รายงานวิจัยฉบับสมบูรณ์

โครงการ: การวิเคราะห์ปฏิสัมพันธ์ของสตาร์ช โปรตีน และไขมัน และการใช้ไมโครเวฟร่วมกับการอบด้วยลมร้อนสำหรับการพัฒนาผลิตภัณฑ์เบเกอรี่จากแป้งข้าวที่มีค่าอัตราการย่อยได้ของแป้งต่ำ

โดย รองศาสตราจารย์นันทวัน เทอดไทย

กุมภาพันธ์ 2559

สัญญาเลขที่ RSA558017

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รองศาสตราจารย์นันทวัน เทอดไทย

ภาควิชาพัฒนาผลิตภัณฑ์
คณะอุตสาหกรรมเกษตร
มหาวิทยาลัยเกษตรศาสตร์

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและมหาวิทยาลัยเกษตรศาสตร์

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ชื่อโครงการ: การวิเคราะห์ปฏิสัมพันธ์ของสตาร์ช โปรตีน และไขมัน และการใช้ไมโครเวฟร่วมกับการอบด้วยลมร้อนสำหรับการพัฒนาผลิตภัณฑ์เบเกอรี่จากแป้งข้าวที่มีค่าอัตราการย่อยได้ของแป้งต่ำ

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ระยะเวลาโครงการ: 3 ปี

โครงการวิจัยนี้ศึกษาการเติมเวย์โปรตีน (WPC, 0 - 6 กรัม/ 100 กรัมแป้ง) การเติมเนย (10 – 30 กรัม/ 100 กรัมแป้ง) และสภาวะการอบ 3 สภาวะ คือ การอบด้วยลมร้อน (HA) การอบด้วยไมโครเวฟ (MW) และ การอบด้วยไมโครเวฟร่วมกับลมร้อน (HA-MW) เพื่อพัฒนาขนมปังที่ปราศจากกลูเตน พบว่า การเพิ่มปริมาณ WPC ทำให้ปริมาตรและปริมาณความชื้นในขนมปังเพิ่มขึ้น การอบแบบ MW และ HA-MW เพิ่มค่าความแข็งของเนื้อขนมปัง และ ค่า L* ของเปลือกขนมปัง ค่าดัชนีน้ำตาลของขนมปังที่อบแบบ HA มีค่า 80.34±0.88. เมื่อเติม WPC 6 กรัม/ 100 กรัมแป้ง พบว่า ค่า GI ลดลงเหลือ 70.46±1.70. นอกจากนี้การเปลี่ยนสภาวะการอบเป็นแบบ MW และ HA-MW ทำให้ค่า GI ลงลงเหลือ 61.67±0.69 โดยการอบบแบบ MW และ HA-MW สามารถลดค่า GI ได้ โดยไม่ขึ้นกับปริมาณเนย ในทางตรงกันข้าม การเพิ่มปริมาณเนย ส่งผลให้ค่า GI ของขนมปังที่อบแบบ HA ลดลง แต่ยังมีค่าสูงกว่าค่า GI ของขนมปังที่อบแบบ MW และ HA-MW การอบแบบ MW และ HA-MW ทำให้เกิดอัตราการให้ความร้อนสูง สามารถเพิ่มความดันไอภายในขนมปัง และทำให้เกิดการสูญเสียความชื้นมากขึ้น การเพิ่มปริมาณเนย ส่งผลให้ค่า a*, b* และ $\triangle$E ของเปลือกขนมปัง และปริมาตรจำเพาะเพิ่มขึ้น ($P \leq 0.05$) แต่ค่า hardness และ chewiness ของเนื้อขนมปังจากการอบทุกแบบลดลง อย่างไรก็ตามการเติมเนยที่มากเกินไป (มากกว่า 20 กรัม/ 100 กรัมแป้ง) ทำให้ปริมาตรจำเพาะของขนมปังที่อบแบบ MW และ HA-MW ลดลง เมื่อเก็บขนมปังปราศจากกลูเตนที่ใส่ WPC 4 กรัม/ 100 กรัมแป้ง กรัม/ 100 กรัมแป้ง และ เนย 20 กรัม/ 100 กรัมแป้ง ที่อุณหภูมิ 4 ºC เป็นเวลา 7 วัน พบว่า enthalpy ของ retrogradation และค่า crystallinity ของขนมปังทุกแบบเพิ่มขึ้น ($P \leq 0.05$) แสดงการเก่าเก็บของขนมปัง ซึ่ง enthalpy และ ค่า B-type crystallinity ของขนมปังจากการอบแบบ MW และ HA-MW สูงกว่าค่าของขนมปังจากการอบแบบ HA.

Keywords: ขนมปัง, ดัชนีน้ำตาล, แป้งข้าว, ไมโครเวฟ, การอบ

ABSTRACT

Project Code: RSA558017

Project Title: Analysis of starch, protein and fat interaction and microwave assisted convective baking for product development of rice flour based bakery with low starch digestibility rate

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Project Period: 3 years

To develop gluten free bread, rice flour dough with the addition of whey protein concentrate (WPC, 0 – 6 g/100g flour) and butter (10 – 30 g/100g flour) was baked under 3 conditions—hot air baking (HA), microwave baking (MW) and microwave-assisted hot air baking (HA-MW). An increase in the WPC content increased the volume and the moisture content of the bread ($P \leq 0.05$). The MW and HA-MW methods increased the hardness of bread crumbs and the L* value of the bread crust ($P \leq 0.05$). The estimated glycemic index (GI) of the HA bread was 80.34$\pm$0.88. With the addition of 6 g WPC/100g flour, the GI was reduced to 70.46$\pm$1.70. By changing from HA to MW, the GI was further reduced to 61.67$\pm$0.69. The MW and HA-MW conditions could reduce ($P \leq 0.05$) GI of the bread, regardless of butter content. In contrast, the increased butter content could reduce GI of the HA bread ($P \leq 0.05$). However, GI of the HA bread was still higher than that of the MW and HA-MW bread. The HA-MW and MW conditions provided a high heating rate, enhanced internal vapor pressure and increased moisture loss. Increasing the butter content could increase a*, b* and ΔE values of the bread crust and specific volume. In contrast, hardness and chewiness of all bread crumbs were decreased. Nevertheless, excessive butter content (more than 20 g/100g flour) could reduce specific volume of the MW and HA-MW bread loaf. When the gluten free bread with 4 g WPC/100 g flour and 20 g butter/100 g flour was stored at 4 ºC for 7 days, the enthalpy of retrogradation (ΔH) and crystallinity of all breads were increased ($P \leq 0.05$), indicating bread staling. The enthalpy of retrogradation and degree of B-type crystallinity of the MW and HA-MW bread were higher than those of the HA bread.

Keywords: bread, glycemic index, rice flour, microwave, baking

INTRODUCTION

Bread is full of nutritional benefit with good flavor and taste. It is one of the most popular bakery products and is a carbohydrate-rich product containing a high amount of rapidly digestible starch, particularly in white bread (Therdthai and Zhou, 2014). The digestibility of a carbohydrate-rich product can be estimated by the glycemic index (GI) where GI is expressed as a percentage of the incremental glucose area under the curve (iAUC) of a test food that is referenced to a standard food containing the same available carbohydrates (Willett *et al*, 2002). Generally white bread has a high GI (above 70). Carbohydrate-based food with a high GI should be easily digested. However, food with too high GI may cause chronic disease such as obesity, diabetes, cardiovascular disease and cancer (Marques *et al*, 2007). Bread is generally made from wheat flour containing high protein content. Gluten is one of the proteins in wheat flour that is important for dough formation and determining bread characteristics. The gluten protein is harmful for people who suffer with celiac disease. The celiac disease is a chronic disease causing an inflammation of the small intestine which damage micro-villi leading to several symptoms such as diarrhea, vomiting, weight loss, digestive problem, skin disorder and osteoporosis. Furthermore, the increasing rate of type I diabetes in celiac disease patients have been reported. Thus, non-gluten flour is used for substitution of wheat flour in bread (Rosell *et al.,* 2007 and Rosell and Marco, 2008).

Rice (*Oryza sativa L.*) is one of the leading food crops in South East Asia (Sivaramakrishnan *et al*, 2004). Recently, rice flour has become increasingly popular as a substitute for wheat flour in the preparation of products for wheat-intolerant or celiac patients. Rice is the most suitable cereal grain flour for the production of gluten-free products due to its bland taste, white color, and hypoallergenic properties (Rosell and Santos, 2010). The absence of gluten which provides dough with viscoelastic properties results in a liquid batter rather than dough. After baking, gluten free bread might have a crumbling texture, poor color and baking quality defects (Onyango *et al,* 2010). Some proteins may be added to improve the quality of the gluten free bread as the proteins can form complexes with starch (particularly amylose) and increase the viscosity of the dough (Onwulata *et al,* 2014).

Whey protein concentrate (WPC) is one of the proteins used in the gluten-free baked products. The protein content of WPC is in a range 25–80% depending on the removal of non-protein solids (Indrani *et al*, 2007). Its thermal stability is above 70^{0}C. Therefore, it has been used in gluten-free bread to increase water absorption to enhance the handling properties of the batter (Gallagher *et al*, 2004). The use of WPC and butter milk powder could improve dough stability and resistance to extension (Madenci and Bilgicli, 2014).

Furthermore, addition of fat into dough is to develop gas-retention of dough by stabilization of gas bubbles. Moreover, small cell size of crumb structure is developed because of inhibition of gas-bubble coalescence (Cauvain and Young, 2006). Therefore, an increase in fat proportion in bread formula enlarges bread volume, improve mouth feel and increase heat transfer (Stauffer, 1996). Additionally, butter is one of the popular fat categories used as an ingredient in the bread product. This might be because of two major advantages, including desirable flavor and melting in the mouth without an unpleasant film coating in the mouth. It is composed of about 80% fat, 15% water and 5% milk solid. It can be classified into salted and unsalted types. The unsalted type is fresher than another. However, the use of salted butter in dough formula may lead to the reduced salt addition in the formulation (Cauvain and Young, 2006).

Baking is one of the steps in bread making. The baking conditions should be properly designed to ensure transformation of dough into bread by considering the steps of starch gelatinisation, protein denaturation, volume expansion, water evaporation and crust formation among others (Therdthai and Zhou, 2014). To speed up the process, microwave heating has been proposed in the baking process of some products, particularly bread and cake. The rapid heat transfer increases the internal pressure and enhances mass transfer from the inner layer to the product surface. However, the temperature within the microwave chamber is not high and consequently, condensation can occur on the product surface which slows down the formation of the bread crust and the Maillard browning reaction may be not completed (Sumnu, 2001). Condensation can be reduced by applying hot air together with microwave baking to increase the surface temperature. Nonetheless, the mechanism of starch gelatinization during the microwave heating was different, compared to the conductive heating. The lack of starch swelling, the granule rupture and the formation of film polymers coating the granule surface were observed during the microwave heating. This could be due to the high heating rate and the vibrational motion (Palav and Seetharaman, 2007). In the case of the chestnut starch, the swelling power was decreased, but the size of starch granule was increased when the microwave treatment time was increased (Shah *et al*, 2016). In addition, the rapid heating rate associated with microwave affected the lamellar structure of the starch granules and enhances the formation of the unpacked helices and disordered ends into a crystalline state (Fan *et al,* 2014). The crystalline form also affects the starch digestibility.

During baking, the supplemented protein in the gluten free dough could be denatured to provide structural support to starch in the bread through the formation of a protein-starch matrix (Ziobro *et al,* 2013). In the pound cake, more matrix protein was developed during the microwave baking than the conventionally baking, resulting in the different hydrolysis. Moreover, the short baking time during the microwave baking yielded the high non-gelatinized starch and retrograded starch. Therefore, the predicted GI of the microwave baked pound cake was lower than that of the conventional baking. The influence of microwave may be varied, depending on dielectric properties of ingredients in the formula (Sanchez-Pardo *et al*, 2007). Therefore, variation in the heating profiles during baking may affect starch gelatinization, retrogradation and quality of the gluten free bread.

As above review, bread characteristics may be varied, depending on ingredients and baking conditions. Therefore this project aims to investigate effect of WPC addition, butter addition and baking condition on characteristics of gluten free bread.

