# RETROGRADATION PROPERTIES OF CASSAVA FLOURS FROM DIFFERENT VARIETIES

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A SPECIAL RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR
THE DEGREE OF MASTER OF ENGINEERING (FOOD ENGINEERING)
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A Special Research Project Submitted in Partial Fulfillment
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Abstract

Cassava flour is among possible alternatives as a wheat-flour replacement ingredient for gluten-free bakery products. This is because cassava flour has an ability to retard retrogradation. Nevertheless, different species of cassava result in cassava flours of different compositions and properties, including the retrogradation behavior. This research therefore aimed to study the retrogradation behavior of flours from cassava different varieties and to compare the results with those of commercial rice and wheat flours. The research consisted of 3 parts, including the analyses of chemical composition and pasting properties of flour samples, analyses of syneresis and textural properties of cassava flour gels during frozen storage and finally investigation of frozen storage of gluten-free chiffon cake prepared from cassava flours; the data were recorded both under unfrozen and frozen storage conditions at 1 day, 7 days, 14 days, 21 days and 28 days. The results indicated that flour from Pirun 4 cassava exhibited higher peak viscosity (309.79 ± 1.36 RVU) and lowest setback viscosity (43.46 ± 2.06 RVU), which imply a higher ability to hold water (i.e., high water holding capacity). Percent syneresis, firmness and cohesiveness values of Pirun 4 cassava flour in a gel model system also suffered less changes during frozen storage. Pirun 4 cassava flour could therefore be used as wheat-flour replacement in a bakery product since it resulted in less change in the cake structure and texture even after 28 days of frozen storage.

Keywords: Cassava Flour/ Frozen Bakery Product/ Gluten-free/ Retrogradation/ Syneresis/ Textural Properties/ X-ray CT Scan

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## บทคัดย่อ

แป้งมันสำปะหลังเป็นส่วนผสมทางเลือกหนึ่งที่อาจนำมาใช้ทดแทนแป้งข้าวสาลีในผลิตภัณฑ์เบเกอรี่ปลอดกลูเต็น เนื่องจากแป้งมันสำปะหลังมีสมบัติบางประการที่สามารถลดการเกิดรีโทรเกรเดชั่น อย่างไรก็ตาม สายพันธุ์ของมันสำปะหลังที่แตกต่างกันส่งผลให้สมบัติ องค์ประกอบ และพฤติกรรมการเกิดรีโทรเกรเดชั่นของแป้งแตกต่างกัน งานวิจัยนี้จึงมุ่งศึกษาพฤติกรรมการเกิดรีโทรเกรเดชั่นของแป้งจากมันสำปะหลังต่างสายพันธุ์ และเปรียบเทียบผลที่ได้กับกรณีแป้งข้าวสาลีและแป้งข้าวเจ้า โดยแบ่งการทดลองออกเป็น 3 ส่วนได้แก่ การวิเคราะห์องค์ประกอบและสมบัติด้านความหนืดของแป้ง การวิเคราะห์เปอร์เซ็นต์ซิเนอริซิสและลักษณะเนื้อสัมผัสของเจลแป้ง และการวิเคราะห์ลักษณะทางกายภาพและลักษณะเนื้อสัมผัสของชิฟฟอนเค้กซึ่งเตรียมจากแป้งมันสำปะหลัง โดยเก็บผลการทดลองทั้งส่วนที่ไม่ผ่านการแช่เยือกแข็งและผ่านการแช่เยือกแข็งเป็นเวลา 1, 7, 14, 21 และ 28 วัน จากผลการทดลอง พบว่า แป้งมันสำปะหลังสายพันธุ์พิรุณ 4 ให้ค่าความหนืดที่สูง (309.79 ± 1.36 RVU) และค่าการคืนตัวต่ำ (43.46 ± 2.06 RVU) ซึ่งหมายถึงความสามารถในการจับตัวกับน้ำของแป้งมันสำปะหลังพิรุณ 4 ที่สูงกว่าแป้งชนิดอื่น ในขณะที่ผลการทดลองในส่วนของเจลแป้ง พบว่า ให้ค่าการเกิดซิเนอริซิส การแข็งตัว รวมไปถึงค่าการยึดเกาะภายในที่ต่ำ ส่งผลให้การเกิดรีโทรเกรเดชั่นต่ำกว่าแป้งชนิดอื่น เมื่อนำผลการทดลองในส่วนของชิฟฟอนเค้กมาวิเคราะห์ พบว่า แป้งมันสำปะหลังสายพันธุ์พิรุณ 4 สามารถใช้ทดแทนแป้งข้าวสาลีในผลิตภัณฑ์เบเกอรี่ได้ เนื่องจากมีความเปลี่ยนแปลง

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# CHAPTER 1 INTRODUCTION

## 1.1 Rationale

Cassava (*Manihot esculenta Crantz*) is one of the most important commercial products in Thailand. Production in Thailand has expanded continuously over the past two decades and by 2016, a total of 9 million rai (or 1.44 million hectares) of cassava were under cultivation, yielding approximately 31 million tons per year. Cassava production is concentrated particularly in Nakhon Ratchasima, Kamphaeng Phet, Chaiyaphum, Kanchanaburi and Ubon Ratchathani and these provinces are therefore also home to businesses such as cassava collection centers and cassava starch factory.

Cassava can be classified into two types which are "sweet" type and "bitter" type, depending on total cyanides content. The bitter type contains high amount of cyanide and normally use in industrial terms are in part of tapioca starch or alcohol beverage. On the other hand, the maximum limit of 50 ppm (fresh weight basis, FAO/WHO, 2005) is the standard for the sweet type. Hanatee and Pirun 4 are two of low-cyanide cassava varieties, bred in Thailand. There has been reported that the cooked root texture of Hanatee variety is mealy (Charoenkul, et al., 2011), and therefore it is commonly used for traditional Thai desserts. Recently, new cassava cultivar, e.g. Pirun 4, has been developed from Hanatee and Huaybon 60 varieties. It is proposed that Pirun 4 variety has the mealy texture for the cooked root and is suitable for gluten-free flour making (NSTDA, 2018). However, researches focusing on the potential use of these two low-cyanide cassava flours as gluten-free flour as well as their retrogradation properties are rarely reported.

