of the native flour samples.

The exWRF had a higher WSI than the exRF which was in line with the results of Guha and Ali (2002) and Sompong *et al.* (2011). They concluded that glutinous rice flour (high amylopectin content) was more degraded during extrusion than rice flour with higher amylose content.

2.5 Glass transition temperature of native and extruded WRF and RF

It is well established that the T_g of a polymer is related to its molecular weight (Kaletunc and Breslauer, 1993). The extrusion-induced fragmentation into smaller molecules represented the primary cause of changes in the T_g and the extent of the fragmentation reduced the T_g of the extruded corn (Kaletunc and Breslauer, 1993). Result from our study showed that T_g of the native WRF and RF were 100.2±6.78°C and 104.1±6.78°C, respectively, which were higher than T_g of those extruded flour. This indicated that the native flour had higher starch molecular weight than extruded flour. Our result was consistent with the finding from Kaletunc and Breslauer (1993). Feeding WRF and RF with a lower water feed rate in the extrusion process led to higher molecular degradation in the starch resulting in a lower T_g in the extruded products (Figure 22). Wen *et al.* (1990) reported that at each screw speed and temperature tested, a decrease in the moisture content from 30% to 20% resulted in an increase in the fragmentation and a decrease in the high molecular weight of the corn starch. Molecular degradation could be the result of thermomechanical stress from the extruder that could produce a low molecular weight in the extruded flour, but it was considered to be the result of mechanical rather than thermal degradation (Kaletunc and Breslauer, 1993). The effect of the water feed rate on molecular degradation was more significant in the exWRF than the exRF which could be seen in the bigger change in the T_g of the exWRF (Figure 22). Chung and Lim (2003) reported that the lower T_g of waxy rice starch could be a result of the presence of branching in waxy rice starch which may obstruct the chain association, whereas the linear amylose molecule facilitates rapid chain association causing a more rigid amorphous structure resulting in higher T_g values. It was clearly observed that the T_g values of the exWRF with a lower water feed rate were lower than those with a higher water feed rate, while this was not clearly seen in the exRF. This T_g result was in line with the result from the WSI where a lower water feed rate caused higher molecular degradation resulting in more water-soluble molecules; moreover, the WSI was higher in the exWRF than in the exRF. Therefore, it could be concluded that extrusion had a stronger effect on the molecular fragmentation of WRF than of RF, which also agreed with the suggestion of Wen *et al.* (1990) that extrusion-induced molecular fragmentation of starch occurred preferentially at the branching point α-(1,6) of the amylopectin fraction, while the amylose polymer passed through extrusion process without significant molecular degradation.

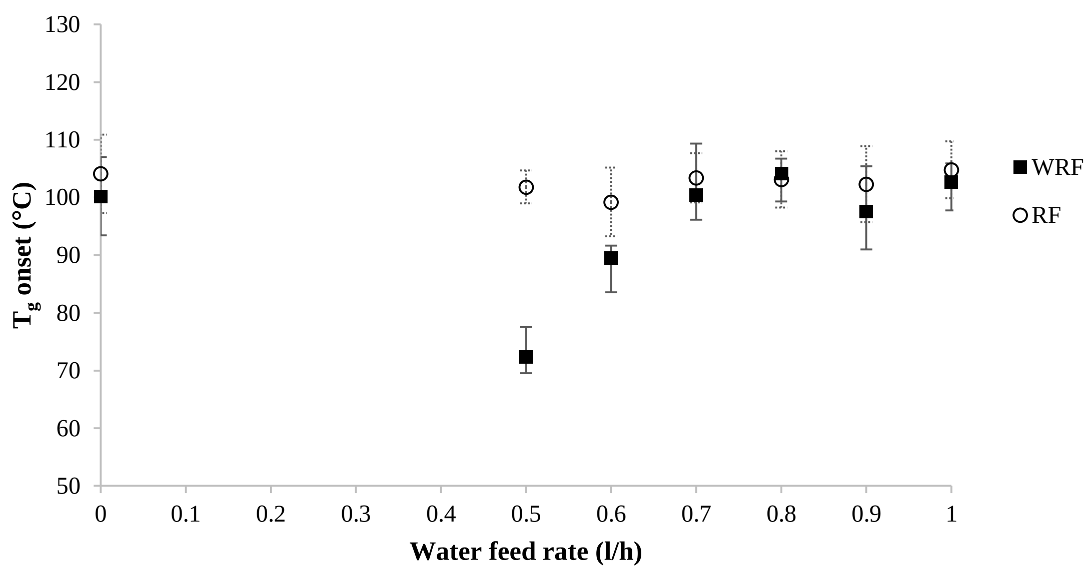


Figure 22 Glass transition temperature (T_g) of native and extruded waxy rice flour (WRF) and rice flour (RF) by extrusion process with water feed rate at 0.5-1.0 l/h. T_g onset values were determined at relative humidity 52.9%, 25°C.

2.6 Pasting properties of native and extruded WRF and RF

The pasting times of native RF and exRF were longer than those of WRF (Figure 23) because the higher amylose content in the RF restricted swelling of the rice granules; therefore, they required a longer time to reach maximum viscosity than did the native WRF and exWRF, respectively. The viscosity of a paste depends on the degree of gelatinization and molecular breakdown (El-Dash *et al.*, 1983). The initial viscosities of the extruded flours were higher than those of the native flour sample which confirmed the properties of pre-gelatinized exWRF and exRF could absorb water and be viscous at room temperature (Wurzburg, 1995). The peak and final viscosity values of the exWRF and exRF were far lower than that those of the native flours (Figure 23), which were characteristics of the extruded samples (Menegassi *et al.*, 2011). Since the exWRF and exRF were pre-gelatinized by thermal gelatinization and mechanically damaged during the extrusion process (Colonna *et al*., 1989), they lost the ability to swell upon heating resulting in a lower viscosity compared with native flours. No gelatinization peaks were observed in the viscogram of the extruded WRF (Figure 23a) but they could be observed in the exRF (Figure 23b). This could imply that under the same conditions of extrusion, increased gelatinization took place during the extrusion of the WRF than of the RF possibly because the higher amylose content in the RF restricted the swelling and gelatinization of the starch granules, as mentioned before. Moreover, the peak viscosity of the exRF increased with increasing water feed rate because, with a high water feed rate, partial gelatinization occurred and some ungelatinized-starch granules were still presented in the extruded flour causing an increase in the viscosity during heating in the RVA. This indicated that a high water feed rate decreased the stress on granule disruption during extrusion. The native and extruded RF had higher setback from the trough compared to the native and extruded WRF, respectively, which showed higher retrogradation of native and extruded RF due to the reassociation of amylose molecules during cooling. Our result was in line with that of Sompong *et al.* (2011).