PART I: EFFECT OF WPC ADDITION AND BAKING CONDITION

ON CHARACTERISTICS OF THE GLUTEN FREE BREAD

MATERIALS AND METHODS

Materials

Rice flour (100 mesh) with 80.31 g starch/100 g sample and 23.04 g amylose/100 g starch bought in Pechpanthong Thailand, was used as a main ingredient in bread making. Other materials included dry yeast (Bruggeman, Belgium), salt (Prung Thip, Thailand), butter (Orchids, Thailand), sugar (Mitr Phol, Thailand), hydroxypropyl methylcellulose (HPMC; Methocel K4M, Vicchi Enterprise, Thailand) and whey protein concentrate (WPC; WPC 80 Instant, Vicchi Enterprise, Thailand).

Preparation of Gluten Free Dough from Rice Flour

Rice flour and other dry ingredients (18 g sugar, 4 g HPMC, 1.6 g yeast and 1 g salt per 100 g rice flour) were mixed with WPC (0, 2, 4 and 6 g/100 g rice flour) and water (the amount of water varied from 85 to 105 g/100 g rice flour to maintain the same consistency for all dough samples) in a mixer (Kitchen Aid, Model 5K 566, USA.) at speed 1 (180 rpm) for 2 min. Then, butter (20 g/100 g rice flour) was added and mixed at speed 2 (240 rpm) for 3 min. The obtained dough was placed in a mould and proved in a prover (Siam Incubator System, Thailand) at 35°C and 85 % relative humidity for 120 min.

After proving, the rice flour dough (700g) was baked in a baking oven (LG MP9489SRC, Thailand) under 3 conditions—hot air baking at 195°C for 30 min (HA), microwave baking at 530 W (tested by the IMPI 2-L method) for 12 min (MW) and combination of the microwave baking at 530 W (tested by the IMPI 2-L method) and hot air baking at 195°C for 10 min (HA-MW). The bread was tested to determine the physical and chemical quality, thermal characteristics and GI.

Determination of Quality of Rice Flour Bread

The obtained bread from the variation in the WPC contents and baking conditions was tested for quality. The moisture content was determined using the oven method (AOAC, 2000). The volume was determined using the rapeseed displacement method (AACC, 2000). The color of the bread crumb and crust were determined using the CIE system (L*, a* and b*) and was measured using a spectrophotometer (Minolta Model CM-3500d, Japan). The texture profile analysis of bread crumbs (a 20 × 20 × 15 mm crumb piece collected from the loaf centre) was investigated using a texture analyzer (TA-XT Plus, Stable Micro System, UK). A probe (P/50) was used with a 20 mm/s test speed. The TPA test was performed at 60% deformation. The pause between the compression cycles was 5 s. The hardness, springiness, cohesiveness and chewiness were estimated from a force-distance profile. At least eight replications of each treatment were carried out and average values were reported.

In-Vitro Starch Digestion and Predicted Glycemic Index

To estimate the GI, an in-vitro starch digestion test was carried out following the method reported in Sopade and Gidley (2009). About 0.5 g of sample was treated with 1 mL of artificial saliva containing porcine a-amylase (Sigma A-3176 Type VI-B) for 15-20 s and incubated with 5 mL of pepsin (Sigma P-6887) at 37°C for 30 min in a shaking water bath (Memmert, Model WB-22, Germany) with 85 rpm reciprocation. The digesta was neutralised with 5 mL of 0.02 M NaOH. Then, 25 mL of 0.2 M sodium acetate buffer was used to adjust the pH to 6. Next, 5 mL of mixture of pancreatin (Sigma P1750) and amyloglucosidase (Sigma A-7420) was added and incubated at 37°C to hydrolyze the digested starch into glucose. The glucose concentration in the digesta during 4 h incubation was measured using a glucometer (Glucocheck blood glucose monitoring system, TD-4230, Germany) at 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min. The amount of digested starch per 100 g dry starch (D) was calculated using Equation (1).

$$D = \frac{0.9 \text{ x } G_G \text{x } 180 \text{ x } V}{W \text{ x } S[100-M]} \tag{1}$$

where G_G is the glucometer reading (mM/L), V is the volume of digesta (mL), 180 is the molecular weight of glucose, W is the weight of the sample (g), S is the starch content of the sample (g/100 g dry sample), M is the moisture content of the sample (g/100 g sample) and 0.9 is the stoichiometric constant for the starch from the glucose contents.

A modified first order exponential model (Mahasukhonthachat *et al,* 2010) was used to simulate digestograms presenting the change in the digested starch content during the in-vitro starch digestion for 240 min. The model included the digested starch at an initial time which represented the rapidly digested starch as shown in Equation 2.

$$D_t = D_0 + D_{\infty-0}(1 - exp[-kt]) \quad (2)$$

where D_t is the digested starch at time t, D_0 is the digested starch at initial time (t=0), D_∞ is the digested starch at time ∞ and k is the rate constant (per minute).

The area under each digestogram (AUCexp) was estimated using Equation (3).

$$AUC_{exp} = \left[D_\infty t + \frac{D_{\infty-0}}{k} exp(-kt)\right]_{t_1}^{t_n} \quad (3)$$

The ratio of the whole area under a digestogram of a sample to the whole area under the digestogram of standard material from white bread was defined as the hydrolysis index (HI). The average glycemic index (GI) was described by Goni *et al* (1997) and estimated using Equation 4.

$$GI = \left[\frac{((39.21+0.803H_{90})+(39.51+0.573HI))}{2}\right] \quad (4)$$

where HI_{90} is the hydrolysis at t=90 min. HI is the hydrolysis at t=120 min.

Determination of Enthalpy of Retrogradation in the Rice Flour Bread

The enthalpy of retrogradation (ΔH) of freshly baked bread and of bread stored at 4°C for 24 and 48 h were evaluated using a differential scanning calorimeter (DSC; 822e, Mettler-Toledo GmbH, Greifensee, Zurich, Switzerland). The test conditions were modified from Ziobro *et al* (2013). Bread crumb (15 mg) was packed into an aluminium pan and hermetically sealed before being analyzed. The sample pan was heated at a rate of 5 $^{\circ}$C/min from 20 to 120°C. The enthalpy of retrogradation expressed in joules per gram of dry sample was estimated by integrating the area under the endothermic peak using the Star^{e} Thermal Analysis Software Ver.8.1. Three replicates for each sample were carried out.

Statistical Analysis

All experiments were carried out with three replications. Quantitative data were analyzed using the statistical package SPSS version 12.0 (SPSS (Thailand) Co., Ltd., Bangkok, Thailand) for 2-way ANOVA and for Duncan's multiple range test to determine significant differences ($P \leq 0.05$) between treatments. In addition, correlations among the moisture content, volume, texture, retrogradation and GI were calculated.

PART I: EFFECT OF WPC ADDITION AND BAKING CONDITION ON CHARACTERISTICS OF THE GLUTEN FREE BREAD

RESULTS AND DISCUSSION

Effect of WPC Addition and Baking Condition on Volume and Moisture Content of the Rice Flour Bread

With the HA method, the rice flour bread without the addition of WPC had the smallest volume (813.5 cm^3). By adding 2–6 g WPC/100 g flour, the volume was significantly ($P \leq 0.05$) improved to 839.2–880.5 cm^3 (Table 1). Similarly, Kittisuban *et al* (2014) reported that the addition of whey protein isolate (2 g/100g), in the presence of HPMC (5 g/100g), increased the specific volume of gluten-free bread from 3.52 cm^3/g (without whey protein isolate) to 5.01 cm^3/g. Shevkani and Singh (2014) studying gluten-free muffins found the specific volume could be improved from 2.4 cm^3/g to 2.9 and 3.0 cm^3/g when protein isolates from amaranth and field pea, respectively, were added.

In addition changing the baking method from HA to MW for dough containing 4–6 g WPC/100 g flour significantly ($P \leq 0.05$) increased the volume from 860.8–880.5 cm^3 to 885.4–909.3 cm^3 because the microwave heating was rapid and thereby induced pressure in the bread loaf. In addition, the crust formed slowly with the MW method due to the low surface temperature and possible condensation on the bread surface. The delay in crust formation allowed the bread to expand for a longer period. However, the HA-MW method tended to yield a lower volume than the MW method, particularly for bread with a high WPC content. It is possible that hot air baking enhanced the crust formation during microwave heating. The crust developed and restricted volume expansion, although pressure was still induced inside the bread loaf.

Nonetheless, the rapid heating in the MW method possibly enhanced the rapid moisture transfer from the inner layer to the surface. Together with the poor barrier resulting from the slow crust formation, the moisture content of the MW bread (63.91–72.76 g water/100 g dried solid) was significantly ($P \leq 0.05$) less than that of the HA bread (72.99–85.82 g water/100 g dried solid). When the HA method was applied together with the MW method, the crust formed earlier than with the MW method. Therefore, the moisture content increased to 67.41–74.78 g water/100 g dried solid, but was still less than that with the HA method due to the enhanced mass transfer from MW heating. By formulating the rice flour dough with WPC, water addition was increased to 85-105 g/100 g flour due to the increased protein content from WPC that could absorb more water. As a result, the bread with WPC addition tended to have a higher moisture content.

Table 1 Volume and moisture content of rice flour bread

Baking method	WPC (g/100 g flour)	Volume (cm^3)	Moisture content (g water/100 g dried solid)
HA	0 (Control)	813.5±2.07^{fg}	79.83±0.64^{b}
	2	839.2±3.98^{d}	72.99±0.16^{e}
	4	860.8±3.31^{c}	78.45±0.09^{c}
	6	880.5±2.45^{b}	85.82±0.04^{a}
MW	0 (Control)	808.6±5.45^{fg}	65.31±0.45^{h}
	2	827.8±6.47^{de}	63.91±0.20^{i}
	4	885.4±1.90^{b}	67.84±0.22^{g}
	6	909.3±6.32^{a}	72.76±0.39^{e}
HA-MW	0 (Control)	802.9±5.53^{g}	70.08±1.22^{f}
	2	817.0±3.88^{ef}	67.41±0.24^{g}
	4	864.8±5.32^{c}	69.47±0.82^{f}
	6	891.5±3.53^{b}	74.78±0.89^{d}
Probability			
	Baking	0.000*	0.000*
	WPC	0.000*	0.000*
	Baking x WPC	0.000*	0.000*

$^{a-i}$ Means within the same column with different letters are significantly different ($P \leq 0.05$).

* is significant at $P \leq 0.05$

Effect of WPC Addition and Baking Condition on Color of the Rice Flour Bread

Figure 1 shows the variation in bread crust color after baking. The 2-way ANOVA indicated that the addition of WPC, the baking condition and their interaction (Baking x WPC) played significant roles in determining the crust color. The influence of WPC addition was much more important for the HA method compared to when microwaves were used. The rice flour dough formulated with the increased WPC content (0–6 g/100 g flour), reduced the lightness (L* value) and yellowness (b* value) of the bread crust, whereas the redness (a* value) increased significantly ($P \leq 0.05$). A similar color change pattern was observed under all baking conditions because an increase in the protein content from the addition of WPC enhanced the Maillard browning reaction (Stanley and Linda, 2006). This coincided with the increased redness in gluten-free muffins from 13.7 to 17.2, 18.0 and 19.9 when protein isolates from kidney bean, amaranth and field pea, respectively were added (Shevkani and Singh, 2014). However, the degree of crust color change with the MW and the HA-MW methods was less than with the HA method because of the short time for the baking process.