With increasing patient with celiac disease over the world, demands as well as research focusing on gluten-free products have also increased rapidly (Araki, et al., 2016; Kadan, et al., 2001; Jensen, et al., 2015; Nindjin, et al., 2011). Number of studies have currently focused on fully substitute wheat flour with rice flour, corn flour, and cassava flour with addition of gums, hydrocolloids, or proteins to prepare gluten-free bakery product (Kadan, et al., 2001). Gums, hydrocolloids, or proteins are normally used in gluten-free bakery product to improve gas retention and water absorbing characteristics usually supplied by wheat gluten (Kadan, et al., 2001). On the other hand, proteins play a role to increase elastic modulus, to improve structure with gelation, to aid in foaming and to improve perceived quality by enhancing Maillard browning and flavor (Arendt, et al., 2008). However, only few studies have focused on the quality change of gluten-free products during storage. Moreover, major problem of gluten-free bread is retrogradation occurring during frozen storage. Retrogradation properties is the one of bread quality during storage which represent hardening at surface and shrinkage of texture. Factors affecting to the retrogradation in bakery product are components of flour (protein and fat), amylose and amylopectin content, storage temperature and water binding capacity (Ottenhof and Farhat, 2004).

Chiffon cake is selected to study the retrogradation in part of textural properties and appearance which account for quality of bakery product. However, there is combination between oil, egg, sugar and salt that affect to the retrogradation. Therefore, the study of flour-gel model system is another section to give the reason about interaction of flour water and heat. Both parts are the macro study of flour in product and model which can indicate the role of flour with other ingredients. Moreover, the chemical compositions and pasting properties of cassava flours from different varieties should be evaluating to confirm the result of retrogradation in term of micro molecules.

There are a few types of research focusing on retrogradation properties of frozen gluten-free flour. Charoenrein and Preechathammawong (2012) demonstrated the effect of waxy rice flour and cassava starch on the freeze-thaw stability of rice starch gels. They reported that rice flour enhanced the retrogradation. It contained a high amount of amylose content. In contrast, wheat flour reduced the retrogradation due to the gluten network. On the other hand, cassava starch exhibited high retrogradation tendency in the freeze-thaw cycle (Charoenrein and Preechathammawong, 2012). Ronda and Roos (2011) reported that the storage temperature below the onset of ice melting temperature (Tm) effectively retarded the starch recrystallization in the gluten-free bread. Although these previous studies presented experimental evidence of starch recrystallization in the gluten-free bakery products during storage, they were done with the rice flour-based gluten-free product. Little information relating to retrogradation in the gluten-free bakery product based on cassava flour is available.

This research aims to study the retrogradation properties from different cassava flours varieties which are Hanatee and Pirun 4 and compare to those commercial products. There are 3-method of this research which is effect of flour type on structural and textural attribute in frozen storage by preparing the chiffon cake from different type of flour, keep in frozen storage (-20 °C) and analysis the retrogradation properties in part of bread characteristic (porosity and texture) (1) chemical compositions and pasting properties of flour samples). Secondly, the determination of retrogradation properties of the flour-gel model by preparing 20 % of gel, frozen then measure the textural properties and syneresis measurement (2) syneresis and textural properties of cassava flour gels during frozen storage). Finally, retrogradation properties of gel model measurements are determined to confirm the result that the retrogradation properties occur because of which composition in flour, for example, starch, fat protein, amylose and moisture content (3) frozen storage of gluten-free chiffon cake based on cassava flour). The results obtained from this research can be useful for gluten-free bakery product which using Thai cassava flour

## 1.2 Objective

To study the retrogradation properties of cassava flour from different varieties and compare to those of commercial rice and wheat flour.

## 1.3 Scopes

1. Low-cyanide cultivars (Hanatee and Pirun 4) were prepared in the laboratory. On the other hand, commercial rice flour and wheat flour were used as the controls.
2. Retrogradation properties are determined in gel model (20 % suspension) and chiffon cake
3. Frozen storage temperature is -20 °C.
4. Analysis between unfrozen to 28 days in frozen storage

## 1.4 Expected Benefits

1. The result of this research is being as the preliminary study in terms of retrogradation properties comparing between gluten-free flour and gluten flour.
2. The result of this research can be useful for gluten free bakery product which using Thai cassava flour.

# CHAPTER 2 THEORY AND LITERATURE REVIEWS

## 2.1 Gluten-Free Product

Celiac disease (CD) is one of the most common food-induced diseases in humans. This is because of the inflammation of the small intestine due to the uptake of gluten and gluten like proteins. Gluten proteins are the trigger for coeliac disease and are found in wheat, rye and barley. Gluten is unique due to its ability to form a visco-elastic network, which can retain gas (Arendt, et al., 2009). In bakery products, the gluten network formation is particularly important. However, the only treatment for patients with CD is the strict adherence to a gluten-free diet. Patients must follow this diet throughout their life, since re-exposure to gluten can re-activate the disease, even after many years (Koehler, et al., 2014). Elimination of gluten increases the role and importance of starch in providing structure and texture to gluten-free products. There are alternatives variety of ingredients that are natural and not contain gluten. Thus, it can be consumed by those on a gluten-free diet if they are labeled gluten-free. Gluten replacement including almond meal flour, brown, white and wild rice, buckwheat, coconut flour, corn, cornstarch, guar gum, potato flour and tapioca flour (Beyond celiac, 2006).

There are many advantages of a gluten-free product including improves cholesterol levels, promotes digestive health, increases energy levels, eliminates unhealthy and processed foods from your diet (oils, fried food, bread, and desserts to name a few) (Chang, 2013). Other benefits are more likely to eat fruits and vegetables because they are all gluten-free, reduced risk of heart disease, certain cancers, and diabetes. Helping ward off viruses and germs as many foods, the consumer will now eat and contain more antioxidants, vitamins, and minerals (Sorensen, 2012), promoting healthy weight-loss, improves the health of people with irritable bowel syndrome and distinctly improved awareness of foods that can have an adverse effect on health.

Many products are declared as gluten-free such as brownie, cake and cookies. Those products are called as ready-to-eat bakery products. The production for ready-to-eat gluten-free bakery products including quality and shelf-life by using the frozen storage to control. The defects in frozen storage are hardness and collapse of cake or called retrogradation.

## 2.2 Cassava

Cassava (scientific name: *Manihot esculent* Crantz) is also called in other names which are tapioca, manioc and yucca depending on the regions of cultivar and processing (Sriroth and Piyachomkwan, 2007). Cassava is a perennial plant widely grown in many tropical and subtropical countries such as Asia and southern Africa because cassava can withstand drought and can grow in low-nutrient soils including Thailand as one of the most important commercial crops.