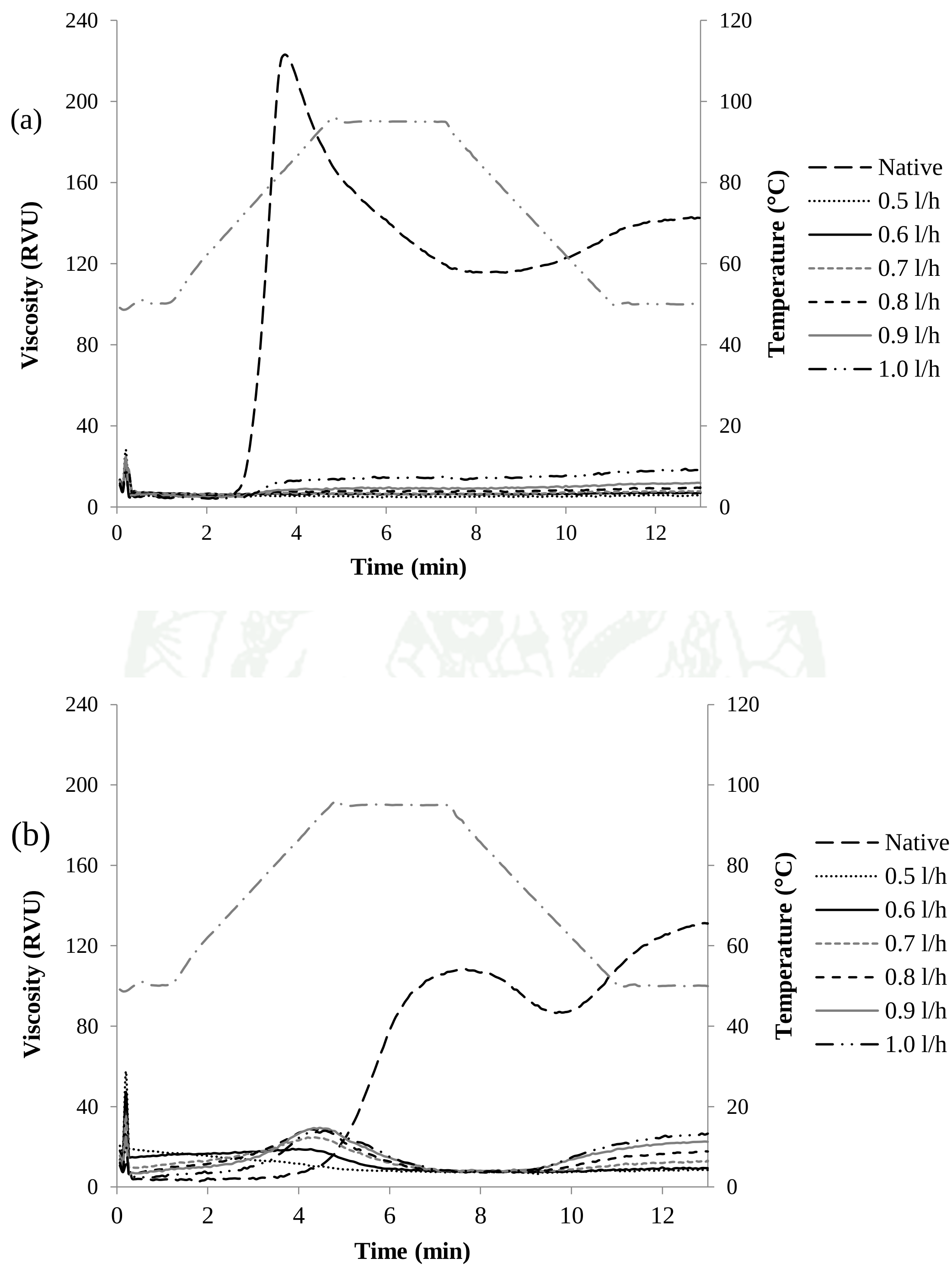


Figure 23 Pasting profile of native and extruded waxy rice flour (a) and rice flour (b) by extrusion process with water feed rate at 0.5-1.0 l/h.

2.7 Gelatinization properties of native and extruded WRF and RF

Native flours had lower T_o and T_p values than the extruded flours; however, there was no significant difference in the T_c values (Table 6). The higher values of T_o and T_p in the extruded flour samples could have been because starch was partially gelatinized by the extrusion process and more heat-stable granules remained in the extruded products causing higher T_o and T_p levels in the extruded flour, compared to those of the native flour samples. The enthalpy of gelatinization or enthalpy of melting endotherm (ΔH_{gel}) values of the native WRF and exWRF were higher than those of the native RF and exRF for all levels of water feed rate because the native WRF and exWRF had higher relative crystallinity than did the RF (Figure 19). ΔH_{gel} reflects the loss of molecular order (Cooke and Gidley, 1992). Since amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the energy for gelatinization (Flipse *et al.*, 1996). The extrusion process of WRF and RF with the lowest water feed rate did not show melting endotherms of crystalline portion which could have been due to complete starch conversion during extrusion. An increase in the water feed rate tended to increase the enthalpy of melting endotherm of the extruded flour which confirmed that partial conversion had occurred when using high water feed rate, which agreed with the result from the RVA. Indeed, these melting enthalpy values corresponded to the results for % crystallinity as the extrusion process with the higher water feed rate resulted in a higher relative crystallinity causing a higher enthalpy of melting endotherm than the process with the lower water feed rate. We also found that the exRF with a water feed rate lower than 0.7l/h had an endotherm at a high temperature (T_p = 114 °C) representing an amylose-lipid complex. This result was consistent with the result from the X-ray diffractogram that indicated that the crystallinity pattern of the exRF changed from the A-type to the V-type (Figure 21).

For this part, it can be concluded that the extrusion process with different water feed rate affected the physicochemical properties of rice flour. With a lower water feed rate, it increased the thermomechanical stress leading to the change in the microstructure, destruction of the crystalline structure, degradation of the starch molecules. Whereas, a higher water feed rate resulted in partial gelatinization and less mechanical damage to the extruded flour (Figure 24).

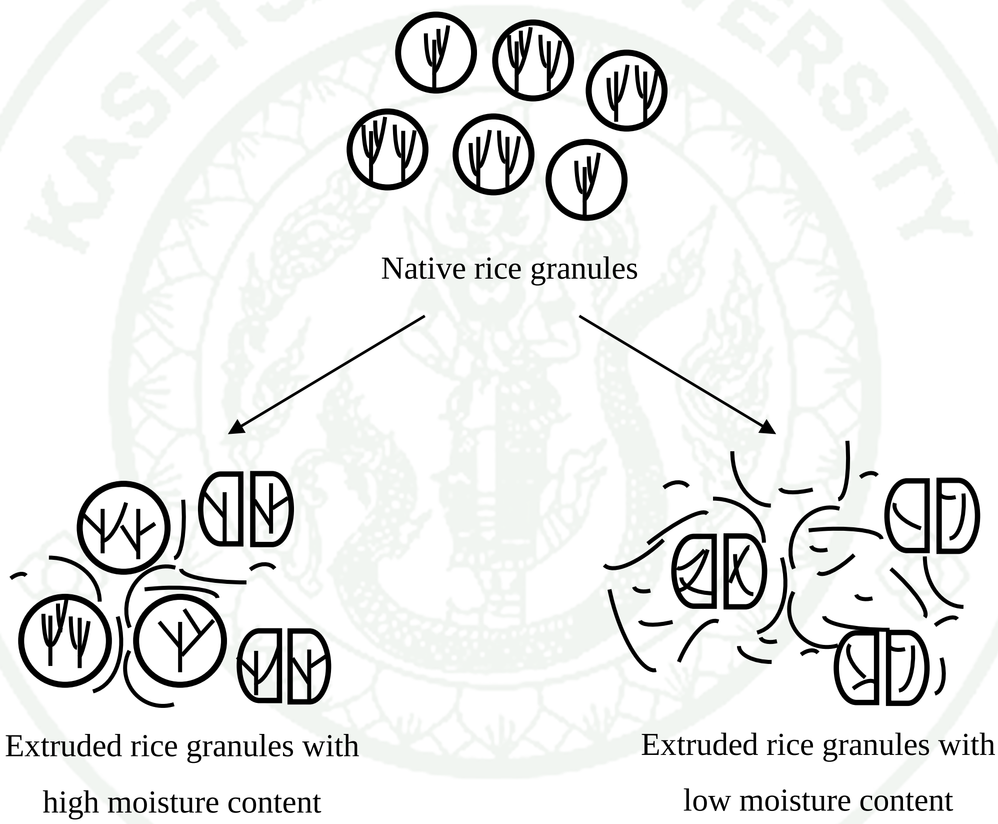


Figure 24 Proposed mechanism represents the change in extruded rice granules with high and low moisture content in feeding material.