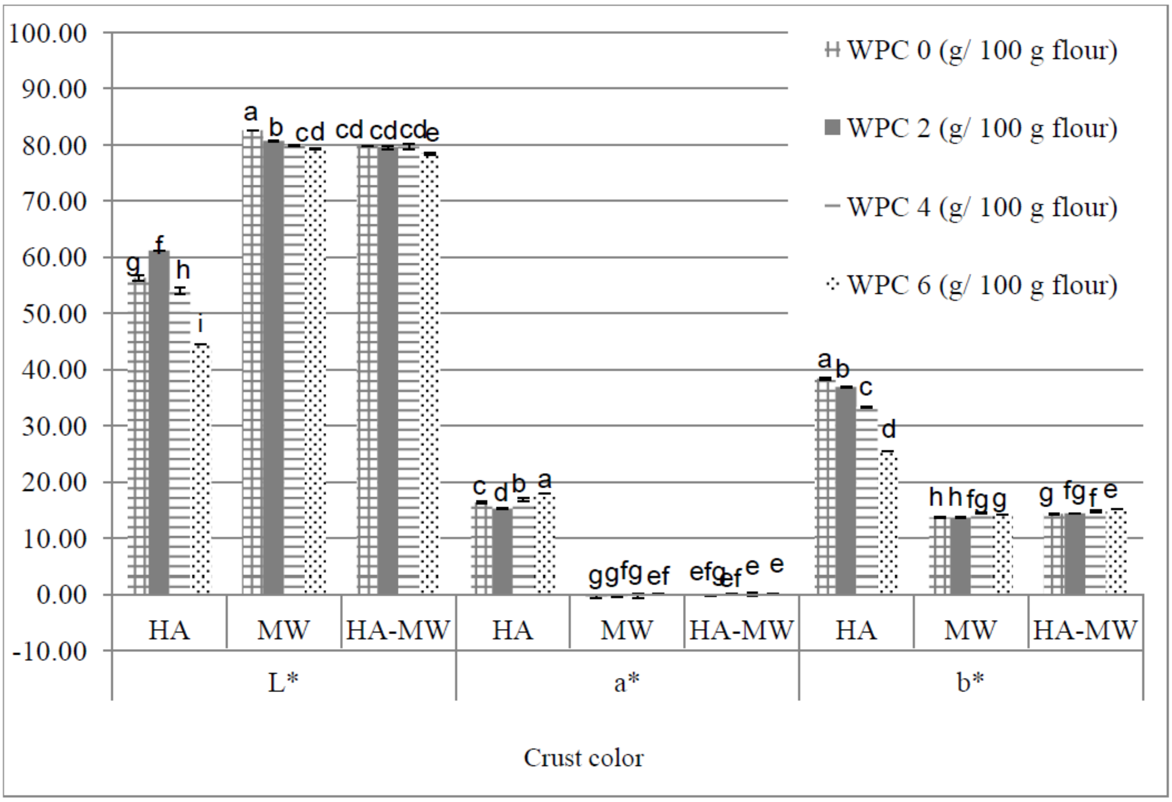

Figure 1 Crust color of rice flour bread.

In addition, MW heating enhanced moisture transfer to the dough surface while the temperature in the microwave chamber was not high. As a result, condensation occurred on the dough surface. This slowed down the Maillard browning reaction. Therefore, the L* value of the MW bread crust was significantly higher than that of the HA bread crust. In contrast, the a* value of the MW bread crust was significantly lower than that of the HA bread crust. Typically MW food is not brown because the surface temperature is too low to enhance the Maillard browning reaction and caramelization (Summu, 2001). MW baking (at 900 W) of Madeira cake resulted in a steady-state surface temperature of 94.1°C which was lower than the steady-state core temperature of 102.7°C (Megahey *et al*, 2005). By using the MW method together with the HA method, the temperature of the dough surface was heated up so that the Maillard browning reaction could be enhanced. Therefore the crust color of the HA-MW bread was darker with a lower L* value than for the MW bread. However, the HA-MW bread crust color was still not comparable with the HA bread.

Nonetheless, variation in the baking conditions did not significantly affect the L* value of bread crumbs (Figure 2). Moreover, there was little variation in the a* and b* values of bread crumbs under the different baking conditions, as all bread crumbs still appeared white.

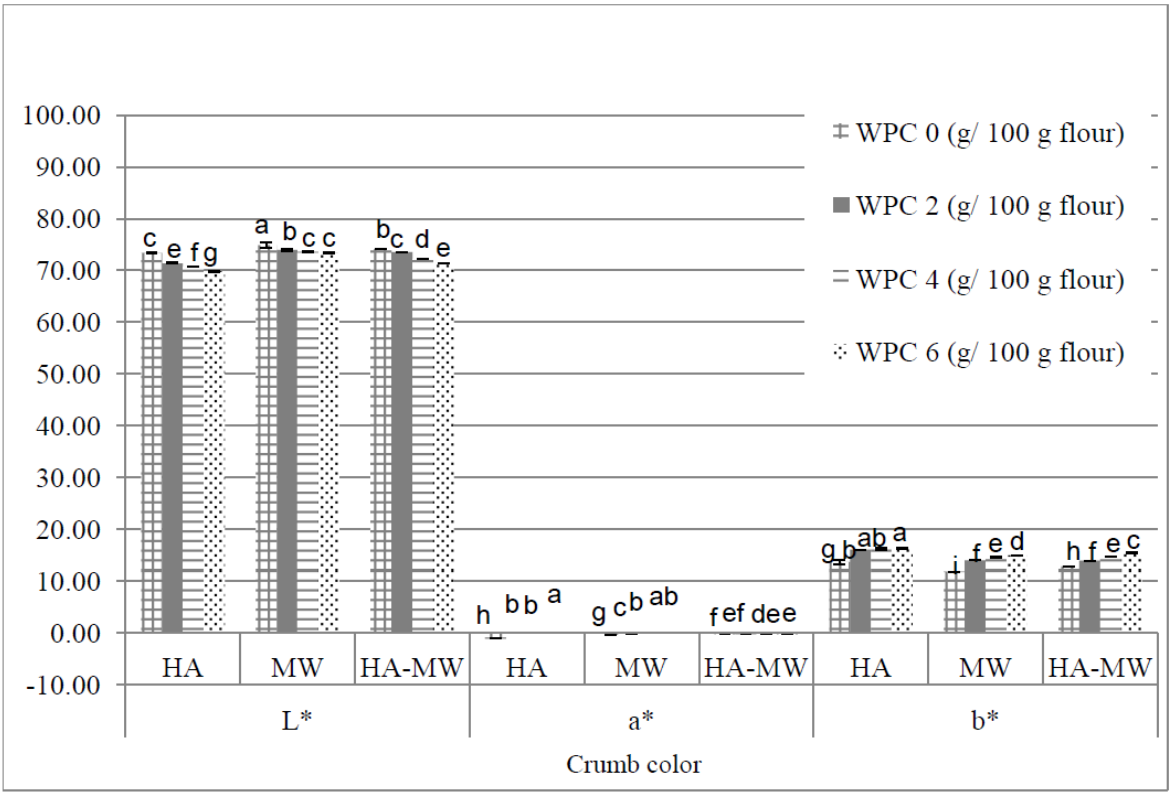

Figure 2 Crumb color of rice flour bread.

Effect of WPC Addition and Baking Condition on Texture of the Rice Flour Bread

The 2-way ANOVA indicated that the addition of WPC, the baking conditions and their interaction (Baking x WPC) played significant roles in determining the hardness, cohesiveness and chewiness of the samples. With the HA method, all bread samples had bread crumb hardness in the range 4.22–5.63 N. The cohesiveness and chewiness tended to increase with the addition of WPC (up to 4 g /100 g flour) because protein formed a network, absorbed more water and increased the dough viscoelasticity, thereby retaining the air cells in the dough. Similarly, Kittisuban *et al* (2014) reported an increase in the cohesiveness and chewiness of gluten free bread from 0.47 and 33.21g to 0.57 and 77.89 g, respectively, when the content of whey protein isolate was increased from 0 to 2 g/100g rice starch. However, too much protein addition (such as 2.5 – 5 g albumen/100g flour) could deteriorate the gluten free bread quality by producing a compact structure (Schoenlechner *et al*, 2010). In the current study, the addition of 6 g WPC/100 g flour caused a decrease in the cohesiveness and chewiness of the HA bread (Table 2).

With bread made using the MW and the HA-MW methods, the WPC addition did not improve springiness. The increased WPC content to 6 g WPC/100 g flour reduced the hardness. However, the hardness of the MW bread was still higher than that of the HA bread because the high heating rate in the MW and HA-MW methods increased the vapor pressure and enhanced moisture migration to the outer layer. As a result, the MW and HA-MW methods caused high moisture loss during baking. The consequent bread crumbs could have been dried by such loss and the hardness increased. This was consistent with the results of Rodriguez-Sandoval *et al* (2014) who reported that the increased hardness of gluten-free cheese bread from 10 N to 90 N was caused by the decreased moisture content in the crumbs. With the MW and HA-MW methods, the bread with a high WPC content had a preferred crumb texture including low chewiness and hardness, compared with the bread with a low WPC content.

Table 2 Texture profile analysis of rice-flour bread crumbs

Baking method	WPC (g/100 g flour)	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
HA	0	4.73±0.65[g]	1.04±0.11[ab]	0.78±0.02[cd]	3.81±0.55[g]
	2	5.63±0.46[f]	1.00±0.03[ab]	0.90±0.06[ab]	5.13±0.74[ef]
	4	5.39±0.30[f]	0.99±0.05[ab]	0.85±0.07[bc]	4.54±0.52[f]
	6	4.22±0.46[g]	0.91±0.11[c]	0.73±0.01[d]	2.80±0.49[h]
MW	0	9.62±0.82[c]	1.02±0.07[a]	0.64±0.10[e]	6.31±1.24[cd]
	2	11.65±0.33[a]	1.04±0.02[ab]	0.99±0.05[a]	12.03±0.75[a]
	4	10.18±0.54[b]	1.02±0.04[ab]	0.85±0.07[bc]	8.76±1.03[b]
	6	6.24±0.99[e]	1.01±0.02[ab]	0.90±0.05[ab]	5.74±1.18[de]
HA-MW	0	7.49±0.99[d]	1.02±0.02[ab]	0.87±0.09[b]	6.60±1.19[c]
	2	10.23±0.68[b]	0.99±0.07[ab]	0.83±0.15[bc]	8.14±0.69[b]
	4	6.19±0.30[e]	0.97±0.08[b]	0.92±0.12[ab]	5.57±0.94[de]
	6	4.27±0.21[g]	0.97±0.10[b]	0.84±0.16[bc]	3.74±0.45[g]
Probability					
	Baking	0.000*	0.032*	0.042*	0.000*
	WPC	0.000*	0.002*	0.000*	0.000*
	Baking x WPC	0.000*	0.162	0.000*	0.000*

[a-h] Means within the same column with different letters are significantly different ($P \leq 0.05$)

* is significant at $P \leq 0.05$

Effect of WPC Addition and Baking Condition on Starch Digestibility of the Rice Flour Bread

The 2-way ANOVA showed that the addition of WPC, baking conditions and their interaction (Baking xWPC) played significant roles in the starch digestibility of bread (Table 3). After adding 6 g WPC/100 g flour, the D_0 value of the HA, MW and HA-MW breads decreased from 35.58, 31.15 and 40.94 g/100 g starch to 23.26, 20.63 and 25.57 g/100 g starch, respectively indicating that the rapidly digested starch was reduced. It is possible that the protein-starch matrix formed during baking could slow down starch digestibility. A decrease in the value of D_0 with increased WPC content was observed under all baking conditions. As a result, the estimated GI decreased, when the protein content increased. Similarly an increase in the solubility of the protein in the sourdough bread reduced the plasma glucose concentration in an in-vivo test from 272 to173 mmol/l per minute (Lappi *et al*, 2010).

Table 3 Hydrolysis index and glycemic index of rice-flour bread

Baking method	WPC (g/100 g flour)	D_0 (g/100 g starch)	K (per min)	GI_{HI90}	GI_{HI}	Avg GI
HA	0	35.58±5.59^{abc}	0.07±0.00^{ab}	67.50±0.68^{b}	93.18±1.07^{b}	80.34±0.88^{b}
	2	33.38±1.25^{abcd}	0.07±0.01^{ab}	66.26±0.50^{bc}	90.96±0.94^{bc}	78.61±0.72^{bc}
	4	26.21±0.90^{def}	0.06±0.00^{ab}	64.86±0.32^{bcd}	88.31±0.62^{cd}	76.59±0.47^{cd}
	6	23.26±4.50^{ef}	0.05±0.02^{b}	60.68±1.23^{de}	80.24±2.17^{de}	70.46±1.70^{e}
MW	0	31.15±1.66^{bcde}	0.09±0.01^{ab}	66.07±1.49^{bc}	90.62±2.83^{bc}	78.34±2.16^{bc}
	2	23.68±0.22^{ef}	0.10±0.00^{ab}	61.11±0.58^{ef}	80.20±0.71^{e}	70.65±0.65^{e}
	4	26.38±0.24^{d}	0.09±0.00^{ab}	56.84±1.31^{ef}	72.62±3.14^{f}	64.73±2.22^{f}
	6	20.63±3.07^{f}	0.02±0.00^{b}	54.24±0.96^{fg}	68.60±1.83^{fg}	61.67±0.69^{g}
HA-MW	0	40.94±3.19^{a}	0.11±0.01^{a}	70.00±1.90^{a}	98.19±3.48^{a}	84.62±1.95^{a}
	2	28.68±4.42^{cde}	0.11±0.11^{a}	63.92±2.75^{cde}	86.51±2.38^{d}	75.22±2.56^{d}
	4	38.16±6.56^{ab}	0.08±0.02^{ab}	62.04±1.78^{de}	81.50±1.98^{e}	71.99±1.88^{e}
	6	25.57±1.63^{def}	0.09±0.01^{ab}	58.37±0.60^{fg}	74.07±0.67^{f}	66.22±0.64^{f}
Probability						
	Baking	0.001*	0.000*	0.000*	0.000*	0.000*
	WPC	0.000*	0.000*	0.000*	0.000*	0.000*
	Baking x WPC	0.090*	0.000*	0.040*	0.000*	0.001*

$^{a-g}$ Means within the same column with different letters are significantly different ($P \leq 0.05$).