### 2.2.1 Cultivars and Chemical Composition

Cassava is classified as either sweet or bitter. Sweet type (such as Hanatee, Rayong 2, Pirun 2 and Pirun 4) has low cyanide content, 50 mg/kg (FAO/WHO, 2005). Soften and chewiness are represented the texture of this type. This is used as a Thai traditional dessert. Bitter type contains high hydrogen cyanide or hydrocyanic acid, bitter flavor, rough texture but it has high starch content. Therefore, the bitter type is suitable for commercial starch production. Bitter varieties of cassava include Rayong 1, Rayong 3, Kasetsart 50 and Huaybong 60.

This cassava Hanatee is used for traditional Thai dessert in terms of steamed, grilled or glazed with syrup because of low cyanide content. Another cassava variety is Pirun 4 (MBR49-2-109). This cassava is contributed between the researcher of Agricultural Research Officer, Institute of Molecular Biosciences, Mahidol University and National Science and Technology Development Agency (NSTDA). Pirun 4 is suitable for wheat flour replacement, which is the main ingredient in bakery products and being a gluten-free ingredient.

The chemical composition and pasting properties of gluten-free flour, cassava flour and rice flour in Thailand are given in table 2.1. The differences in the chemical composition of flour derived from different varieties of cassava can affect the properties of flour.

**Table 2.1** Chemical compositions (%d.b.) of flour from different botanical sources in Thailand

| Flour types | Varieties | Protein | Lipid | Ash | Fiber | Reference |
|---|---|---|---|---|---|---|
| Cassava | Hanatee | 0.94 ± 0.00 | 0.34 ± 0.01 | 2.02 ± 0.10 | 2.18 ± 0.08 | Charoenkul, et al., 2011 |
| | Rayong 2 | 1.18 ± 0.01 | 0.34 ± 0.04 | 2.34 ± 0.04 | 2.48 ± 0.01 | Charoenkul, et al., 2011 |
| | KU50 | 0.80 ± 0.02 | 0.25 ± 0.02 | 1.10 ± 0.06 | 2.01 ± 0.10 | Chotineeranat, et al., 2006 |
| | unknown | 0.05 ± 0.00 | 0.04 ± 0.01 | 0.12 ± 0.01 | 0.68 ± 0.97 | Ritthiruangdej and Suwonsichon, 2007 |
| Rice | Hom-Mali | 9.89 ± 0.18 | 0.39 ± 0.04 | 0.34 ± 0.04 | N/A | Varavinit, et al., 2003 |
| | Luang 11 | 9.56 ± 0.15 | 0.34 ± 0.05 | 0.39 ± 0.03 | N/A | Varavinit, et al., 2003 |
| | Pathumthani 1 | 12.00 ± 0.20 | 0.32 ± 0.02 | 0.43 ± 0.04 | N/A | Varavinit, et al., 2003 |
| | Chainat | 12.6 ± 0.23 | 0.35 ± 0.02 | 0.36 ± 0.02 | N/A | Varavinit, et al., 2003 |
| | Plaingam-Prajeenburi | 7.61 ± 0.16 | 0.44 ± 0.05 | 0.45 ± 0.03 | N/A | Varavinit, et al., 2003 |
| | Kordeaw | 9.26 ± 0.15 | 0.42 ± 0.06 | 0.43 ± 0.02 | N/A | Varavinit, et al., 2003 |
| | Kor-Khor 10 | 9.15 ± 0.10 | 0.33 ± 0.03 | 0.45 ± 0.02 | N/A | Varavinit, et al., 2003 |
| | Kor-Khor 6 | 9.10 ± 0.25 | 0.36 ± 0.01 | 0.35 ± 0.01 | N/A | Varavinit, et al., 2003 |
| | San-Patong | 13.19 ± 0.33 | 0.44 ± 0.04 | 0.36 ± 0.03 | N/A | Varavinit, et al., 2003 |
| | Kheaw-Prajeenburi | 8.08 ± 0.17 | 0.31 ± 0.02 | 0.37 ± 0.02 | N/A | Varavinit, et al., 2003 |
| | Khaw Tahang | 9.25 ± 0.22 | 0.36 ± 0.02 | 0.33 ± 0.03 | N/A | Varavinit, et al., 2003 |

According to cultivars, cassava flour composes of 0.32-1.18 % d.b. of protein and 7-13 % d.b. proteins in rice flour. There are 0.04-0.34 % lipid in cassava flours and approximate 0.3-0.4 % lipid in rice flours, respectively. These compositions, especially for protein and lipid contents, may affect the retrogradation properties of cassava flour.

### 2.2.2 Pasting Properties

Pasting properties are an important characteristic of commercial flour. Pasting properties of cassava flour gel is an excellent characteristic when it is compared with other flours. Generally, the pasting properties of flour paste are measured by Brabender Visco-Amylograph or Rapid Visco-Analyser (RVA) (Shiroth and Piyachomkwan, 2007).

The Rapid Visco Analyser (RVA) is a recording viscometer that may be used to determine the pasting properties of flour-water suspension during heating and cooling. The peak viscosity is defined as the maximum viscosity that occurs prior to the initial sample cooling. The minimum viscosity is the lowest viscosity recorded after the peak viscosity. The final viscosity is the viscosity at the end of the test. All viscosities are measured in Rapid Visco Unit (RVU).