As can be seen from the results that extrusion could modify the WRF to have higher water absorption ability than native WRF. The moisture of feeding material at 0.8-1.0l/h could improve the water absorption ability of modified WRF and the maximum value was the moisture of feeding material at 0.8l/h.

Part 3: Application of extruded rice flour for quality improvement of freeze-thawed cakes

According to the highest water absorption ability from the result in part 2, the extruded flour with water feed rate at 0.8l/h was used to substitute for WF at 10% w/w for making frozen cake. The characteristics of batter and fresh and frozen cakes prepared with substituting extruded flour were investigated.

3.1 Batter characteristics

3.1.1 Viscosity and density of batter

The viscosity of batter prepared with WF tended to be higher than those of batters substituted with native WRF and RF (Table 7). This could have been because of a reduction in wheat gluten which is responsible for viscosity development in batter (Loewe, 1993). This result agreed with the result in part 1.3.1 that substitution of WRF for WF cause a decrease of batter viscosity (Table 3). However, batters substituted with exWRF and exRF had the highest viscosity. This could have been due to the pre-gelatinization characteristic of extruded flour that could absorb water and provide viscosity at a room temperature (Wurzburg, 1995). There was no significant ($p>0.05$) difference in density between the WF batter and samples substituted with WRF, RF, exWRF and exRF which might have been because the viscosity of each batter did not differ enough to making an appreciable difference in density, as mentioned in part 1.3.1. However, density of batters substituted with extruded flour tend to be lower because they had higher viscosity which could prevent loss of air and increase batter stability, compare to WF batter and those substituted with native WRF and RF.

3.1.2 Microstructure of batter

The microstructure of the cake batters with different flour substitutions for WF was observed under a light microscope. Figure 25 (a-e) shows that there was more uniform distribution of air bubbles in the batter substituted with extruded flours which could have been a result of the higher viscosity of those batter compared to other flour substitutions. Low viscosity of batter could lead to the coalescence of bubbles into larger bubbles (Allais *et al.*, 2006); thus, larger bubbles were observed in batter substituted with WF, WRF and RF.

Table 7 Properties of batter and cake prepared with 10% w/w substituted flour; wheat flour (WF), waxy rice flour (WRF), rice flour (RF), extruded waxy rice flour (exWRF) and extruded rice flour (exRF)

Substitution	Batter		Cake	
	Viscosity (Pa.s)	Density (g/cm^3)	Baking loss (%)	Height (mm)
WF	9.05 ± 0.19^a	0.54 ± 0.00^a	17.31 ± 0.26^c	43.50 ± 0.71^a
WF+WRF	8.89 ± 0.29^a	0.56 ± 0.01^a	16.57 ± 0.31^b	41.75 ± 0.35^a
WF+RF	8.87 ± 0.76^a	0.56 ± 0.06^a	16.41 ± 0.05^{ab}	43.25 ± 1.06^a
WF+exWRF	9.42 ± 0.75^{ab}	0.52 ± 0.03^a	16.22 ± 0.21^a	42.65 ± 0.49^a
WF+exRF	9.60 ± 0.56^b	0.51 ± 0.02^a	16.20 ± 0.16^a	43.25 ± 0.35^a

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

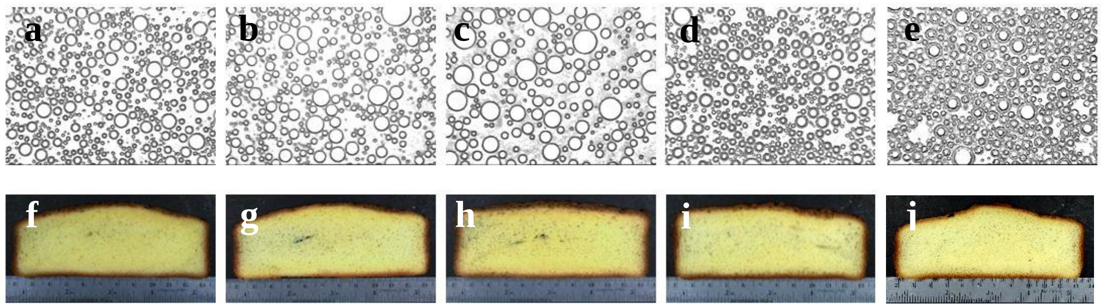


Figure 25 Light micrographs (40×) of aerated cake batter (a-e) and images of cakes (f-j) prepared with wheat flour (a,f) and wheat flour with 10% substituted waxy rice flour, rice flour, extruded waxy rice flour, and extruded rice flour ((b,g), (c,h), (d,i) and (e,j), respectively).

3.2 Cake properties

The overall appearance of fresh-baked cakes prepared with different flour substitutions was similar to that of WF cake (Figure 25f-j). For the fresh-baked cakes, any height (41.75-43.50 mm) between WF cake and cakes substituted with WRF, RF, exWRF and exRF was not significant ($p>0.05$). After freeze-thawing process, there was no significant ($p>0.05$) difference in the height between freeze-thawed cakes and fresh-baked cakes (data was not shown).

The result from Table 7 showed that WF cake had a significant ($p\leq0.05$) higher baking loss, followed by cakes substituted with WRF and RF, whereas cakes prepared with extruded flour substitution had the lowest baking loss. This could have been a result of higher water absorption ability of extruded flour that was caused by extrusion-induced gelatinization (Colonna *et al*., 1989) and mechanical damage from shearing in extrusion process, as discussed in part 1.4. Therefore, cakes substituted with extruded flour could absorb higher water and could reduce the moisture loss during baking and cooling, consequently, they had significantly ($p\leq0.05$) higher moisture content than those prepared with substituted WRF and RF and WF cake.

3.3 Effect of flour substitutions on freeze-thawed cake

3.3.1 Moisture content of cake

Fresh baked cake substituted with extruded flour had higher moisture content than that of WF cake which agreed with the result of baking loss that it was higher in cake substituted with extruded flour than WF cake. The moisture content of freeze-thawed WF cake and cakes substituted with WRF and RF decreased significantly ($p \leq 0.05$) after the third freeze-thaw cycle (Table 8) which was in line with the result in part 1.5.1. However, for cake substituted with exWRF and exRF, no significant ($p > 0.05$) difference was observed in the moisture content between fresh-baked cake and cake subjected to five freeze-thaw cycles, which could also have been a consequence of high water absorption ability of extruded flour. WAI of WF was 2.04 g/g which was lower than WAI of exWRF and exRF (3.04 and 7.47g/g, respectively). Therefore, extruded flour could prevent moisture loss in frozen cake during repeatedly freeze-thawing process and storage.

Table 8 Properties of fresh and freeze-thawed cake prepared with substituted flour at 10% w/w