* is significant at $P \leq 0.05$

In addition, applying a rapid heating rate with the MW method possibly destroyed the lamellar structure within the starch granules and the double helices were loosened, followed by the unpacked helices and disordered ends being packed into a crystalline structure (Fan *et al,* 2014). This could slow down starch digestion; as a result, the MW bread contained a lower estimated GI than the HA bread. Although the HA-MW method had a faster heating rate, the estimated GI was not lower because a crust was formed by the hot air heating and this formed a barrier to outside moisture loss. Therefore, the starch gelatinization profile with the HA-MW method may not differ from that with the HA method. The MW method only produced a high heating rate in the inner layer and so the crust formed slowly. The rate of moisture migration to the outer layer was increased and thus, the water available for starch gelatinization might have become limited. The deformation of starch granules was different from that in the HA bread and the HA-MW bread. Therefore, its estimated GI was the least.

Effect of WPC Addition and Baking Condition on Retrogradation of the Rice Flour Bread

Table 4 shows the enthalpy of retrogradation in the rice flour bread with variation in the WPC content and baking conditions. Of all the baking conditions for dough without WPC addition, the MW method yielded bread with a higher enthalpy of retrogradation than from the HA and HA-MW methods. This could be explained by a rapid heating rate and high moisture loss with the MW method. After the bread was cooled, retrogradation occurred. At an early stage, crystallinity of the starch gel was dominated by the recrystallization of amylose whereas crystallinity in the later stage was dominated by the recrystallization of amylopectin (Singh *et al*, 2012). An increase in the heating rate from 6^{0}C/min to 25^{0}C/min during baking could significantly increase the amylopectin recrystallization enthalpy from nearly 0 J/g to almost 1 J/g after 2 h storage. The correlation between the heating rate and amylopectin recrystallization enthalpy was significant up to 7 d of storage (Patel *et al,* 2005). The increased enthalpy indicated that more perfect recrystallization resulted with the MW method. This is important for enzymatic starch hydrolysis and coincided with the lower GI in the MW bread than in bread baked using the other methods.

Without the addition of WPC, the enthalpy of retrogradation was in the range 0.44–0.55 J/g. A main structural backbone in the gluten-free bread would arise from the three dimensional starch network (Barbiroli *et al,* 2013). With the addition of WPC, the enthalpy of retrogradation increased to 0.79–0.92 J/g due to the interaction between starch and protein that possibly occurred through hydrophobic interactions. Therefore protein was adsorbed onto the starch granule interface. In the protein-starch mixture, the amount of casein adsorption into starch slowly reached a plateau value of 0.35 mg/m^{2} when 0.25% NaCAS was used. An increase in the concentration of NaCAS to 10% could speed up protein adsorption and increase the plateau value of 0.7 mg/m^2 depending on the concentration of NaCAS (Noisuwan *et al,* 2011). The adsorption of protein on the starch could affect the crystallization of amylose and amylopectin. After keeping bread at 4°C for 24 h and 48 h, the enthalpy of the bread with WPC addition increased to 0.93–1.05 J/g and 1.10–1.81 J/g, respectively. In addition, the MW bread had a higher enthalpy of retrogradation than did HA-MW bread and HA baked bread.

Table 4 Enthalpy of retrogradation in rice-flour bread

Baking method	WPC (g/100 g flour)	ΔH (J/g) Fresh bread	24 h storage	48 h storage
HA	0	0.44±0.03^{d}	0.66±0.02^{c}	1.01±0.16^{c}
	4	0.86±0.21^{ab}	1.05±0.23^{a}	1.10±0.09^{c}
MW	0	0.55±0.06^{c}	0.76±0.13^{b}	1.55±0.13^{b}
	4	0.92±0.32^{a}	0.98±0.10^{a}	1.81±0.24^{a}
HA-MW	0	0.48±0.04^{d}	0.77±0.18^{b}	1.07±0.13^{c}
	4	0.79±0.40^{b}	0.93±0.01^{a}	1.43±0.09^{b}

$^{a\text{-}d}$ Means within the same column with different letters are significantly different ($P \leq 0.05$).

Correlations among Bread Quality Attributes

Variation in the baking conditions and the WPC addition could produce gluten-free bread from rice flour with varying quality attributes. Some correlations among those attributes were significant (Table 5). Bread containing a high moisture content tended to have high values of a* and b* for the bread crust but a low value of L* for the bread crust. Moreover, that bread would have low values of hardness, springiness and chewiness. The rice flour bread with a high enthalpy of recrystallization would have a reduced average GI. Therefore, the baking conditions and WPC addition should be optimized, to develop the required quality of gluten-free bread.

Table 5 Correlation matrix among baking quality characteristics of rice flour bread

	Moisture content	Volume (cm^3)	Crust L*	Crust a*	Crust b*	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Enthalpy (J/g)	Avg GI
Moisture	1	0.334	-0.891*	0.819*	0.662*	-0.855**	-0.608*	-0.297	-0.827**	0.297	0.020
Volume (cm^3)		1	-0.092	-0.004	-0.128	-0.384	-0.504	0.211	-0.279	0.943**	-0.873**
Crust L*			1	-0.956**	-0.820**	0.644*	0.517	0.264	0.626*	-0.091	-0.203
Crust a*				1	0.942**	-0.616*	-0.339	-0.206	-0.588*	-0.070	0.323
Crust b*					1	-0.579*	-0.078	-0.114	-0.529	-0.214	0.414
Hardness (N)						1	0.563	0.154	0.935**	-0.303	-0.030
Springiness							1	0.231	0.595*	-0.486	0.251
Cohesiveness								1	0.470	0.087	-0.264
Chewiness (N)									1	-0.253	-0.120
Enthalpy (J/g)										1	0.806**
Avg GI											1

** Correlation is significant at $P \leq 0.01$

* Correlation is significant at $P \leq 0.05$

PART II: EFFECT OF BUTTER CONTENT AND BAKING CONDITION ON CHARACTERISTICS OF THE GLUTEN FREE DOUGH AND BREAD

MATERIALS AND METHODS

Materials

Rice flour (100 mesh) with 80.31 g starch/100 g sample and 23.04 g amylose/100 g starch bought in Pechpanthong Thailand, was used as a main ingredient in bread making. Other materials included dry yeast (Bruggeman, Belgium), salt (Prung Thip, Thailand), butter (Orchids, Thailand), sugar (Mitr Phol, Thailand), hydroxypropyl methylcellulose (HPMC; Methocel K4M, Vicchi Enterprise, Thailand) and whey protein concentrate (WPC; WPC 80 Instant, Vicchi Enterprise, Thailand).

Preparation of gluten free dough and bread

Rice flour and other dry ingredients (18 g sugar, 4 g HPMC, 1.6 g yeast and 1 g salt per 100 g rice flour) were mixed with WPC (4 g/100 g rice flour) and water (95 g/100 g rice flour) in a mixer (Kitchen Aid, Model 5K 566, USA.) at speed 1 (180 rpm) for 2 min. Then, butter (0, 10, 15, 20, 25 and 30 g/100 g rice flour) was added and mixed at speed 2 (240 rpm) for 3 min. The obtained dough was placed in a mould (260×125×70 mm) and proved in a prover (Siam Incubator System, Thailand) at 35°C and 85 % relative humidity for 120 min.

After proving, the rice flour dough (700g) was baked in a baking oven (LG MP9489SRC, Thailand) under 3 conditions—hot air baking at 195°C for 30 min (HA), microwave baking at 530 W (tested by the IMPI 2-L method) for 12 min (MW) and combination of the microwave baking at 530 W (tested by the IMPI 2-L method) and hot air baking at 195°C for 19 min (HA-MW).

Determination of gluten free dough characteristics

Dough rheology was determined using a rotational rheometer (Malvern-Bohlin Instruments, Gemini 200 HR Nano, UK). For dough making, the gluten free dough was prepared with variation of butter contents (but without adding any yeast to avoid interference of bubble formation). After that, the dough rheology properties corresponding to the both elastic modulus (G') and viscous modulus (G'') were evaluated in triplicates. All samples were determined at 25 °C, using serrated parallel plate geometry (40 mm diameter) with 2000 micron-gap. The samples were placed between the plates. The edges were trimmed with a spatula and then covered with paraffin oil. The test methods carried out the samples were: 1) dynamic frequency sweep range 0.1 - 20 Hz with 0.1% strain and 2) dynamic stress sweep range 1 – 10,000 Pa with 1 Hz- frequency (adapted from Demirkesen *et al.*, 2011).

Determination of gluten free bread quality

The obtained bread from the variation in the butter contents and baking conditions was tested for quality. The moisture content of the bread crumb at the center (with 40 mm. diameter) was determined using the oven method (AOAC, 2000). The volume was determined using the rapeseed displacement method (AACC, 2000). Then, specific volume was defined as volume per g sample.

The color of the bread crumb and crust were determined using the CIE system (L*, a* and b*) and was measured using a spectrophotometer (Minolta Model CM-3500d, Japan) with color mode: L*a*b*, observer: 10° and primary illuminant: D65. Total color change (ΔE) was calculated by Equation 5 (adapted from Demirkesen *et al.*, 2011).

$$\Delta E = [(L^*-L_0)^2 + (a^*-a_0)^2 + (b^*-b_0)^2]^{1/2} \qquad (5)$$

where L*, a* and b* values of the initial dough

The texture profile analysis of bread crumbs (a 20 × 20 × 15 mm crumb piece collected from the loaf centre) was investigated using a texture analyzer (TA-XT Plus, Stable Micro System, UK). A probe (P/50) was used with a 20 mm/s test speed. The TPA test was performed at 60% deformation. The pause between the compression cycles was 5 s (adapted from Barrett *et al.,* 2000). The hardness, springiness, cohesiveness and chewiness were estimated from a force-distance profile. At least eight replications of each treatment were carried out and average values were reported.

In-vitro starch digestion test was carried out following the method reported in Sopade and Gidley (2009) (Detail was listed in Part I).

Determination of gluten free bread quality change during storage

Rice flour bread with 20 g butter/100 g flour baked by HA, MW and HA-MW was stored at 4 ºC for 7 days. Then the bread was sampling to determine quality change.

Thermal properties of bread with addition of 20 g butter/100 g flour were determined by Differential Scanning Calorimeter (DSC) using a DSC instrument (Mettler Toledo, Star^e System, DSC 821^e). Both temperature and enthalpy (ΔH) of retrograded amylopectin of bread crumb during storage for 7 days at 4°C were evaluated, according to the method of Bosmans *et al.* (2013). The bread crumb (4.0 - 5.0 mg) was freeze-dried and exactly weighed in quadruplicate in aluminum pan. Excess de-ionized water was approximately added in a ratio of 1:3 (w/w, sample dry matter: water). After the pan was hermetically sealed, the both sample and the empty reference pan were heated from 0 to 120°C at a rate of 4 °C/min.

Crystallinity of starch in the gluten free bread crumb was determined by X-ray diffractometer (Bruker AXS Model D8 Discover, Germany) using the method of Rattanamechaiskul *et al* (2014) with some modification. Bread crumb (5 mg) was put into a sample holder in the machine operated at 40 kV and 40 mA with Cu-anode source (Cu Ka-radiation of wavelength k = 1.54 A). The scanning range of diffraction angle was started from 5 to 40 and detected every 0.02 of diffraction angle (2θ). The degree of crystallinity (Xc) of the bread sample was calculated by Equation 6.

$$X_c = \left(\frac{A_c}{A_a + A_c}\right) x\ 100 \qquad (6)$$

where A_c and A_a are the areas of crystalline and amorphous regions, respectively.

Statistical analysis

Experiment was designed using a completely randomized design (CRD) to determine variation of gluten free bread characteristics due to variation of butter content and baking conditions. Difference between means of all treatments was analyzed using a one-way ANOVA. Duncan’s multiple range test was used to identify a significant difference (P $\leq$ 0.05) by SPSS statistics version 12.0.