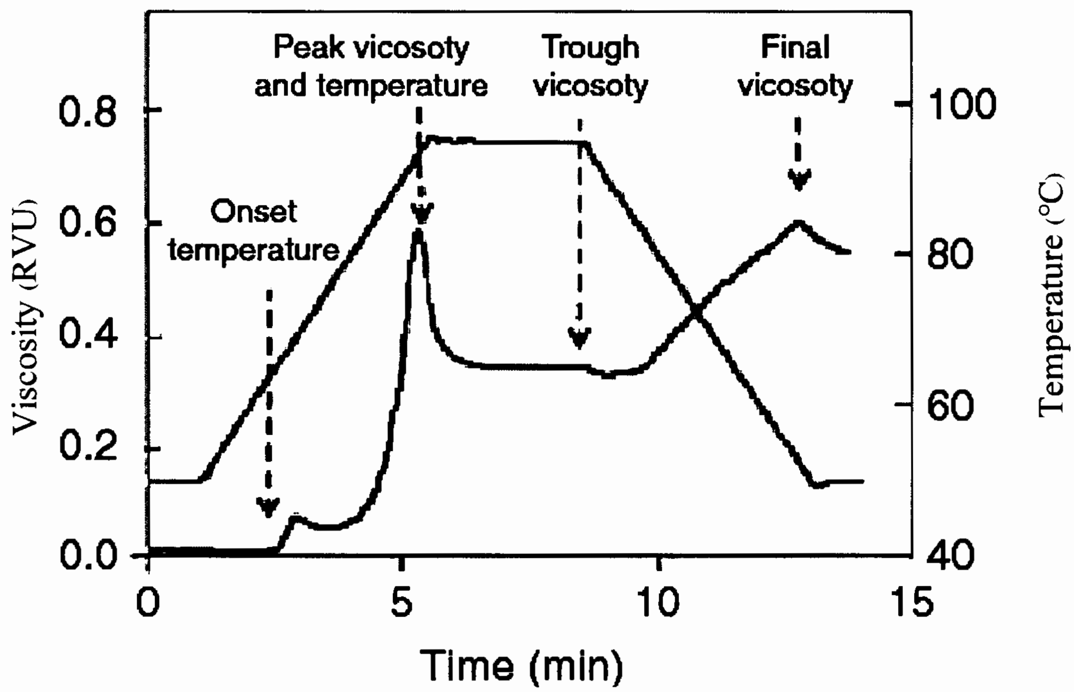



**Figure 2.1** Typical Rapid Visco Amylograph from the Rapid Visco Analyser (RVA) (Source: Li, et al., 2008)

Figure 2.1 shows Rapid Visco Amylogrph (RVA), which represents peak viscosity, through viscosity, breakdown, final viscosity and setback from trough in the RVU unit. Peak viscosity has strongly relative with flour or starch properties. It can describe the water-binding efficient of flour or starch. According to Table 2.2, wheat flour gives lower peak viscosity (133.17 RVU) than cassava flour in range of 161.17-296.25 RVU as same as wheat starch (233.58 RVU) and cassava starch(244.91 RVU). Through viscosity is the lowest viscosity from heat supply and stirred-force. The result indicated that all of the starch has higher through viscosity among other flours types. On the other hand, the comparison between highest viscosity and lowes viscosity or breakdown for longer stirred at constant temperature conclude that all flour and starch has specific characteristic then give breakdown viscosity between 37.92-171 RVU. Final viscosity is the parameter for the quality of flour and starch and results in part of cooling characteristic (gelling or viscous). In addition, pasting properties variables, which are peak viscosity and setback viscosity can estimate the trend of retrogradation when comparing to those starch types. Peak viscosity is referred to as the trend of amylose or amylopectin content. Therefore, cassava flour has a low setback from trough (19.17-40 RVU) than other flour and starch. This may conclude that cassava flour has a lower degree of retrogradation when comparing to those flour and starch.

**Table 2.2** Pasting properties of cassava flour from different varieties

| Flour/ Starch | Peak viscosity (RVU) | Through viscosity (RVU) | Breakdown (RVU) | Final viscosity (RVU) | Setback from trough (RVU) | Sources |
|---|---|---|---|---|---|---|
| Cassava flour | 266 ± 5 | 95 ± 8 | 171 ± 5 | 135 ± 8 | 40 ± 1 | Chotineeranat, et al., 2006 |
| | 296.25 ± 1.08 | 147.83 ± 1.83 | 148.33 ± 0.92 | 191.83 ± 1.33 | 44 ± 0.58 | Dudu, et al., 2018 |
| | 164.5 ± 1.5 | 48.66 ± 2.08 | 115.83 ± 2.67 | 67.83 ± 0.41 | 19.17 ± 0.83 | Dudu, et al., 2018 |
| | 161.17 | 123.25 | 37.92 | 247.66 | 36 | Jensen, et al., 2014 |
| Cassava Starch | 244.91 ± 0.17 | 175.61 ± 0.12 | 69.30 ± 0.11 | 315.10 ± 0.10 | 115.37 ± 0.18 | Ojo, et al., 2017 |
| Wheat flour | 133.17 | 94.83 | 38.33 | 130.83 | 124.08 | Blazek and Copeland, 2008 |
| Wheat starch | 233.58 ± 0.03 | 164.08 ± 0.03 | 69.5 ± 0.10 | 279.17 ± 1.23 | 115.08 ± 0.01 | Sun, et al., 2015 |
| Rice starch | 215.00 ± 2.5 | 158 ± 1.00 | 56.8 ± 1.5 | 298 ± 1.00 | 139.42 ± 0.01 | King, 2006 |

## 2.3 Retrogradation

### 2.3.1 Definition and Determination Method

Retrogradation is a reaction that takes place when amylose and amylopectin chain structure are recrystallization after heat and water supplied or in a gelatinization state. When native starch is heated and dissolved in water, the crystalline structure of amylose and amylopectin molecules is lost, and hydrate to form a viscous solution. If the viscous solution is cooled for a long enough period, the linear molecules, amylose, and linear parts of amylopectin branch molecules retrograde and rearrange themselves again to a more crystalline structure. The linear chains place themselves parallel and form hydrogen bonds. In viscous solutions, the viscosity increases to form a gel (Wang, et al., 2015).

Retrogradation is the change after the gelatinization of starch from an amorphous state to the crystalline state. This occurred because pastes or gels of gelatinized starch are not in thermodynamic equilibrium. The rheological properties change is the increase of firmness or rigidity. Loss of water binding capacity and restoration of crystallinity also become evident and increase in aging. These phenomena are called retrogradation and are thought to exert a major influence on texture leading to decrease acceptability of many bakery products (Gudmundsson, 1994).

Retrogradation occurs when foods are cooked and then cooled. Generally, there is a negative relationship between the amylose content of starch, the peak viscosity and setback from through that are the value of pasting properties (Fredriksson, et al., 1998). High amylose starches (such as those from various maize cultivars) require higher temperatures and pressures than high amylopectin starches to be gelatinized (Colonna and Mercier, 1985). Thus, high amylose starches are intrinsically more resistant than those higher in amylopectin and retrograde more readily (Aparicio and Saguilán, 2005).

Starch retrogradation is mostly taken to be an undesirable process that occurs during the storage of starchy foods. Retardation or inhibition of starch retrogradation is of special interest, a challenge for the food industry, and an area where great efforts have been made to study the influencing factors. As discussed subsequently, water content, starch source, and storage conditions are all well-known factors that can greatly influence starch retrogradation. The presence of food components such as lipids, carbohydrates, salts, proteins and peptides have also been shown to play a significant role in retarding the rate of starch retrogradation.

Therefore, the interesting factors affecting retrogradation including 3 parts which are the component of starch (amylose content, proteins, lipid, etc.), storage temperature and water-binding capacity.