Properties	Substitution	Number of freeze-thaw cycle			
		0	1	3	5
Moisture content (%)	WF	41.92 ± 0.25^{bA}	41.80 ± 0.51^{bA}	39.65 ± 0.37^{aA}	39.08 ± 0.37^{aA}
	WF+WRF	43.53 ± 0.62^{bB}	43.42 ± 0.66^{bB}	42.09 ± 0.03^{aB}	42.03 ± 0.01^{aB}
	WF+RF	43.62 ± 0.42^{bB}	43.39 ± 0.35^{bB}	42.24 ± 0.39^{aB}	41.83 ± 0.41^{aB}
	WF+exWRF	43.94 ± 0.30^{aB}	43.71 ± 0.00^{aB}	42.70 ± 0.53^{aB}	42.26 ± 1.17^{aB}
	WF+exRF	43.98 ± 0.81^{aB}	43.74 ± 0.22^{aB}	42.51 ± 1.15^{aB}	42.19 ± 1.40^{aB}
Firmness value by texture analyzer (g)	WF	260.47 ± 17.44^{aA}	385.87 ± 5.03^{bA}	447.81 ± 27.84^{bA}	540.37 ± 45.09^{cC}
	WF+WRF	238.93 ± 19.19^{aA}	344.93 ± 55.48^{bA}	408.56 ± 2.95^{bA}	503.00 ± 19.84^{cBC}
	WF+RF	232.68 ± 10.95^{aA}	379.37 ± 1.77^{bA}	424.35 ± 56.25^{bA}	458.66 ± 12.84^{bAB}
	WF+exWRF	242.86 ± 25.19^{aA}	268.15 ± 38.25^{aA}	380.33 ± 4.42^{bA}	416.47 ± 14.45^{bA}
	WF+exRF	256.84 ± 9.60^{aA}	320.20 ± 6.37^{bA}	406.23 ± 11.04^{cA}	435.15 ± 34.60^{cAB}
Firmness score by intensity test	WF	5.59 ± 2.63^{a}	5.16 ± 3.00^{aA}	n.d.	4.85 ± 3.04^{aA}
	WF+WRF	n.d.	3.88 ± 1.69^{aA}	n.d.	4.87 ± 2.34^{aA}
	WF+exWRF	n.d.	4.17 ± 2.47^{aA}	n.d.	5.41 ± 3.40^{aA}
Firmness score by acceptance test	WF	5.8 ± 1.52^{a}	5.8 ± 1.98^{aA}	n.d.	5.2 ± 1.73^{aA}
	WF+WRF	n.d.	6.5 ± 1.70^{bA}	n.d.	5.7 ± 1.74^{aAB}
	WF+exWRF	n.d.	6.7 ± 1.47^{aA}	n.d.	6.4 ± 1.61^{aB}

n.d. = not determined

Mean values in each row with different lower case superscript letters (a-c) are significantly ($p\leq0.05$) different

Mean values in each column of each attribute with different upper case superscript letters (A-C) are significantly ($p\leq0.05$) different

3.3.2 Firmness of cake

The firmness of cakes measured by texture was shown in Table 8. The firmness of fresh-baked cakes were not significantly ($p>0.05$) different among each treatment. However, the firmness of all cake increased significantly ($p\leq0.05$) after the first freeze-thaw cycle, with an exception of the cake substituted with exWRF that its firmness increased significantly ($p\leq0.05$) after the third freeze-thaw cycle. This could explain by the same mechanism as WRF in Figure 17 that the swollen waxy rice starch granules spread cover wheat starch granules; moreover, the extruded waxy rice starch granules was pre-gelatinized by the extrusion process (as discussed in Part 2) which could let them to be easier for water absorption, swelling and spreading cover wheat starch granules, compare to native WRF. Thus, exWRF had more pronounced effect in retardation of an increase in firmness than native WRF. During frozen storage, the increase in the firmness values of cake was caused by moisture migration from the inner to the outer part and then by loss to the atmosphere, and caused by starch retrogradation (Cauvain, 1998; Kerr, 2004). After the fifth freeze-thaw cycle, the firmness of WF cake was raised by 107.46% causing the highest firmness value; whereas, that of cake substituted with exWRF was increased by 71.49% leading to the lowest value. This result showed that substitution of exWRF for WF could improve the firmness of freeze-thawed cake. However, there was no significant ($p>0.05$) difference in firmness between freeze-thawed cakes substituted with WRF, RF and exRF.

The firmness values of WF cake in part 1.5.1 (Table 4) and the result in this part (Table 8) was slightly different which could have been a result from the variation in raw material. The firmness values of both fresh-baked and freeze-thawed cakes in part 1.5.1 were higher than those of cakes in this part which could have been due to the different egg sources. Therefore, we verified this hypothesis by comparing the firmness value of fresh-baked and freeze-thawed cakes prepared with different egg sources. The results were showed in Appendix Table 1. The fresh-baked cake made with supermarket eggs and that cake subjected to the first freeze-thawed cycle tended to have higher firmness value than those cakes made with KU eggs. After the third freeze-thawed cycle, the firmness of cakes made with KU eggs was significantly higher than that made from supermarket eggs. The difference in firmness values could have been due to the difference in egg freshness from these two sources resulting in the different egg viscosity. The fresher the egg, the more viscous the egg white (John *et al*., 2012). KU eggs were purchased and used on the day that was laid, whereas, for supermarket eggs, we could not control the laying date which sometimes was 3-5 days before we purchased. As a result, KU eggs were fresher than supermarket eggs. Therefore, the batter prepared from KU eggs could keep more air bubbles than that prepared from supermarket eggs (Figures 14a and 25a) and could reduce the loss of air during baking. Consequently, the firmness of cake prepared with KU eggs (Table 8) was lower than that of cake prepared supermarket eggs (Table 4). Therefore, in this part, the fresh eggs was used for the experiment.

Base on the best textural result, exWRF was selected for the further study on the sensory analysis, microstructure and retrogradation properties. In order to compare the influence of extruded flour on freeze-thaw cake properties, the (non-extruded) WRF were also continuously studied.

3.3.3 Sensory evaluation of fresh and freeze-thawed cakes

In this part, the panelists could not detect the different between fresh-baked WF cake and WF cake subjected to five freeze-thaw cycles; while, they could detect the difference in part 1.5.3 (Table 4). This could have been due to change in firmness value detected by texture analyzer between fresh-baked cake and cake subjected to five freeze-thaw cycles was only 51.8% (Table 8) which was smaller than 62.17% of the change in part 1.5.3. Therefore, in this part, the firmness intensity scored between fresh baked and freeze-thawed WF cake given by panelists did not significant differ. Furthermore, the firmness of freeze-thawed WF cake was not significantly ($p>0.05$) different from those of cakes substituted with WRF, RF, exWRF and exRF. This could imply that the difference in firmness of cake among each treatments and the change in firmness between fresh and freeze-thawed cakes were too small to be detected by human perception, but the texture analyzer has a higher sensitivity to detect those differences. However, in acceptance test, freeze-thaw cycles tended to lower the acceptance score of cake firmness. This effect significant pronounced in WF cake. After the fifth freeze-thaw cycle, cake substituted with exWRF had the highest acceptance score, followed by cake substituted with WRF and WF cake had the lowest acceptance score. The results from the acceptance test on firmness agreed with the firmness value evaluated by the texture analyzer that the fifth freeze-thaw cycle resulted in higher firmness in the cake and lower acceptance by the panelists. It can conclude that substitution of exWRF provided freeze-thawed cake with lower firmness and a higher acceptance score than the WRF and WF cakes.

3.4 Microstructure of fresh and freeze-thawed cakes evaluated by SEM

The microstructure of cake samples was observed using SEM at 35× (Figure 26) and 500× magnifications (Figure 27) which could show the differences in microstructure between fresh-baked and freeze-thawed WF cakes. Similar result as part 1.6 was observed. Repeating the freeze-thaw cycle caused a thinner and rougher matrix than that of fresh-baked cake (Figure 26). After the first freeze-thaw cycle, channel-like structure were clearly observed (Figure 27) and the matrix seemed to be more compact than in fresh-baked cakes. Furthermore, the matrix was distinctly thinner in cakes subjected to the fifth freeze-thaw cycle than fresh-baked cakes, and the largest and deepest channels were well defined which was due to amylose retrogradation (Jongsutjarittam and Charoenrein, 2013). 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 8). After the first freeze-thaw cycle, substitutions of WRF and exWRF led to less compact matrix compared to cakes without substitution (Figure 26). The matrix of cake substituted with exWRF were thickest and that of WRF cake were thicker than that of WF cake. The same trend also showed in cakes subjected to five freeze-thaw cycles. This resulted from the lower amylose content and the higher swollen granules of WRF compared to WF (Jongsutjarittam and Charoenrein, 2013). Moreover, a thicker matrix of exWRF substituted cake than WRF substituted cake could have been resulted from higher moisture content of exWRF substituted cake. Water molecules could bind to the starch molecules which could prevent the formation of hydrogen bonds within and between starch molecules; therefore, starch molecules could not get closer to each others resulting in thicker matrix of cake substituted with exWRF than that substituted with WRF. Hence, the firmness of freeze-thawed cakes with exWRF substitution was significantly ($p \leq 0.05$) lower than in WRF and WF (Table 8). These structural findings corresponded well with the firmness results from the texture analyzer.