PART II: EFFECT OF BUTTER CONTENT AND BAKING CONDITION ON CHARACTERISTICS OF THE GLUTEN FREE DOUGH AND BREAD

RESULTS AND DISCUSSION

Effect of butter contents on dynamic moduli (G′ and G″) and viscosity of gluten free dough.

The rheological characteristics of the dough (such as elasticity, mixing tolerance and gas holding ability), which related to the gluten matrix, was useful for prediction the appearance and crumb structure of gluten free bread (Lazaridou and Biliaderis, 2009). The dynamic moduli of the gluten free bread consisted of G' (Elastic modulus or shear storage modulus) and G" (Viscous modulus or loss modulus). G' exhibited the non-dissipative component or mechanical properties. Moreover, the G' spectrum, which was independent of frequency and greater than G" over a certain range of frequency, identified the viscoelastic solid or rubber-like property.

In the current study, dynamic moduli of the gluten free dough with variation of butter contents was shown in the Figure 3 - 5. For dough with the butter content (from 0 to 30 g/100 g flour), the value of G' exceeded the G" in all frequency ranges, indicating a viscoelastic property. The G' of dough with addition of butter content 0, 10, 15, 20, 25 and 30 g/ 100 g flour was in the range of 7503 to 2110 Pa, 10760 to 29200 Pa, 13860 to 38460 Pa, 10820 to 30050 Pa, 8057 to 22870 Pa and 6919 to 20040 Pa, respectively. The G" of dough with addition of butter content 0, 10, 15, 20, 25 and 30 g/ 100 g flour was in the range of 2312 to 8126 Pa, 3528 to 10990 Pa, 4770 to 14810 Pa, 3739 to 11030 Pa, 3020 to 8807 Pa and 2491 to 7767 Pa, respectively. The range of dough rheological properties of these samples was coincided with the other gluten free bread and wheat bread. Additionally, Sciacarini *et al.* (2012) reported the gluten free bread with addition of several additives (emulsifiers, hydrocolloids and enzymes) had G' value of around 21640 to 165500 Pa. and G" value of around 3900 to 41100 Pa. Balestra *et al.* (2011) reported G' value of the wheat bread containing ginger powder was in the range of 9267 to 12878 Pa. while G" value was in the range of 5004 to 6526 Pa.

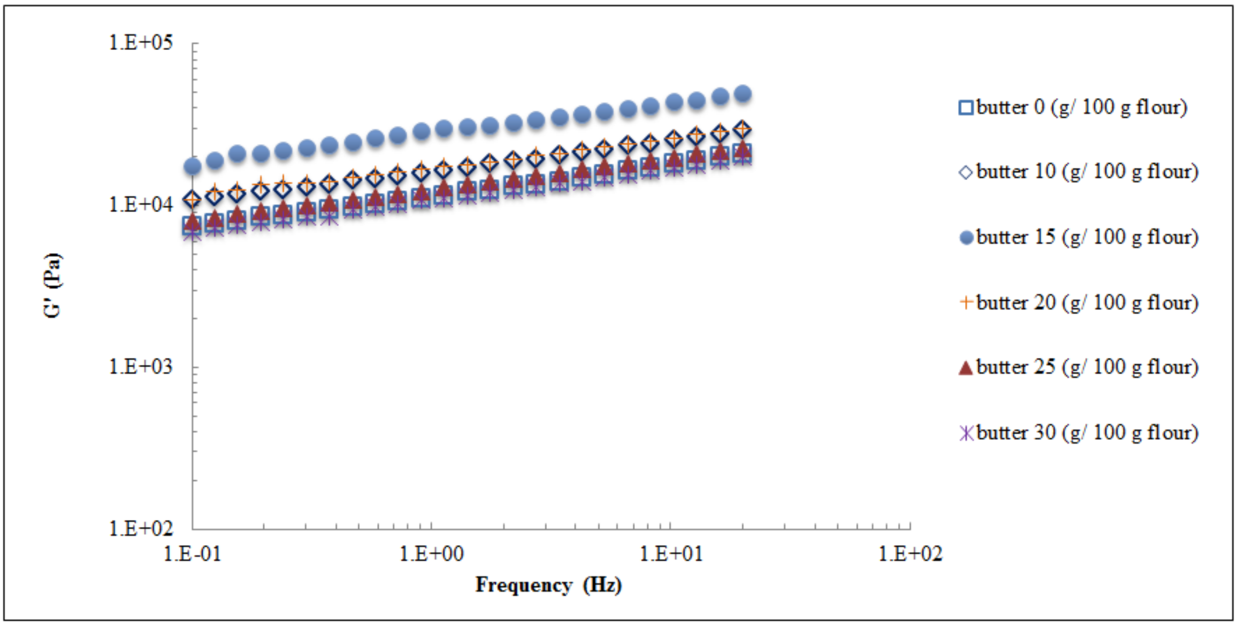

Figure 3 Elastic modulus (G′) of gluten free dough with variation of butter content.

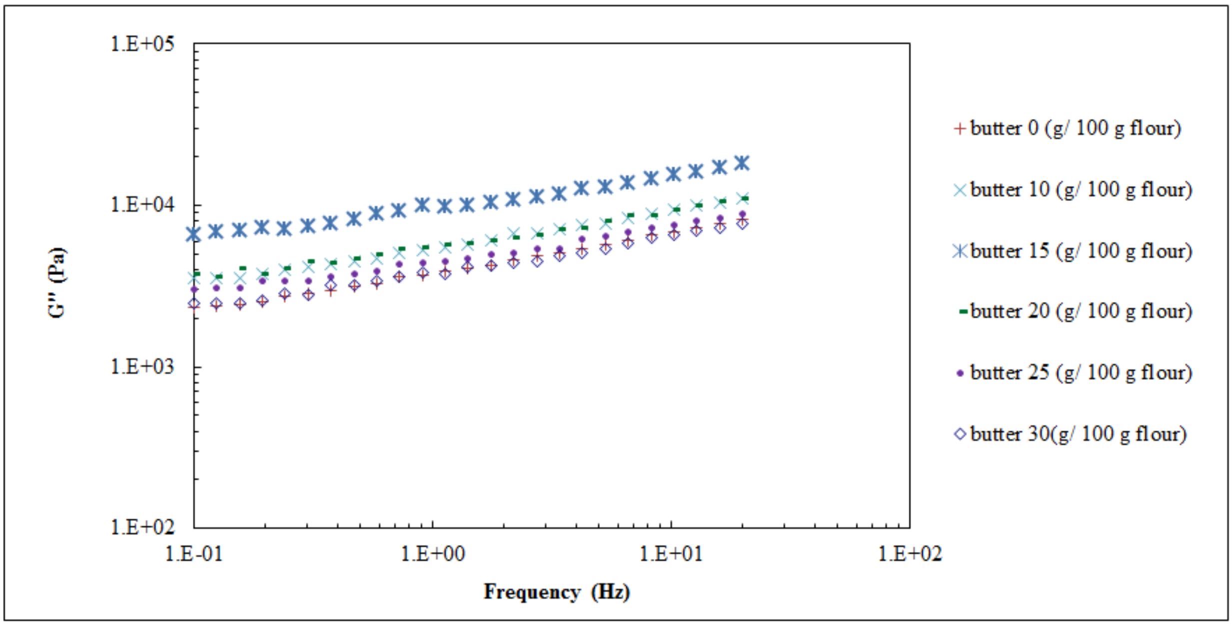

Figure 4 Viscous modulus (G″) of gluten free dough with variation of butter content.

In addition, phase angle tangent (tan δ) of all samples (dough with addition of butter from 0 to 30 g/100 g flour) ranged from 0.2 to 0.4. The tan δ value that was lower than 1 could confirm that the elastic properties predominated over the viscous properties (Hesso *et al,* 2015). By increasing fat content in the formula, their viscoelastic property would tend to slightly increase. This result might contribute to the increased specific volume of their baked bread because of hydrophilic interaction or emulsion between fat and water leading to trapping of gas bubbles in the mixing stage. Then, gas bubbles were maintained by emulsion property during baking process. However, the viscoelastic property was decreased when the butter content was too high (more than 20 g/ 100g flour).

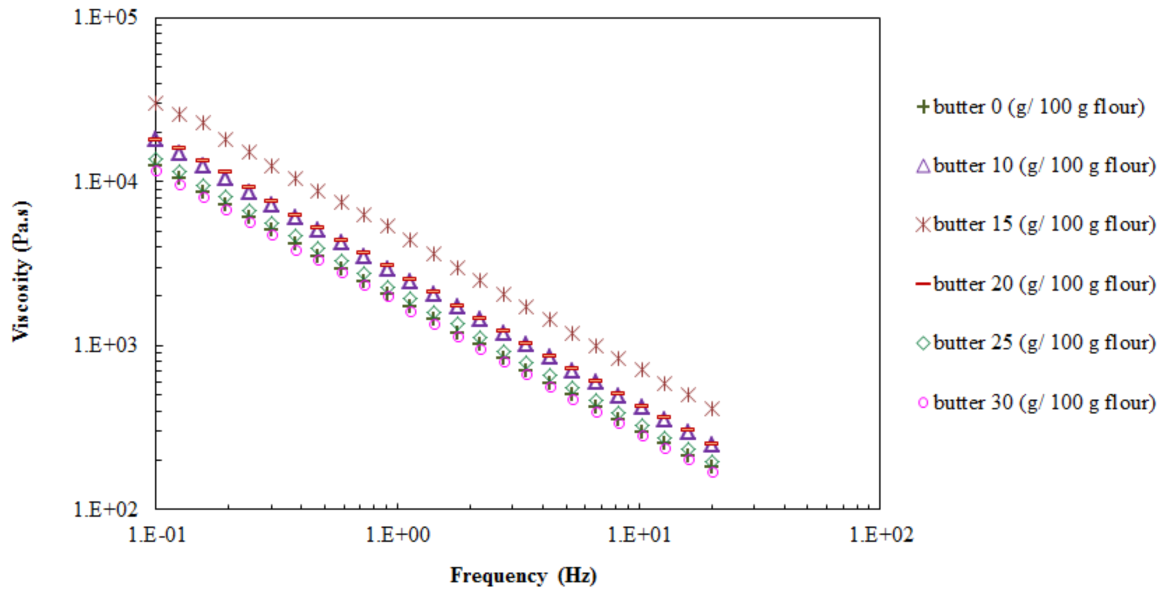

Figure 5 Viscosity of gluten free dough with variation of butter content.

Effect of butter content and baking condition on moisture content and specific volume of gluten free bread

Moisture content of the gluten free bread was significantly different ($P \leq 0.05$) depending on butter content and baking condition (Table 6). An increase in butter content from 10 to 30 g/100 g flour in the HA bread, MW bread and HA-MW bread decreased the moisture content from 81.84 to 73.66 %db, from 69.45 to 66.39 %db and from 76.49 to 68.17 %db, respectively. This was because of the increased butter content that reduced water proportion in the dough formula. Additionally, the moisture content of the MW and HA-MW bread was lower than those of the HA bread because of a late crust formation during MW heating. There was not enough baking time to generate proper crust color. The water would migrate from the center of bread to its surface leading to high moisture loss and dried crumb (Keskin *et al,* 2004).

Table 6 Moisture content and specific volume of the bread with variation of butter content and baking condition.

Baking condition	Butter content (g/ 100g flour)	Moisture content (%db)	Specific volume (cm^3/g)
HA	0	87.27±0.68[a]	1.72+0.04[fg]
	10	81.84±0.17[b]	1.70+0.05[g]
	15	81.58±1.39[b]	1.73+0.08[fg]
	20	78.98± 0.31[c]	1.91+0.01[cde]
	25	77.24±0.78[d]	1.78+0.13[efg]
	30	73.66±0.79[e]	1.78+0.05[efg]
MW	0	65.03±0.42[h]	1.74+0.02[efg]
	10	69.45±0.55[f]	1.85+0.05[def]
	15	69.10±0.66[fg]	1.80+0.02[efg]
	20	68.95±0.49[fg]	2.05+0.03[ab]
	25	67.91±0.48[g]	1.77+0.17[fg]
	30	66.39±0.62[h]	1.71+0.03[fg]
HA-MW	0	68.01±1.16[g]	1.94+0.01[bcd]
	10	76.49±0.18[d]	1.98+0.03[abc]
	15	76.01±0.37[d]	2.08+0.09[a]
	20	69.47±0.82[f]	2.01+0.03[abc]
	25	68.07±0.56[g]	1.81+0.01[efg]
	30	68.17±1.26[fg]	1.74+0.09[fg]

Different letters within a column mean significant different ($P \leq 0.05$)

Regarding volume expansion, specific volume of all samples was significantly different ($P \leq 0.05$). The increased butter content from 10 to 20 g/100 g flour in the HA, MW and HA-MW bread increased their specific volume. This was coincided with O'Brien *et al* (2003) that report the improvement of volume was derived from functional properties of fat. Fat could improve gas-retention properties of dough and oven spring relating to height, volume and softness of bread

(Cauvain and Young, 2006). However, when the butter content was more than 20 g/100 g flour in dough, dough strength was reduced. Then it was collapsed during transfer from the prover to the oven. Both MW and MW-HA gluten free bread had the high specific volume. This was because their rapid heating rate generated the high vapor pressure, resulting in the high oven-spring. The late crust formation also caused the high volume expansion during the baking.