#### 2.3.1.1 Staling in Bread

Starch is the main component of bakery products. The staling of bread essentially involves the retrogradation of amylose and amylopectin but not the amylopectin fraction (Schoch and French, 1947). Many investigations have been carried out to determine the respective role of amylose, amylopectin and their combined effect in the retrogradation of starch gel and the staling of baked products. The role of amylose is the major in part of swelling in the gelatinization of starch granule. Seventy-percent of flour is starch content which is regarded as the main flour component involved in staling.

Due to an hour of cooling after baking, the initial crumb structure is set by amylose gelatinization, creating a network in which the gelatinized starch granules are embedded. Re-crystallization of amylopectin chains leads to the increasing rigidity of the starch granules. Overall strengthening of the crumb structure can be measured as an increase in crumb firmness. However, the distribution of water between protein and starch also has an impact on bread firming due to an important role in baking and cooling. Starch retrogradation is the main factor with respect to time determined changes in crumb softness. Functional ingredients that limit retrogradation behavior are instrumental in improving crumb softness (Saral, 2015).

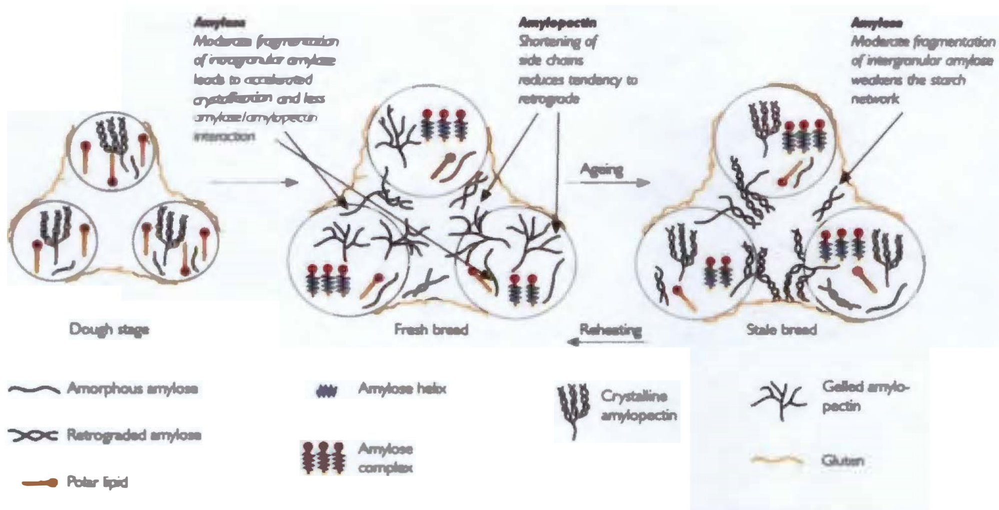



**Figure 2.2** Change in starch during baking, cooling and storage

(Source: Saral, 2015)

Figure 2.2 shows the changes that occur from the dough stage to fresh bread and, finally, stale bread. The restoration of bread freshness by heating (toasting) is also indicated. In the dough stage, un-swollen starch granules contain crystalline amylopectin, amorphous amylose, and polar lipids. The granules are embedded in gluten, which forms the continuous phase. During baking, the starch granules absorb water and swell. The amylopectin crystals are gradually disrupted at temperatures above 60 °C, and gelatinization takes place. Some of the amylopectin molecules expand into the inter-granular space and, at a somewhat higher temperature around 80 °C, some of the amyloses that have not formed complexes with polar lipids leaks from the swollen granules. The amylose molecules restructure the network, and a sliceable crumb structure is formed, giving the fresh bread its initial firmness within an hour. During aging, the reformation of the amylopectin is a double helical structure and reorganization into crystalline regions takes place. While the re-association of amylose occurs within hours, the retrogradation of amylopectin takes days.

### 2.3.1.2 Retrogradation Properties Assays

The retrogradation properties measurement can be divided into 2 sections which are macromolecules view and micro molecules view. Texture analyzer and syneresis measurement for starch gel or bakery products are an important method for macromolecules observing. In part of the micro molecule view, the study of pasting properties and components of starch or flour, for example, are lipids, proteins, moisture, ash, starch and amylose content are selected.

### 2.3.1.2.1 Texture Analyzer

Texture Profile Analysis is a popular double compression test for determining the textural properties of foods. It is occasionally used in other industries, such as pharmaceuticals, gels, and personal care. During a TPA test samples are compressed twice using a texture analyzer to provide insight into how samples behave when chewed. The TPA test was often called the "two-bite test" because the texture analyzer mimics the mouth's biting action (Texture Technologies Corporation and Stable Micro Systems, 2019).

The textural is an important parameter to identify the retrogradation properties in a bakery product. For example, the hardness of bakery products will be increasing after bread or cake starting to cool and the cohesiveness of texture will be decreasing after cooling in storage temperature because of loosing of moisture content in a bakery product.

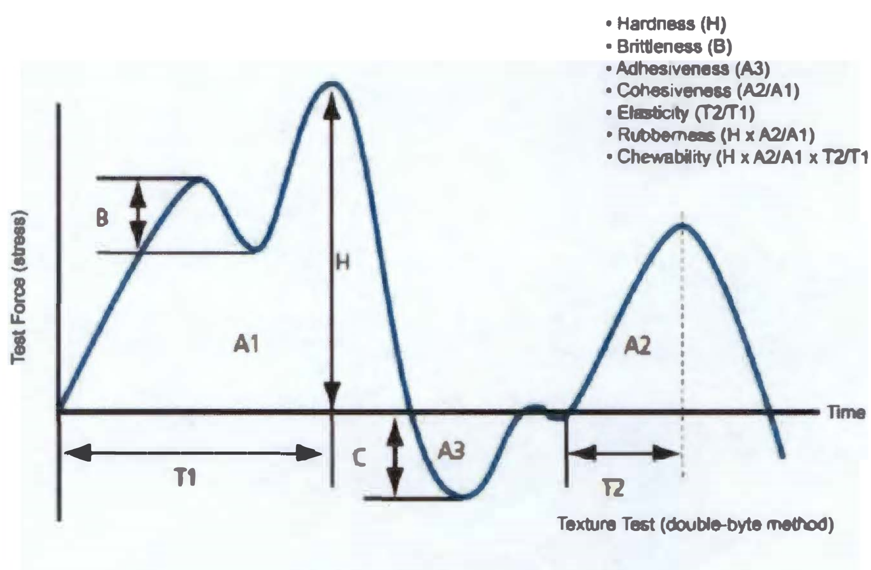



**Figure 2.3** Textural profile

(Source: Chinzorig and Hwang, 2018)

Parameters of textural profile (Figure 2.3) consist of hardness, brittleness, cohesiveness, springiness, gumminess and adhesiveness. Hardness or firmness value is the peak force that occurs during the first compression. Next, not all products brittles; but when they do brittles the brittleness point occurs where the plot has its first significant peak (where the force falls off) during the probe's first compression of the product. Cohesiveness is how well the product withstands a second deformation relative to its resistance under the first deformation. Springiness is how well a product physically springs back after it has been deformed during the first compression and has been allowed to wait for the target wait time between strokes. The spring back is measured at the down-stroke of the second compression. In some cases, excessively long wait time will allow a product to spring back more than it might under the conditions being researched.