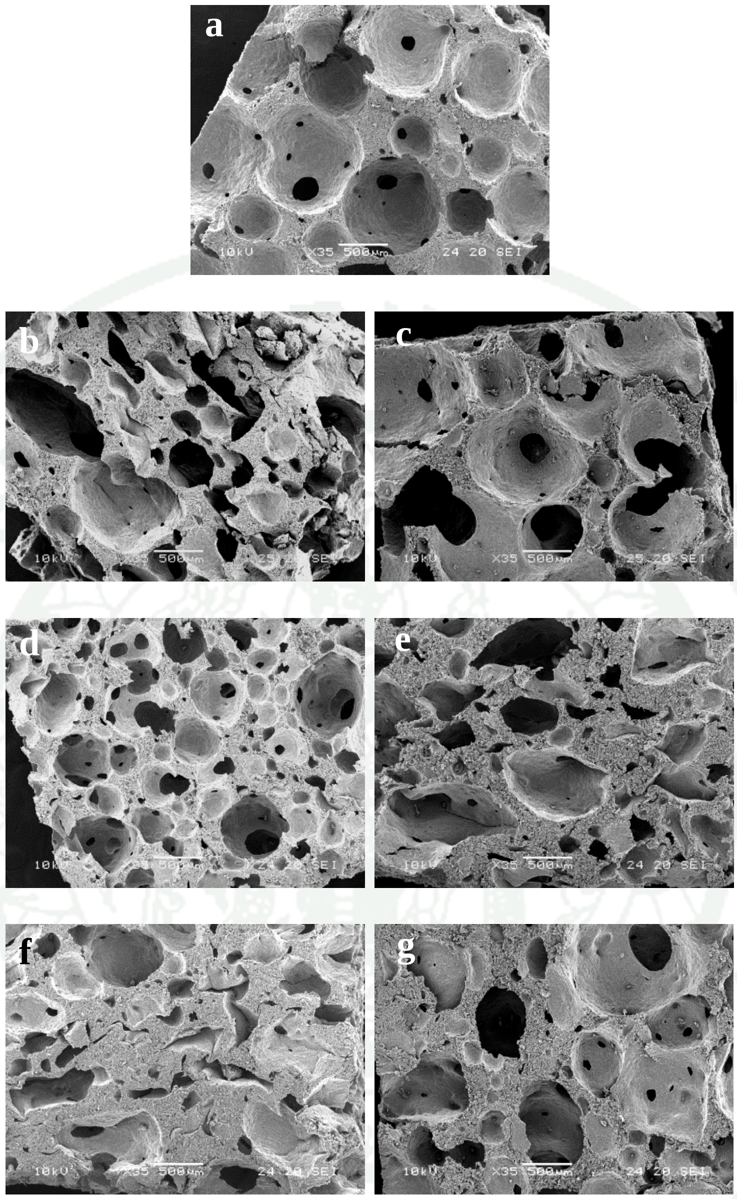


Figure 26 Scanning electron microscope images (35×) of fresh cake (a) and cakes with the first (b, d and f) and fifth (c, e and g) freeze-thaw cycle prepared with wheat flour (WF) (a-c), 10% w/w waxy rice flour (d-e) and extruded waxy rice flour (f-g) substitutions. Bars = 500µm.

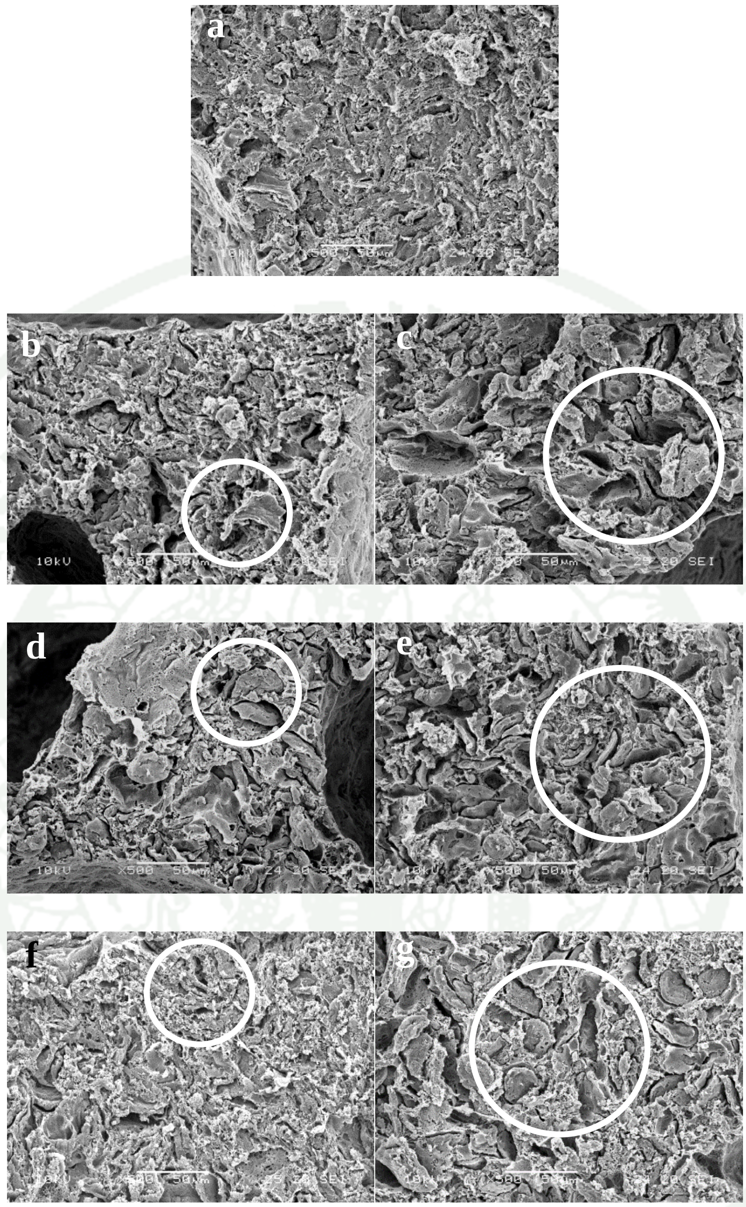


Figure 27 Scanning electron microscope images (500×) of fresh cake (a) and cakes with the first (b, d and f) and fifth (c, e and g) freeze-thaw cycle prepared with wheat flour (WF) (a-c), 10% w/w waxy rice flour (d-e) and extruded waxy rice flour (f-g) substitutions. The circles indicates channel-like structure. Bars = 50µm.

3.5 Retrogradation properties of freeze-thawed cakes

The enthalpy of melting retrograded amylopectin in fresh and freeze-thawed cake samples was measured using DSC. The example of thermograms of cake was displayed in Appendix Figure 5. A peak occurred approximately at 54.3°C which is a characteristic of the melting of retrograded amylopectin that was detected in the cake samples subjected to five freeze-thaw cycles (Table 9). The differences in T_o, T_p and T_c was not found neither between fresh-baked and freeze-thawed cakes nor between each treatment. While, the enthalpy of melting retrograded amylopectin (ΔH) of these cakes significant ($p \geq 0.05$) increased with increasing the number of freeze-thawed cycles. The results of DSC consistent with 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-baked cakes and cakes subjected to the first freeze-thaw cycle (Table 8). Furthermore, it was consistent with microstructure of cake subjected to 5 freeze-thaw cycles that had the most compact structure (Figure 26), which indicated that repeated freezing and thawing resulted in an increase in amylopectin retrogradation leading to a higher firmness value and consequently, a lower acceptance score. The ΔH value of cake subjected to five freeze-thaw cycles showed that cakes substituted with WRF and exWRF had significantly ($p \leq 0.05$) lower ΔH than WF cake which indicated that WRF and exWRF substitutions could reduce the firmness of freeze-thawed cake.