Effect of butter content and baking condition on bread crust color

Crust color of the gluten free bread was presented in Table 7. L* (lightness-darkness), a* (redness-greenness), b* (yellowness-blueness) and ΔE (total color change) of all bread were significantly different ($P \leq 0.05$). When the butter content was increased from 10 to 30 g/100 g flour into the HA bread, the L*-value decreased from 66.54 to 53.97 while ΔE values increased from 28.80 to 44.39. It is possible that high butter content increased heat transferring rate. Then sample temperature would be increased and could enhance Maillard reaction. Therefore, redness was increased. The a*-value of the HA bread was increased from 15.00 to 18.32 when the butter content was above 15 g/100 g flour. However, the increased a*-value of the MW and MW-HA bread was little (from -0.70 to -0.41 and from 1.37 to 2.10, respectively). The b*-value of the HA, MW and MW-HA bread crust increased from 34.73 to 41.03, from 13.36 to 15.75 and from 20.94 to 25.32, respectively. It is possible that butter consisted of carotenoid pigment (Rattanapanone, 2010) leading to the improvement of yellow color intensity of gluten free bread crust.

For baking conditions, L*-values of the MW bread was higher than those of the HA HA-MW bread. On the other hand, a*, b* and ΔE of the HA and HA-MW bread were higher than those of the MW bread (Figure 6 - 7). This was coincided with Demirkesen *et al.* (2014) reporting that ΔE value of the HA bread was significantly higher than those of the MW bread. Additionally, when HA was combined with MW heating, both L* and ΔE values of HA-MW bread surface was darker than those of individual MW bread consistent with Keskin *et al* (2004) which investigated effect of halogen lamp-microwave combined baking on quality of bread. The crust color of bread from the combined baking would be similar to the HA bread. The halogen lamp transferred the heat through radiation mechanisms. Thus products were heated from surface to inner layer which is similar to HA heating. In addition, Sumnu and Sahin (2005) reported the ΔE value of the MW bread was the lowest and similar to the white standard value. This was because of the short baking time and low surface temperature during MW that could not promote the proper browning reaction and crust formation.

Table 7 Crust color of bread with variation of butter content and baking condition.

Baking condition	Butter content (g/ 100g flour)	L*	a*	b*
HA	0	71.74±0.00^{g}	13.67±0.53^{f}	39.25±1.28^{f}
	10	66.54±0.79^{j}	9.65±0.70^{e}	34.73±0.52^{e}
	15	65.26±0.32^{k}	8.35±0.66^{d}	35.75±0.94^{d}
	20	57.96±0.51^{l}	15.00±0.28^{c}	38.65±0.40^{c}
	25	55.13±0.38^{m}	15.90±0.02^{b}	39.91±0.44^{b}
	30	53.97±0.15^{n}	18.32±0.42^{a}	41.03±0.56^{a}
MW	0	76.40±0.04^{bc}	-0.29±0.02^{j}	13.36±0.15^{k}
	10	76.94±0.24^{a}	-0.70±0.04^{k}	12.50±0.15^{n}
	15	76.52±0.27^{ab}	-0.56±0.05^{k}	12.37±0.09^{n}
	20	76.62±0.08^{ab}	-0.46±0.03^{k}	14.43±0.17^{m}
	25	76.02±0.05^{c}	-0.43±0.02^{k}	14.65±0.18^{m}
	30	74.07±0.22^{d}	-0.41±0.02^{k}	15.75±0.28^{l}
HA-MW	0	73.27±0.01^{e}	0.44±0.01^{hi}	17.12±0.00^{j}
	10	72.56±0.02^{f}	1.37±0.01^{i}	20.94±0.01^{j}
	15	71.55±0.14^{g}	1.47±0.02^{hi}	21.52±0.02^{j}
	20	70.79±0.03^{h}	1.62±0.00^{hi}	23.98±0.01^{i}
	25	70.50±0.03^{h}	1.78±0.01^{gh}	24.66±0.02^{h}
	30	67.99±0.01^{i}	2.10±0.00^{g}	25.32±0.02^{k}

Different letters within a column mean significant different ($P \leq 0.05$)

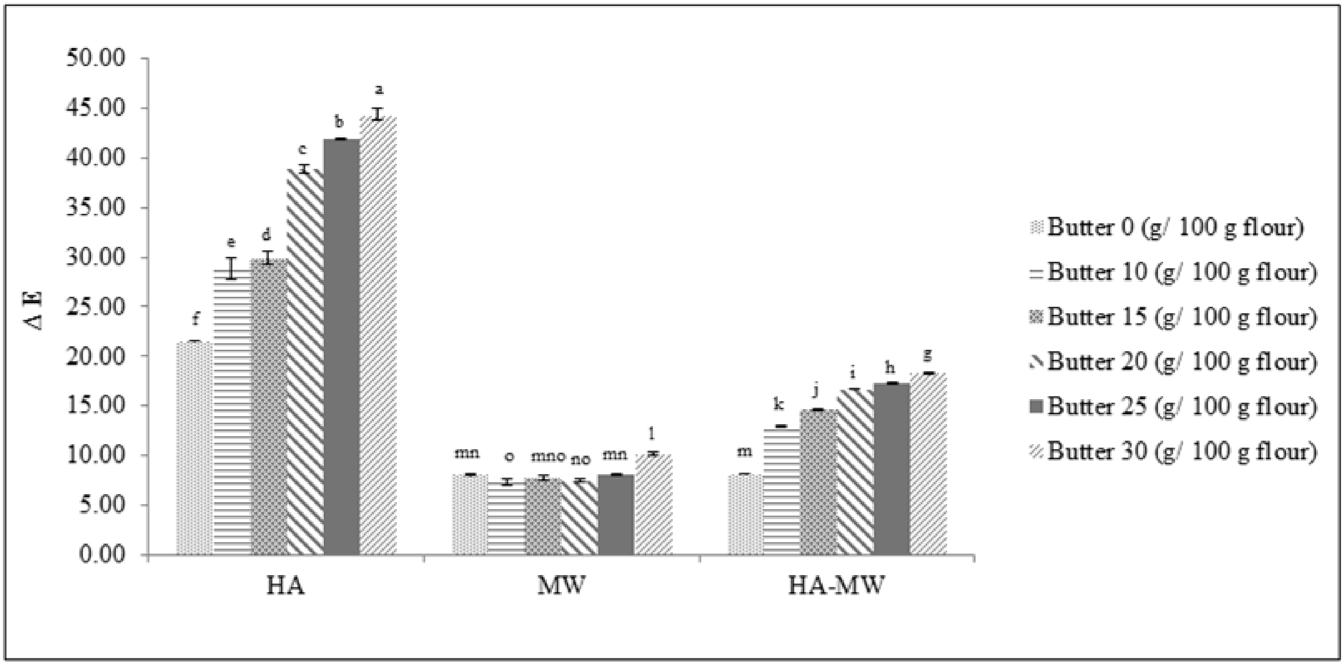

Figure 6 Total color change (ΔE) of the gluten free bread with variation of butter contents and baking conditions.

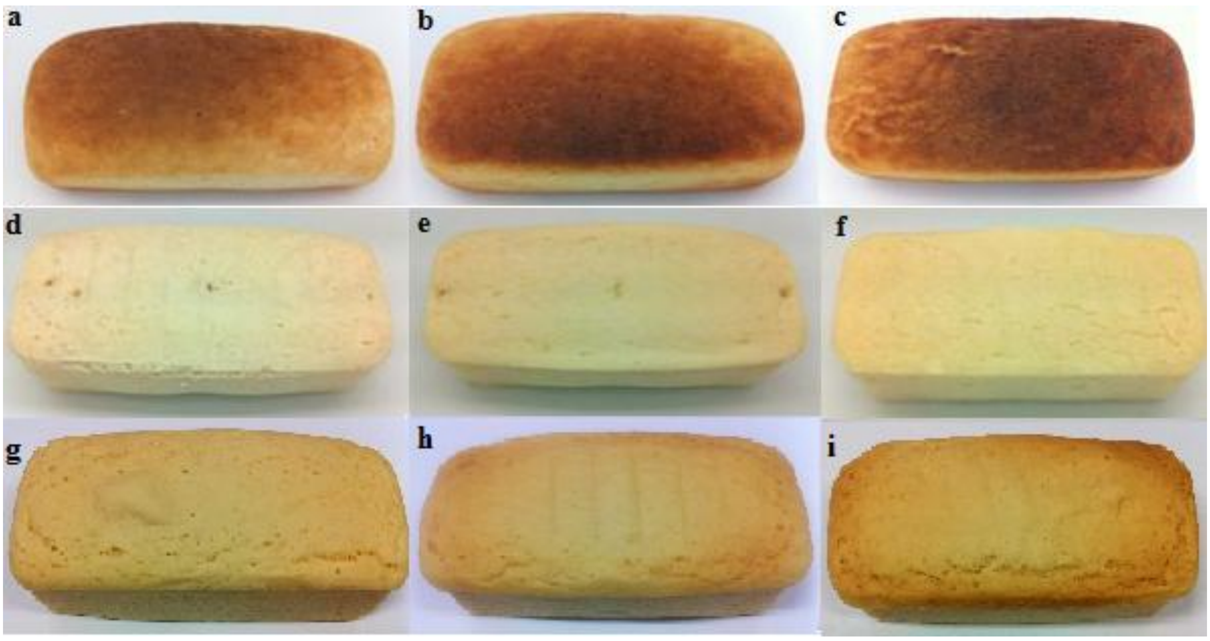

Figure 7 Crust characteristics of some gluten free bread: HA bread with 10 g butter / 100 g flour (a), HA bread with 20 g butter/ 100 g flour (b), HA bread with 30 g butter/ 100 g flour (c), MW bread with 10 g butter/ 100 g flour (d), MW bread with 20 g butter/ 100 g flour (e), MW bread with 30 g butter/ 100 g flour (f), HA-MW bread with 10 g butter/ 100 g flour (g), HA-MW bread with 10 g butter/ 100 g flour (h) and HA-MW bread with 30 g butter/ 100 g flour (i).

Effect of butter content and baking condition on texture of the gluten free bread

Texture profile analysis (hardness, springiness, cohesiveness and chewiness) of bread crumb was presented in Table 8.

Table 8 Textural profiles of the bread with variation of butter contents and baking conditions.

Baking condition	Butter content (g/ 100g flour)	Hardness (N)	Chewiness (N)
HA	0	9.05 ± 0.54^{b}	5.48 ± 0.51^{cd}
	10	5.24 ± 0.88^{def}	3.28 ± 0.79^{fghi}
	15	4.84 ± 1.09^{fg}	3.10 ± 0.66^{ghi}
	20	4.06 ± 0.71^{g}	2.51 ± 0.48^{hij}
	25	4.17 ± 0.37^{g}	2.29 ± 0.34^{ij}
	30	4.05 ± 0.60^{g}	1.94 ± 0.36^{ij}
MW	0	14.17 ± 0.97^{a}	11.43 ± 0.63^{a}
	10	7.65 ± 0.92^{c}	6.84 ± 1.34^{b}
	15	7.70 ± 0.90^{c}	5.54 ± 0.71^{cd}
	20	5.73 ± 1.18^{de}	4.75 ± 1.13^{c}
	25	4.17 ± 0.82^{g}	3.07 ± 0.77^{hij}
	30	4.11 ± 0.60^{g}	2.75 ± 0.43^{hij}
HA-MW	0	13.39 ± 2.42^{a}	10.99 ± 2.04^{a}
	10	6.21 ± 0.70^{d}	4.54 ± 0.72^{de}
	15	6.09 ± 0.35^{de}	4.24 ± 0.99^{ef}
	20	5.73 ± 2.31^{def}	3.93 ± 1.70^{de}
	25	5.64 ± 0.66^{def}	3.90 ± 0.88^{efg}
	30	5.14 ± 0.65^{ef}	3.52 ± 0.46^{efg}

Different letters within a column mean significant different ($P \leq 0.05$).