#### 2.3.1.2.2 Syneresis Measurement in Water-flour Gel Model

Syneresis is a process in which a gel contact on standing and exudes liquid. Although syneresis is a physical characteristic of most gels, it can be used to assess the freeze-thaw stability of starch by measuring the water exuded from a gel on standing or after freezing and thawing. Syneresis increases with the number of freezes-thaw cycles, in part due to enhanced amylopectin retrogradation in the starch-rich phase (Yuan and Thompson, 1998). A low syneresis value on freezing and thawing is indicative of slow retrogradation of starch gels due to strong interactions between dispersed amylose/amylopectin and water molecules.

### 2.3.2 Factors Affecting Retrogradation

#### 2.3.2.1 Component of Starch

Amylose and amylopectin summation represents approximately 98–99 % of the dry weight of starch granules. The ratio of the two polysaccharides varies according to the botanical origin of the starch. Amylopectin is the major component of most starches, with the amylopectin content in starch varying from close to 100% (e.g., in waxy starches from wheat, barley, maize, and rice) to less than 30 % amylopectin (e.g. in the wrinkled pea and in amylomaizes) (Lansky, et al., 1949).

Amylose and amylopectin have different structures and properties (Figure 2.4). Amylose is a relatively long, linear α-glucan with few branches, containing around 99% (1→4)-α- and up to 1% of (1→6)-α- linkages and differing in size and structure depending on botanical origin (Buleon et al., 1998; Mua and Jackson, 1997).

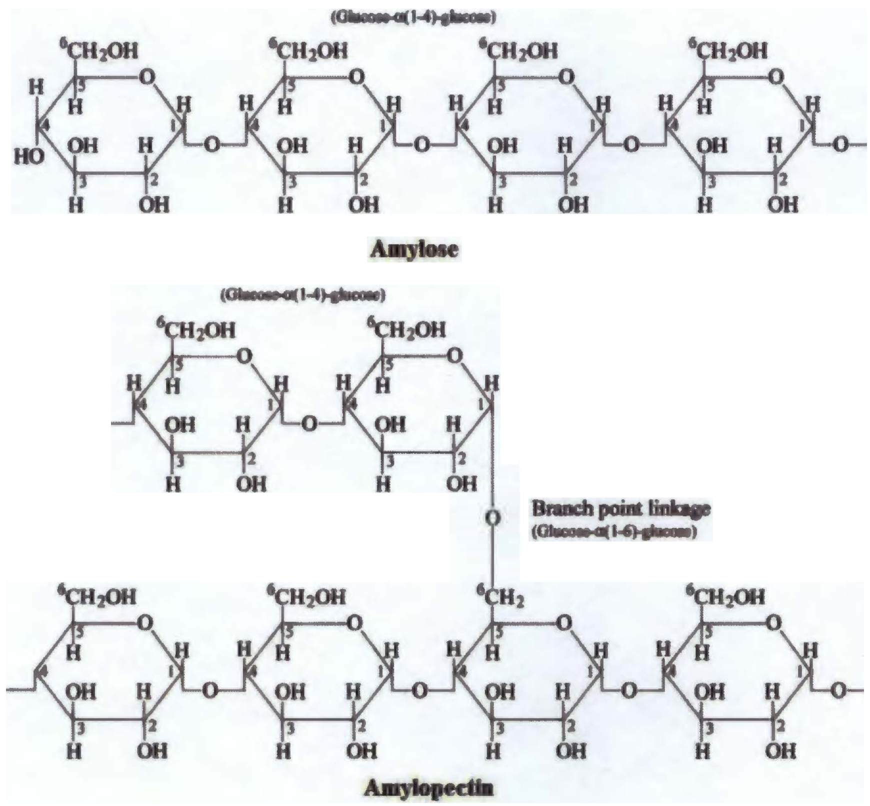



**Figure 2.4** The structural of amylose and amylopectin
(Source: Paul, 2016)

Polar lipids are known to have an anti-staling effect on bread and to extend its shelf-life (Krog and Jensen, 1970). The retarding mechanism of lipids or surfactants on retrogradation is thought to be dissimilar from that of other solutions, i.e. an anti-plasticizing effect, being instead related to their ability to form complexes with the amylose fraction (Lagendijk and Pennings, 1970). Since Schoch and French (1947) proposed that the amylose fraction and the amylopectin fraction were responsible for the retrogradation. Many investigations have attributed the long-term effects associated with retrogradation to the amylose fraction (Mikus, et al., 1946). The question then arises of how lipids or surfactants affect the retrogradation as they are thought to form complexes only with the amylopectin molecule, and not with the amylose fraction (Kugimiya, et al., 1982).

A few possible of retardation of retrogradation is the intact amylose-lipid complex, i.e. as one entity, interferes with the crystallization of the amylopectin in some unknown way and retards the retrogradation, the amylose-lipid complex interferes indirectly by changing or retarding the water distribution and hence the retrogradation (D'Appolonia and Morad, 1981). Co-crystallization of amylose and amylopectin are possible to some extent, and substances that form complexes with amylose eliminate the contribution of amylose in the recrystallization process (Russel, 1987). Finally, lipids and surfactants interact directly with the amylopectin fraction, at least to a small extent, and retard retrogradation through the formation of an amylopectin-lipid complex (Gudmudsson and Eliasson, 1990).

Amylose lipid complexes (ALCs) are defined as the inclusion of lipid molecules in amylose (Figure 2.5). Single-helix amyloses that are co-crystallised with compounds (e.g., fatty acids or alcohols) are given the generic name V-amylose (Godet, et al., 1993). ALCs have six glycosyl residues per turn and a guest molecule inside the helix. V-amylose exists in dry and hydrated forms.