Table 9 Retrogradation properties of flour prepared from fresh and freeze-thawed cakes

Retrogradation properties	Substitution	Fresh	1FTC	5FTC
T_o (°C)	WF	49.0±1.18^{a}	49.0±1.43^{aA}	48.4±0.59^{aA}
	WF+WRF	n.d.	48.9±1.46^{aA}	48.1±0.53^{aA}
	WF+exWRF	n.d.	49.1±0.95^{aA}	45.5±1.42^{aA}
T_p (°C)	WF	54.0±1.27^{a}	54.5±1.27^{aA}	55.6±0.35^{aA}
	WF+WRF	n.d.	54.2±0.92^{aA}	56.1±0.59^{aA}
	WF+exWRF	n.d.	54.2±0.81^{aA}	55.0±1.27^{aA}
T_c (°C)	WF	58.6±1.62^{a}	61.4±2.97^{aA}	63.5±1.16^{aA}
	WF+WRF	n.d.	61.0±1.69^{aA}	62.7±0.88^{aA}
	WF+exWRF	n.d.	61.1±0.56^{aA}	62.1±1.64^{aA}
ΔH (J/g dried flour)	WF	1.34±0.06^{a}	1.50±0.14^{aA}	3.99±0.14^{bB}
	WF+WRF	n.d.	1.77±0.01^{aB}	3.05±0.17^{bA}
	WF+exWRF	n.d.	1.94±0.02^{aB}	3.25±0.02^{bA}

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, ΔH_{ret} = enthalpy of melting retrograded flour

n.d. = not determined

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.

CONCLUSION AND RECOMMENDATIONS

Conclusion

1. 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. WRF could retard the starch retrogradation by spreading swollen waxy rice starch granules to cover wheat starch granules, lower amylose content and the highly swollen characteristics of WRF during baking which produced a subsequent lowering of the firmness of freeze-thawed cake.

2. The structure and physicochemical properties of extruded rice flour were influenced by the amylose and amylopectin content and the moisture content of the feeding material. Extrusion had a stronger effect on the WRF due to the higher amylopectin content of the WRF than in the RF; thus, the extruded WRF showed higher molecular degradation. The extrusion process with a lower moisture content in the feeding flour increased the thermomechanical stress which resulted in a change in the microstructure, destruction of the crystalline structure, degradation of the starch molecules. In contrast, a higher moisture content in the feeding flour caused partial gelatinization and less mechanical damage to the extruded flour.

3. Substitution of exWRF at level of 10% w/w could reduce baking loss of fresh-baked cake and lowered the moisture loss of freeze-thawed cake. Moreover, it could retard the starch retrogradation and lowering of the firmness of freeze-thawed cake which could have been a result of the lower amylose content and highly water absorption ability of exWRF. While, substitution of exRF for WF could also reduce the moisture loss and lower a change in firmness of freeze-thawed cake but it had less significant affect than exWRF.

It can be concluded that WRF could be used as a substituting flour for WF in order to improve the firmness of freeze-thawed cake. Furthermore, the physically modification by extrusion could be applied to produce the exWRF that had higher water absorption ability which could reduce the baking loss and moisture loss of frozen cake. From this study, we suggest that exWRF has a potential to replace the usage of WF not only in frozen cake, but also could be used in other commercial frozen bakery products. In addition, the exWRF could apply in other WF-based products; however, it depends on the required properties of flour and modifying conditions of extrusion. By using of exWRF for replacement of WF, it could add the value to waxy rice and would reduce the amount of imported wheat.

Recommendations

1. The substitution of WRF or exWRF for WF in other frozen bakery products or other WF-based products should be further investigated.

2. Measurement of T_g is an indirect method to investigate the change in molecular weight of the polymer. For further study, a direct method, such as size exclusion chromatography, should be apply to measure the change in molecular weight of extruded flour.

3. From this study, WRF can substituted for WF at 10% w/w in frozen cake. However, it could be substituted at higher level by adjusting the cake formula, such increasing amount of cake improver, or applying to other frozen bakery products, such as bread which could be substituted at the higher amount because of higher gluten content in the system than that of cake.

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APPENDIX

แบบทดสอบทางประสาทสัมผัสแบบ Intensity test

ชื่อผู้ทดสอบ.. **วันที่**....................................

เพศ ☐ ชาย ☐ หญิง อายุ...................ปี

ผลิตภัณฑ์ เค้กแช่เยือกแข็ง

คำชี้แจง โปรดทำเครื่องหมายเส้นตรงและเขียนรหัสของแต่ละตัวอย่างบนเครื่องหมายเส้นตรงนั้น เพื่อแสดงตำแหน่งที่ท่านได้ให้แก่แต่ละตัวอย่างในด้าน ความแน่นเนื้อ (Firmness) ของแต่ละผลิตภัณฑ์ตามที่ท่านคิดว่าเหมาะสมที่สุด โปรดทำการทดสอบตัวอย่างจากซ้ายไปขวาและกรุณาบ้วนปากระหว่างตัวอย่าง

สเกลการให้คะแนน

1 5 9

นิ่มมากที่สุด แน่นเนื้อมากที่สุด

ขอบคุณค่ะ

Appendix 1 Sensory evaluation form for firmness intensity test

แบบทดสอบทางประสาทสัมผัสแบบ Hedonic scale

ชื่อผู้ทดสอบ.. **วันที่**....................................

เพศ ☐ ชาย ☐ หญิง อายุ..................ปี

ผลิตภัณฑ์ เค้กแช่เยือกแข็ง

คำชี้แจง โปรดเขียนรหัสตัวอย่างของเค้กแช่เยือกแข็งที่ได้รับ ชิมตัวอย่างเค้กตามลำดับที่นำเสนอจากซ้ายไปขวาและให้คะแนนความชอบของตัวอย่างในด้าน ความแน่นเนื้อ (Firmness) ของผลิตภัณฑ์ตามที่ท่านคิดว่าเหมาะสมที่สุด กรุณาบ้วนปากระหว่างตัวอย่าง

1 = ไม่ชอบมากที่สุด	4 = ไม่ชอบเล็กน้อย	7 = ชอบปานกลาง
2 = ไม่ชอบมาก	5 = เฉยๆ	8 = ชอบมาก
3 = ไม่ชอบปานกลาง	6 = ชอบเล็กน้อย	9 = ชอบมากที่สุด

คะแนน	**รหัสตัวอย่าง**			
ความแน่นเนื้อ				

ความคิดเห็น ตัวอย่างที่ชอบที่สุดคือ..........................เพราะ..

ตัวอย่างที่ไม่ชอบที่สุดคือ........................เพราะ..