An increase in butter content in the gluten free dough significantly ($P \leq 0.05$) decreased hardness and chewiness of the bread crumb. When butter content was increased from 10 to 30 g/100 g flour, hardness of the HA, MW and HA-MW bread was decreased from 5.24 to 4.05 N, from 7.65 to 4.11 N and from 6.21 to 5.12 N, respectively. Chewiness of the HA, MW and HA-MW bread was decreased from 3.28 to 1.95 N, 6.84 to 2.75 N and 4.54 to 3.49 N. Therefore, adding fat into the dough could soften the crumb texture. In addition to baking condition, the hardness and chewiness values of the MW and HA-MW bread were higher than the HA bread. It was consistent with high moisture loss and dry crumb in the case of the microwave baking.

Effect of butter content and baking condition on GI of the gluten free bread

From In-vitro digestibility test, GI values of HA (76.80 - 89.70), MW (68.96 - 69.55) and HA-MW (68.58 - 69.38) bread were lower than the wheat flour bread (93.45) (Table 9). By using the HA, an increase in butter content from 10 to 30 g/ 100 g flour significantly ($P \leq 0.05$) decreased the GI value of the HA bread from 89.70 to 76.80. It is possible that adding fat to carbohydrate reduced postprandial glycemic response in for diabetes patients by delaying gastric emptying (Bland - Miller *et al.* 2000; Pi –Sunyer *et al,* 2002). The delaying in gastric emptying may lead to the both acute and chronic effect of fat on stimulating gut hormones such as gastric inhibitory peptide (GIP) (Collier and O'Dea, 1983). In addition, Moghaddam *et al.* (2006) stated that adding fat to carbohydrate enhanced insulin in the healthy or hyperinsulinemic people. The stimulating insulin secretion of fat affected postprandial blood glucose. However, this effect could be varied, depending on fat source and cooking method. In the current study, effect of fat addition on GI was significant when dough was only baked in the hot air oven. When dough was baked in the microwave oven, the effect of the increased fat on GI became non-significant ($P > 0.05$).

Nonetheless, GI of the gluten free bread baked in the MW and the HA-MW was significant lower than the HA. It is possible that MW heating increased resistance starch which could not be digested in the small intestine; however, it could fermented in large intestine leading to the reduced GI (Zhang *et al*., 2009). During MW heating, the interaction between -OH polar groups of flour and –NH polar group of protein was occurred leading to change molecule structure of flour component, then the resistance starch in bread was increased (Zhou *et al.,* 2006). Moreover, Maria *et al.* (2007) found that the predicted GI of the microwave baked pound cake (59.13) was less than the conventionally baked one (77.92).

Table 9 GI of wheat flour bread and gluten free bread with variation of butter content and baking condition.

Baking condition	Butter (g/ 100g flour)	Average GI
Control (HA)	0	89.70 ± 3.57^{a}
HA	10	83.10 ± 0.91^{b}
	20	80.27 ± 0.02^{bc}
	30	76.80 ± 2.12^{c}
MW	10	68.96 ± 0.17^{d}
	20	69.15 ± 0.10^{d}
	30	69.65 ± 1.01^{d}
HA-MW	10	68.68 ± 0.72^{d}
	20	68.56 ± 0.75^{d}
	30	69.38 ± 0.28^{d}

Different letters within a column mean significant different ($P \leq 0.05$).

Effect of baking condition and storage time on retrogradation of the gluten free bread crumb

As shown on Figure 8, when the samples were stored at 4 ºC for 7 days, the enthalpy (ΔH) of retrogradation of HA, MW and HA-MW bread with addition of 20 g butter/100 g flour was occurred. At the beginning of storage time, the enthalpy of both fresh HA bread and HA bread stored for a day was not detected. However, the enthalpy of the HA bread was slightly increased from 1.12 to 2.90 J/g of dry mass sample when the bread was stored for 3 - 7 days. Similarly, the enthalpy of both fresh MW and fresh HA-MW bread was not detected. However, after 1 – 7 day storage, the enthalpy of MW and HA-MW bread was gradually increased from 2.26 to 4.31 and 1.63 to 3.03 J/g of sample dry mass. It is possible that the retrogradation occurred during storage time. The amylopectin recrystallization, which was a nucleation-limited process and occurred in heterogeneous formation, would be quickly developed when the bread was stored at low temperature (4 - 14 ºC) (Bosmans *et al,* 2013). The amylopectin generally recrystallized in a range of 50 to 90˚C (Curti *et al*, 2014).

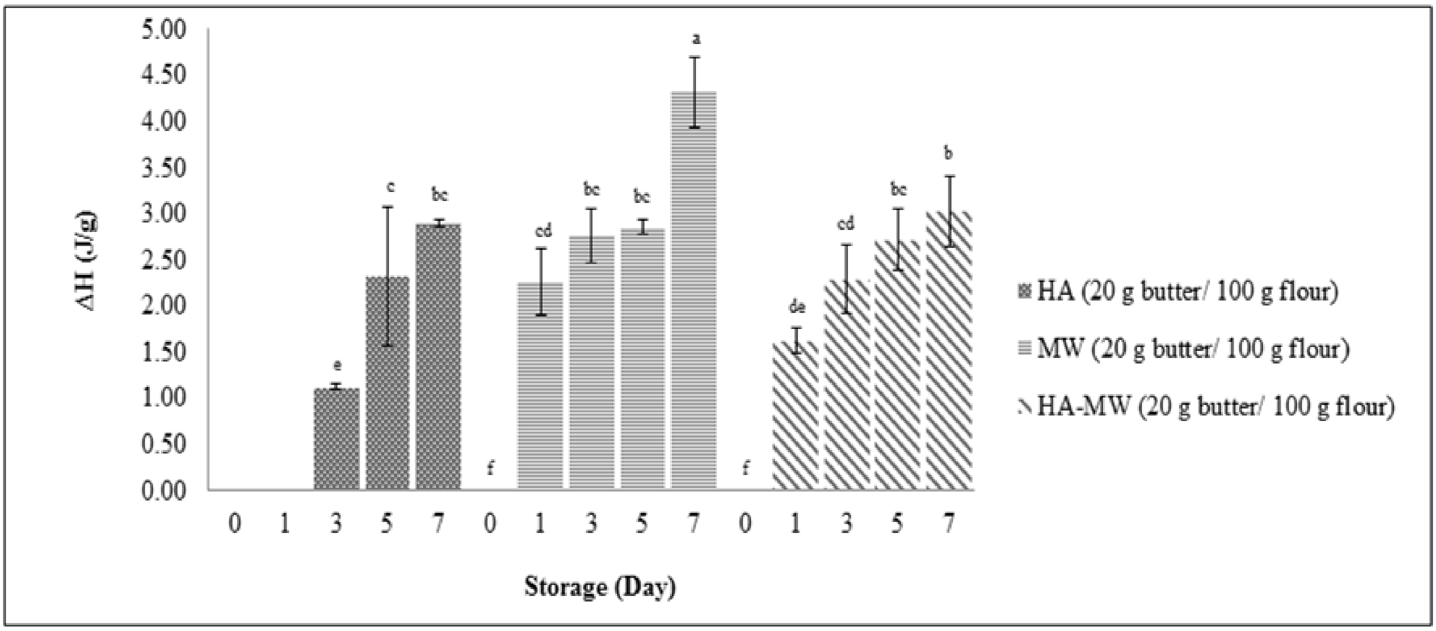

Figure 8 Enthalpy of retrograded amylopectin of bread crumb during storage at 4˚C.

Values are means ± standard deviations. Different letters mean significant different ($P \leq 0.05$)

In the current study, the melting temperature of amylopectin recrystallization of all samples was in a range of 39.00˚C to 56.79˚C (Table 10). The enthalpy of the MW bread and the HA-MW bread was not only predominate over but also faster than the HA bread. This was because light-crust formation of the MW bread led to fast moisture migration and staling reaction. These results were also related to the diffraction pattern of those samples.

Table 10 Melting temperature of retrograded amylopectin of bread crumb during storage at 4˚C for 7 days.

Baking condition	Storage (Day)	T_0 (°C)$^{n.s.}$	T_p (°C)$^{n.s.}$	T_e (°C)
HA	0	-	-	-
	1	-	-	-
	3	44.17±1.36	50.16±0.05	55.68±0.16^{ab}
	5	40.88±0.59	47.64±0.38	54.55±0.65^{bc}
	7	42.29±0.19	49.22±0.62	56.79±0.24^{a}
MW	0	-	-	-
	1	39.00±0.47	45.84±0.76	52.31±0.87^{def}
	3	39.48±0.04	44.08±0.23	51.48±0.33^{f}
	5	39.70±0.07	45.71±0.01	53.24±0.86^{cde}
	7	42.54±3.85	47.52±3.39	53.85±1.63^{cd}
HA-MW	0	-	-	-
	1	41.72±1.05	44.78±3.28	53.97±0.42^{cd}
	3	40.40±0.01	42.63±3.13	51.21±0.56^{f}
	5	45.38±6.29	42.91±4.36	51.05±0.64^{ef}
	7	40.25±0.02	43.05±3.99	51.66±0.01^{ef}

Different letters mean significant different ($P \leq 0.05$).

n.s. mean significant different ($P > 0.05$).

Effect of baking condition and storage time on X-ray diffraction pattern of the gluten free bread

The diffraction pattern of gluten free bread, which related to crystalline structure, could be analyzed by X-ray diffraction as shown in Table 11. After the gluten free bread was stored for 7 days, total crystallinity of the HA, MW and HA-MW bread was significantly increased from 4.06 to 26.52 %, 4.72 to 27.25 % and 4.96 to 27.43 %, respectively. This was coincided with Karim *et al.* (2000) that the starch component of the fresh bread was mostly exhibited amorphous structure, then it was gradually became crystalline structure associated with staling during storage. In general, when crystallization of amylose was completely carried out, then crystallization of amylopectin was developed. The degree of A-type crystalline structure, which presented 4 peaks at the diffraction angles of 14.2º, 17.0º, 18.0º and 23.1º. In addition, the portion of crystalline sucrose was also shown as the A type at the diffraction angles of 24.8º and 25.27º. However, the loss of A-type structure of bread would be observed (Figures 9 - 11). In the current study, bread was stored at 4 ºC for 7 days, the peaks of A-type structure at the diffraction angles of 14.2º, 17º, 18º and 23.1º were also reduced or disappeared. This indicated that the A-type crystalline structure of the bread crumb was destructed.

Table 11 Degree of crystallinity of the gluten free bread during storage at 4ºC for 7 days.

TRT	Storage (Days)	Degree of crystallinity (%)			
		A-type[n.s.]	B-type[n.s.]	V-type[n.s.]	Total
HA	0	ND	ND	ND	4.06±0.06[c]
	7	10.66±0.48	2.87±0.07	5.16±0.47	25.39±0.35[b]
MW	0	ND	ND	ND	4.72±0.73[c]
	7	14.49±0.03	3.49±0.64	5.38±1.21	27.25±0.22[a]
HA-MW	0	ND	ND	ND	4.96±0.04[c]
	7	13.10±1.89	3.48±0.87	6.06±0.45	27.43±0.37[a]

Different letters within a column mean significant different ($P \leq 0.05$)

n.s. mean significant different ($P > 0.05$).

On the other hand, the degree of crystallinity of B-type structure (at the diffraction angle of 17º.) of the HA, MW and HA-MW bread was increased to 2.87 %, 3.49% and 3.48 %, respectively when those breads were stored for 7 days (Table 6). This was coincided with the reports of Osella *et al.* (2005) that presented the low B-type crystallinity of the fresh bread, whereas the B-type crystallinity was gradually increased when the bread was stored for 7 days. This was because of amylopectin recrystallization.

In addition, the crystallinity of V-type structure (at the diffraction angle of 20º) of the HA, MW and HA-MW bread, was not detected in the fresh bread, but the degree of crystallinity was observed at 5.16 %, 5.38% and 6.06 %, respectively, after 7 day storage. This indicated the development of amylose-lipid complex of the bread crumb during the storage. This was coincided with Zobel and Kulp (1996) who reported the low crystallinity of V-type structure at the beginning, and then it was increased during storage for 4 days.