Several types of lipids, including monoglycerides and fatty acids and their esters, can complex with amylose, particularly with the hydrocarbon portion inserted into the helical cavity of amylose (López, et al., 2012). The lipid methylene groups and glycosidic linkages line the inner surface of ALCs. Hydrophilic hydroxyl groups point away from the helix (Immel and Lichtenthaler, 2000).

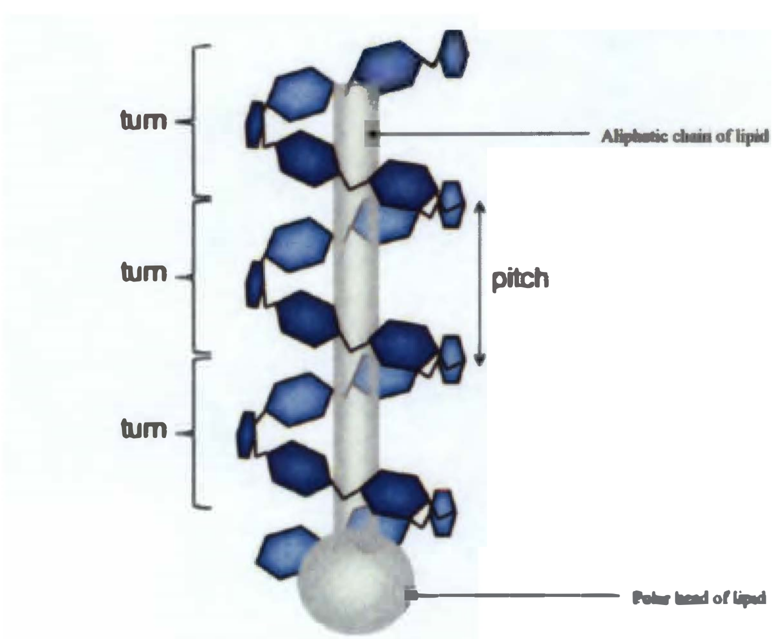



**Figure 2.5** Structural of amylose-lipid complex
(Source: Panyoo and Emmambux, 2016)

V-amylose is classified as type I or type II depending on the melting temperature of the crystalline components (Biliaderis and Galloway, 1989). Type I generally has melting temperatures between 94 °C and 104 °C, while type II melts between 115 and 121 °C (Biliaderis and Seneviratne, 1990). Type I complexes consist of a partially ordered structure with no distinct crystalline regions, while type II complexes are composed of distinct crystalline/semicrystalline structures. Type II can be subdivided into types IIa and IIb. The two differ slightly in the degree of crystallinity or the perfection of the ordered domains. The type IIa complexes melt at approximately 115 °C, and type IIb complexes melt at approximately 121 °C (Karkalas, et al., 1995). Biliaderis and Galloway suggested that type II complexes are superstructures of several type I complexes crystallized together. Non-dissociated type I complexes serve as nuclei for crystallization. Rappenecker and Zugenmaier suggested that type IIb is the most stable form of the amylose-lipid complex.

The example of amylose and amylopectin content are showed in Table 2.3. The result from previous study indicated that amylose can interact with amylose because of the linear structure. To consider in starch and amylose content in cassava flours from different varieties, there are 67 - 91% of starch and 10 – 23% of amylose content. In part of amylose content in rice flours, it was found that 1 – 33% of amylose content in flours which effect to retrogradation properties. Moreover, rice flours are separated into 3 types which are high amylose (amylose content is more than 20%), medium amylose (amylose content is between 20% - 6%) and low amylose or waxy rice (amylose content is lower than 6%) with respect to the amount of amylose content. Therefore, rice flour was used to be a wheat flour replacement according to non-gluten flour. However, high amylose content in rice flour may increase retrogradation properties. For bakery products, amylose and amylopectin content play in different roles. Linear amylose interacts with lipid and form amylose-lipid complex while branch amylopectin unfolds and bond with water. After cooling, the amylose-lipid complex structure does not restructure but amylopectin will restructure which is the cause of stalling bread. Therefore, the main of retrogradation in starch structure is amylopectin content. In this table, rice starch/flour will occur fast of retrogradation follow by cassava starch/flour and the last is wheat starch/flour. Amylopectin has a lesser tendency to gelation, retrogradation, and syneresis because of the branched structure.

**Table 2.3** Starch and amylose contents of flours from different botanical sources

| Flour types | Variety | Country | Starch content (%) | Amylose content (%) | References |
|---|---|---|---|---|---|
| Cassava | Hanatee | Thailand | $82.41 \pm 0.42^{A}$ | | Charoenkul, et al., 2011 |
| | Rayong 2 | Thailand | $81.38 \pm 0.35^{A}$ | | Charoenkul, et al., 2011 |
| | KU50 | Thailand | $91.10 \pm 1.10^{A}$ | | Chotineeranat, et al., 2006 |
| | Unknown | Thailand | | $23.07 \pm 0.07^{A}$ | Ritthiruangdej and Suwonsichon, 2007 |
| | Thirty-one varieties | Ghana | | 10.9 - 44.3 | Aryee, et al., 2006 |
| | Yellow cassava | Nigeria | $67.1 - 82.4^{A}$ | 17.4 - 22.3 | Alamu, et al., 2017 |
| Rice | Kor-Khor 6 | Thailand | | $4.47 \pm 0.16$ | Varavinit, et al., 2003 |
| | Kor-Khor 10 | Thailand | | $4.87 \pm 0.12$ | Varavinit, et al., 2003 |
| | San-Patong | Thailand | | $5.28 \pm 0.15$ | Varavinit, et al., 2003 |
| | Hom-Mali | Thailand | | $14.63 \pm 0.38$ | Varavinit, et al., 2003 |
| | Pathumthani 1 | Thailand | | $15.45 \pm 0.33$ | Varavinit, et al., 2003 |
| | Luang 11 | Thailand | | $21.95 \pm 0.45$ | Varavinit, et al., 2003 |
| | Khaw Tahang | Thailand | | $22.76 \pm 0.30$ | Varavinit, et al., 2003 |
| | Chainat | Thailand | | $23.98 \pm 0.28$ | Varavinit, et al., 2003 |
| | Kordeaw | Thailand | | $24.39 \pm 0.18$ | Varavinit, et al., 2003 |
| | Plaingam-Prajeenburi | Thailand | | $26.02 \pm 0.37$ | Varavinit, et al., 2003 |
| | Kheaw-Prajeenburi | Thailand | | $26.42 \pm 0.29$ | Varavinit, et al., 2003 |