ขอบคุณค่ะ

Appendix 2 Sensory evaluation form for firmness acceptance test

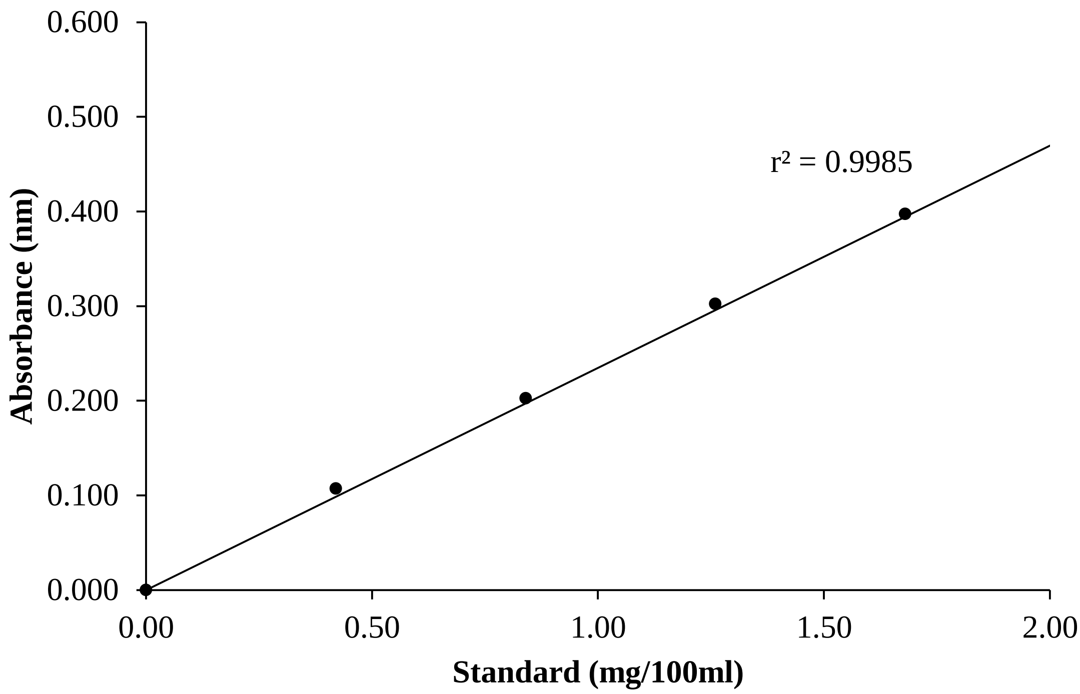
Appendix Table 1 Firmness measured by texture analyzer of fresh and freeze-thawed cakes prepared with egg from different sources

Egg source	Number of freeze-thaw cycles			
	0	1	3	5
Supermarket	301.88±11.95^{aA}	382.84±11.85^{bA}	592.56±29.50^{cB}	707.96±3.49^{dB}
KU	269.55±13.19^{aA}	347.04±6.03^{bA}	449.59±0.80^{cA}	551.95±17.94^{dA}

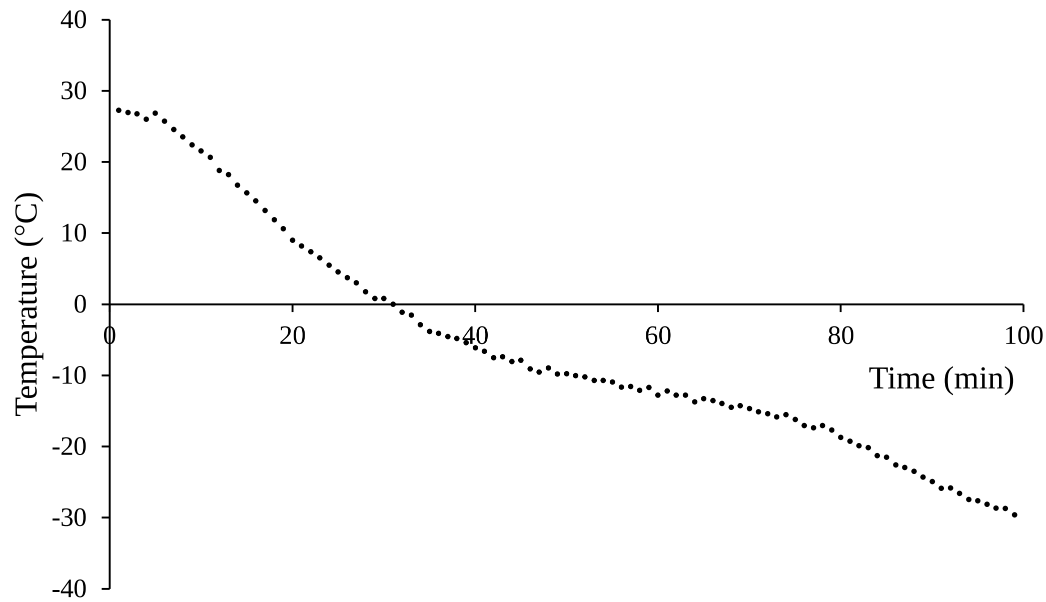
KU: Chicken Farm, Faculty of Agriculture, Kasetsart University, Thailand

Mean values in each row of each with different lower case superscript letters (a-c) are significantly ($p \leq 0.05$) different.

Mean values in each column attribute with different upper case letter superscripts (A-C) are significantly ($p \leq 0.05$) different.



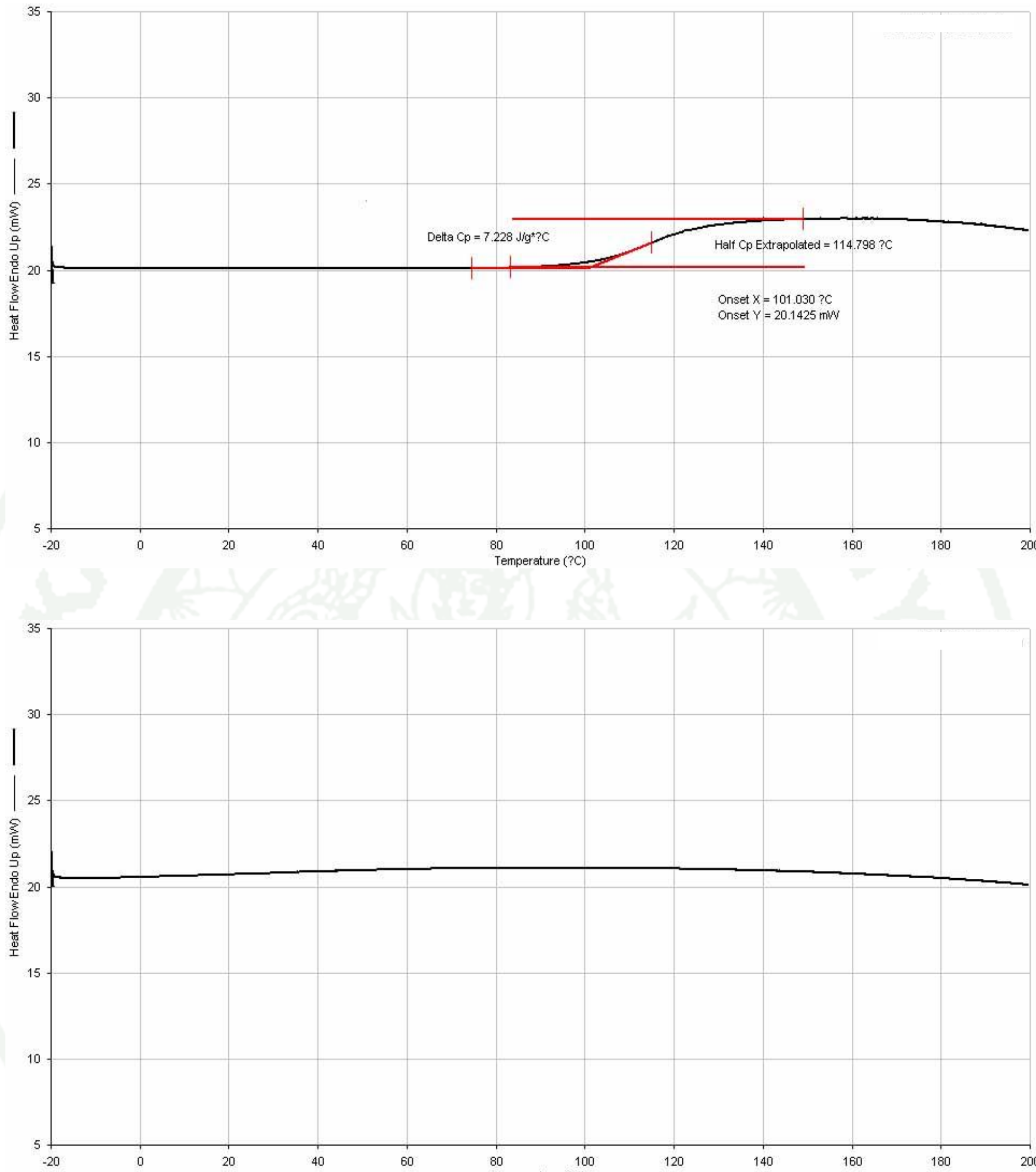
Appendix Figure 1 Standard curve for analysis of amylose content