Regarding baking conditions, the total degree of crystallinity, crystallinity of B-type structure and crystallinity of V-type structure of the HA bread was lower than those of the MW and HA-MW bread. These might be because the high temperature and long-time baking could exterminate the amylose-lipid complex. Similar to the case of grain, the high temperature of grain after drying (111 - 118 ºC) could destroy the amylose-lipid complex (Srisang *et al,* 2011).

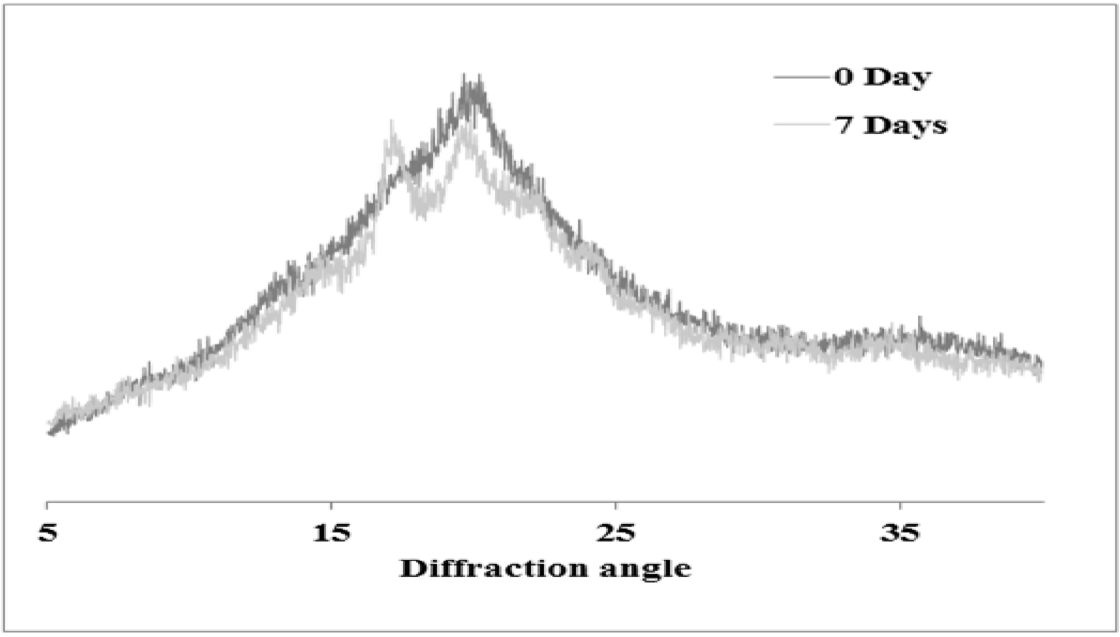

Figure 9 X-ray diffratogram of HA bread during storage at 4ºC for 7 days.

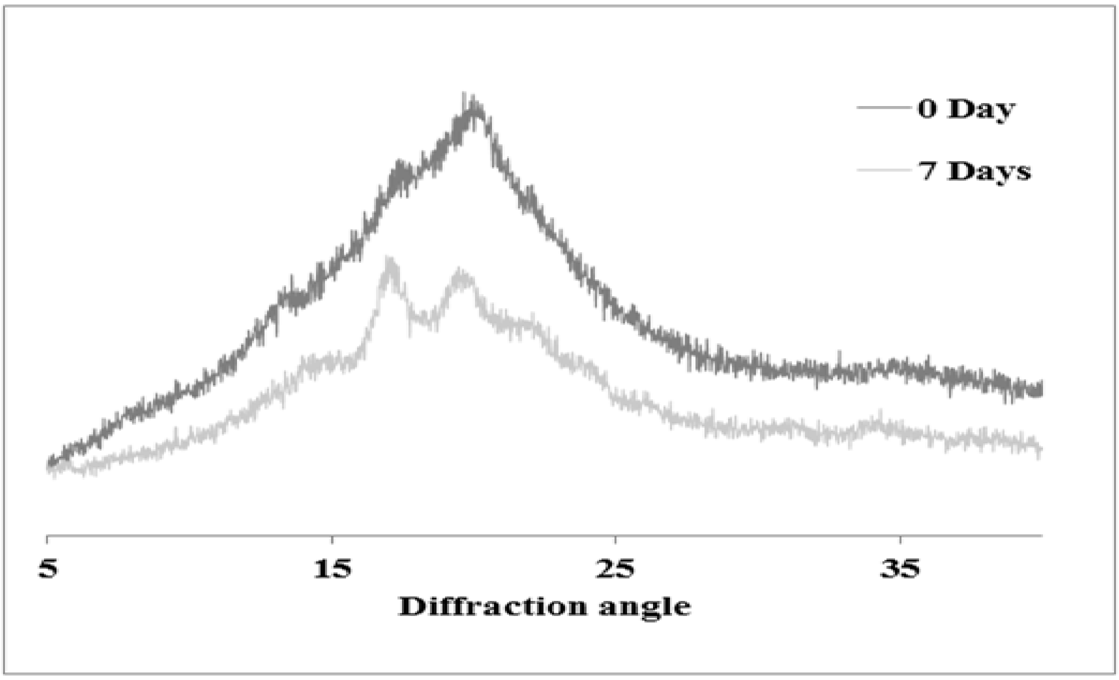

Figure 10 X-ray diffratogram of MW bread during storage at 4ºC for 7 days.

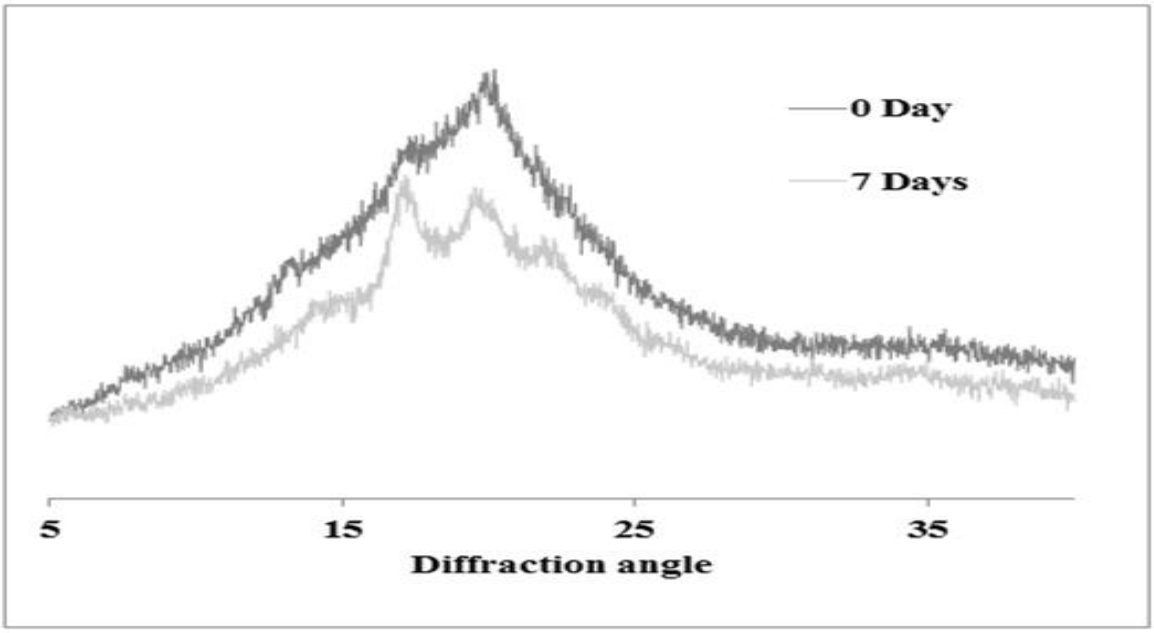

Figure 11 X-ray diffratogram of HA-MW bread during storage at 4ºC for 7 days.

CONCLUSION

Addition of WPC (0–6 g/100g flour) into the rice flour dough increased the volume and moisture content of the gluten free bread. Then, bread crumbs with 6 g WPC/100g flour were the softest. Increasing butter content (0–30 g/100g flour) caused an increase in dynamic moduli of dough. However, the excessive butter content would reduce the dynamic moduli. All the gluten free dough from the rice flour with the increased butter content indicated a viscoelastic characteristic. The increased content of butter could improve characteristics of the gluten free bread. Hardness and chewiness of the gluten free bread crumb were reduced. The MW method could decrease the baking time and improve the volume. However, the bread crust was pale with significantly ($P \geq 0.05$) increased values of L*. Increasing the butter content could increase ΔE of the gluten free bread crust. However, the crust color of the MW and HA-MW bread were still less than those of HA bread, due to the late crust formation. Additionally, the increased butter content could reduce GI of the HA bread. Nonetheless, it was not helpful to reduce GI of the HA-MW and MW bread. This might be because effect of the MW heating on the GI was significant. During storage the bread with addition of 4 g WPC and 20 g butter /100 g flour bread at 4ºC for 7 days, the enthalpy of retrogradation and the crystallinity were gradually increased. The enthalpy of retrogradation and degree of crystallinity of B-type crystalline structure of the MW and HA-MW bread were predominated over those of the HA bread.

Hence, to develop the gluten free bread with the reduced GI to less than 70 (medium GI), the dough should have addition of the 4 g WPC and 20 g butter/ 100g flour and baked by either HA-MW or MW condition. The HA-MW or MW condition could reduce GI of the gluten free bread, regardless of fat content. Therefore, amount of butter was added into formula to only improve bread characteristics.

RECOMMENDATION

1. The gluten free bread should have sensory evaluation and consumer testing to confirm acceptability of bread quality.
2. The GI of the gluten free bread may be further reduced by addition of sourdough. The gluten free bread will be unique and healthier from fermentation of lactic acid bacteria.

OUTPUTS

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1. International Journal Publication
   1.1 Therdthai, N., Tanvarakom, T., Ritthiruangdej, P. and Zhou, W. Effect of microwave assisted baking on quality of rice flour bread. Journal of Food Quality (Accepted)
   1.2 Srikanlaya, C., Therdthai, N., Ritthiruangdej, P. and Zhou, W. Effect of butter content and baking condition on characteristics of the gluten free dough and bread. (Submitted)
2. Application
   2.1 Commercial Application
   The bread with WPC has been slightly modified to produce the high protein bread for weight training consumers (It is requested by a private company.).
   2.2 Policy Application
   -
   2.3 Public Application
   The gluten-free product has been one of the interesting topics worldwide. The outcome of this project is not only gluten free, but also low GI.
   2.4 Academic Application
   The project could support 2 Master degree students and 1 PhD student.
3. Others
   3.1 Jitrakbumrung S and Therdthai N. 2014. Effect of addition of sourdough on physicochemical characteristics of wheat and rice flour bread. Kasetsart Journal (Natural Science). 48:964-969.
   3.2 Tanvarakom, T. and Therdthai, N. 2015. Effect of egg and microwave baking on quality of rice-flour bread. Food and Applied Bioscience Journal. 3(1): 59-68.
   3.3 Zhou,W., Therdthai,N. and Hui, Y. H. 2014. Chapter 1: Introduction to baking and bakery products. *In* Zhou, W. and Hui, Y. H. Eds. Bakery Products Science and Technology, 2nd Edition. Wiley-Blackwell (an imprint of John Wiley & Sons, Ltd.) 776 p. ISBN: 978-1-119-96715-6.
   3.4 Therdthai,N. 2014. Chapter 18: Fermentation. *In* Zhou,W. and Hui, Y. H. Eds. Bakery Products Science and Technology, 2nd Edition. Wiley-Blackwell (an imprint of John Wiley & Sons, Ltd.) 776 p. ISBN: 978-1-119-96715-6.

3.5 Therdthai,N. and Zhou, W. 2014. Chapter 27: Manufacture. *In* Zhou,W. and Hui, Y. H. Eds. Bakery Products Science and Technology, 2nd Edition. Wiley-Blackwell (an imprint of John Wiley & Sons, Ltd.)776 p ISBN:978-1-119-96715-6.

3.6 Zhou, W. and Therdthai, N. 2012. Fermented bread. *In* Hui, Y.H. and Evranuz, O. eds. Handbook of Plant-Based Fermented Food and Beverage Technology. 2nd edition. CRC Press, Taylor and Francis, LLC.

3.7 Srikanlaya, C. Therdthai, N. and Ritthiruangdej, P. 2015. Effect of butter content and baking conditions on characteristics of the guten fee bread. The proceedings of the 17th Food Innovation Asia Conference 2015 (FIAC 2015) Bangkok, Thailand, 18-19 June 2015.

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