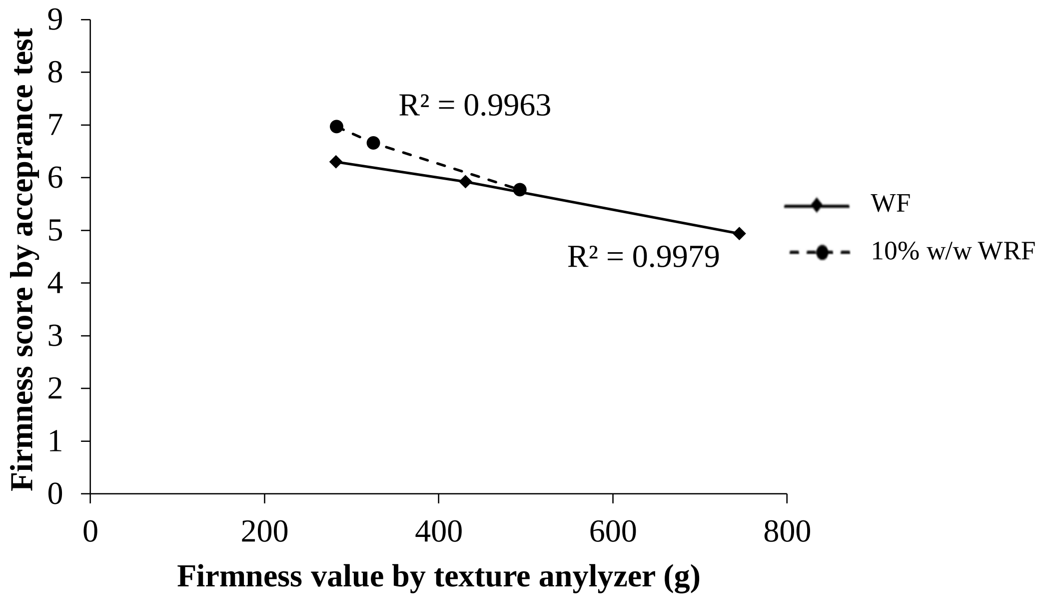
Appendix Figure 2 Freezing profile of cake in cryogenic freezer using air temperature at -40°C

(a)

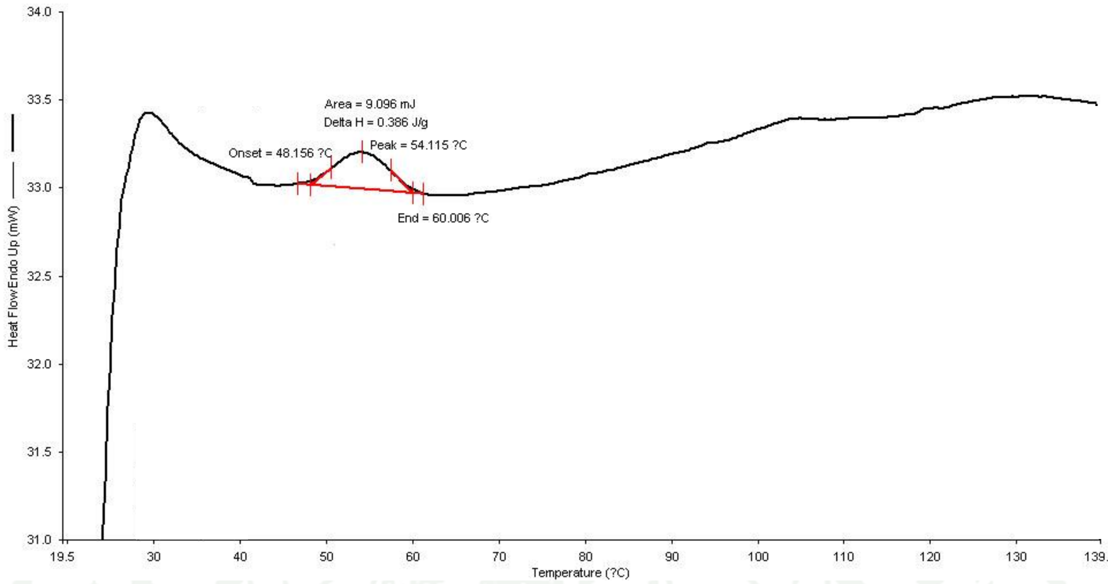
(b)



Appendix Figure 3 The example of thermogram showed glass transition temperature of extruded waxy rice flour observed as a change in heat flow rate at the first scan (a), whereas it did not show at the second scan (b)



Appendix Figure 4 The correlation between the firmness values evaluated by the texture analyzer and sensory evaluation of cakes prepared with wheat flour (WF) and 10% w/w waxy rice flour (WRF) substitution



Appendix Figure 5 The example of thermogram showing enthalpy of melting retrograded amylopectin in cake

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- Scholarship for Ph.D. Program from Kasetsart University and the Faculty of Agro-Industry, Kasetsart University.
- Scholarship for research program from the Carbohydrate Center, Graduate Institute of Food Science and Technology, National Taiwan University.
- Certificate of Merit "A Finalist in IFT Refrigerated & Frozen Foods Division: Graduate Paper Competition 2013 Annual Meeting of IFT".

PUBLICATIONS

1) Jongsutjarittam, O. and S. Charoenrein. 2014. The effect of moisture content on physicochemical properties of extruded waxy and non-waxy rice flour. **Carbohyd. Polym.** 114: 133-140.

2) Jongsutjarittam, N. and Charoenrein, S. 2013. Influence of waxy rice flour substitution for wheat flour on characteristics of batter and freeze-thawed cake. **Carbohyd. Polym.** 97: 306-314.

PRESENTATIONS

1) Jongsutjarittam, O. and S. Charoenrein. 2014. Glass transition temperature of extruded waxy and non-waxy rice flours. Poster presentation. **12th International Hydrocolloids Conference, Functional hydrocolloids: The key to human health**. Taipei, Taiwan, 5-9 May 2014.

2) Jongsutjarittam, O. and S. Charoenrein. 2014. Application of extruded waxy rice flour in frozen cake. Poster presentation. **12th International Hydrocolloids Conference, Functional hydrocolloids: The key to human health**. Taipei, Taiwan, 5-9 May 2014.

3) Jongsutjarittam, N. and S. Charoenrein. 2013. The influence of extrusion cooking on physicochemical properties of waxy and non-waxy rice flours. Oral presentation. **The 7th International Conference on Starch Technology**. Bangkok, Thailand, 21-22 November 2013.

4) Jongsutjarittam, N. and S. Charoenrein. 2013. The effect of waxy rice flour substitution for wheat flour on staling retardation of freeze-thawed cakes. Poster Presentation. **International Food Technologists (IFT) Annual Meeting and Food Expo 2013**. Chicago, Illinois, USA, 13-16 July 2013.

5) Jongsutjarittam, N. and S. Charoenrein. 2012. Improvement of freeze-thawed cake's quality by waxy rice flour substitution for wheat flour. Oral presentation. **The 7^{th} Taiwan-Thailand Bilateral Conference Multifunctional and Food Make Life Better**, Pingtung, Taiwan, 18-19 October 2012.