$^{A}$ = the values were reported in %dry basis

**Table 2.3** Starch and amylose contents of flours from different botanical sources. (Cont'd)

| Flour types | Variety | Country | Starch content (%) | Amylose content (%) | References |
|---|---|---|---|---|---|
| Rice | Koganemochi | Japan | | 1.95 | Iwashita, et al., 2011 |
| | Milky queen | Japan | | 8.5, 11.5 | Araki, et al., 2016; Iwashita et al., 2011 |
| | Koshihikari | Japan | | 18.0, 18.9 | Araki, et al., 2016; Iwashita et al., 2011 |
| | Nipponbare | Japan | | 19.5, 22.3 | Araki, et al., 2016; Iwashita et al., 2011 |
| | Hinohikari | Japan | | 23.1 | Iwashita, et al., 2011 |
| | Kinuhikari | Japan | | 22.4 | Iwashita, et al., 2011 |
| | Harnuki | Japan | | 23 | Iwashita, et al., 2011 |
| | Akitakomachi | Japan | | 22.4 | Iwashita, et al., 2011 |
| | Hitomebore | Japan | | 22.3 | Iwashita, et al., 2011 |
| | Kirara397 | Japan | | 22.5 | Iwashita, et al., 2011 |
| | Yumetoiro | Japan | | 33.2, 37.1 | Araki, et al., 2016; Iwashita et al., 2011 |
| White wheat flour | | Belgium | 69.68[A] | 25.9 | Nindjin, et al., 2011 |

[A] = the values were reported in %dry basis

### 2.3.2.2 Storage Temperature

The effects of starch gelatinization and retrogradation on the texture of bakery products are well established (Russell, 1983a, 1983b, 1987; Zeleznak and Hoseney, 1986; Krog, et al., 1989). Starch is a partially crystalline, partially amorphous polymer of glucose. Amylose and the branching of amylopectin contribute to the amorphous region and the outer chains of amylopectin contribute to the crystalline region. Baking is the result of a completely amorphous starch (Slade and Levine, 1991). When the gelatinized product is cooled, the amylose fraction retrogrades immediately while the amylopectin remains in an amorphous state (Miles, et al., 1985). During storage, firmness and opacity increase in the baked product. These changes have been correlated with the re-association and crystal formation of the amylopectin fraction of gelatinized starch (Ring, et al., 1987). Keetels, et al. (1996) correlated the changes in the mechanical properties of wheat and potato bread with changes in sponge structure and amylopectin recrystallization.

The quality of cooked rice is affected by its starch properties, mainly amylose content. Cooked rice with low amylose is soft and sticky, while rice with high amylose is firm and fluffy. Like bread products, cooked rice texture changes with storage (Lima and Singh, 1993). The firmness of cooked rice gradually increases during storage, and this has been attributed to starch retrogradation. Most studies relating texture or mechanical change to changes in starch properties have focused on gels from rice flour.

The study of starch retrogradation and texture of cooked milled rice during storage was reported that starch retrogradation had a direct linear relationship with firmness (Figure 2.6), Firmness of cooked rice decrease with increased temperature in varies storage temperature (36, 20, 3 and -13 °C). The relationship between starch retrogradation and stickiness was more complex and was dependent on varieties and storage temperature (Perdon, et al., 1999).

Same as other cereal-based food, the degree of starch retrogradation in cooked milled rice affected to texture. In general, as the degree of starch retrogradation increased during storage, rice firmness is increased, and stickiness decreased. These results were similar to those reported previously (Perez, et al., 1993; Lu, et al., 1997; Villareal, et al., 1997). As in starch gels, cultivar differences affecting the rate of texture change and starch retrogradation may be explained by differences in the amylose to amylopectin ratios.

Ottenhof and Farhat (2004) studied the effect of gluten on the retrogradation of wheat starch in 25 °C storage temperature. Generally, starch consist of amylose and amylopectin content by the way gluten in wheat-protein, since this research mixed it together with variation of starch and wheat and analysis retrogradation properties by XRD, DSC and NMR. their results indicated that the presence of gluten at this concentration (10%) did have a significant effect on the kinetics of retrogradation, the extent or the polymorphism of amylopectin retrogradation during storage. According to the amylose-lipid complex structure in the study, the change of firmness has not been affecting to increase retrogradation rather than amylopectin content.

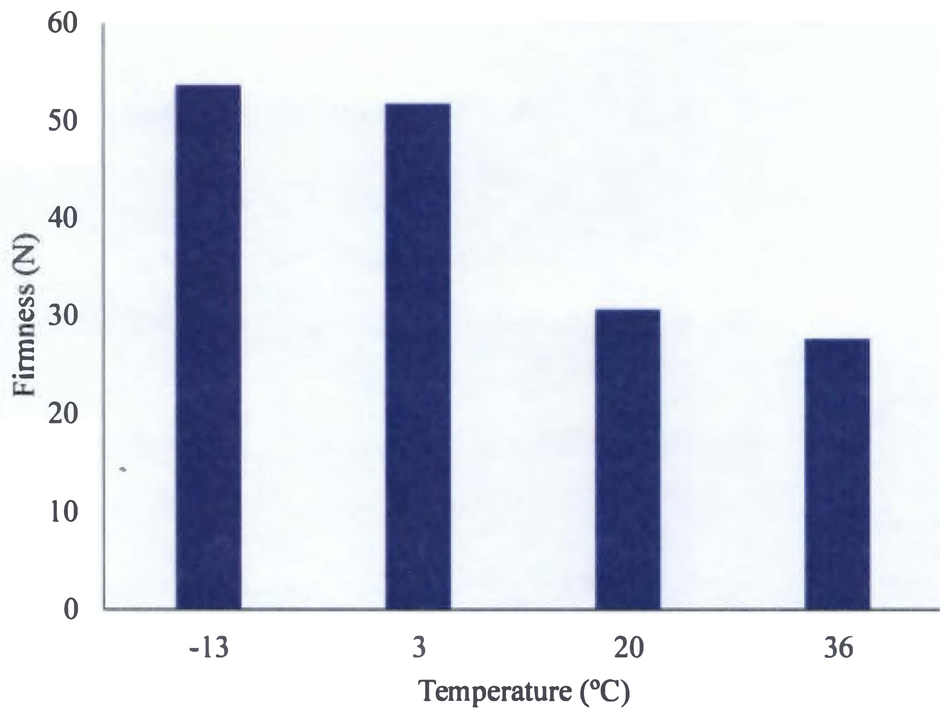



**Figure 2.6** The firmness of rice cookies with different storage temperature for 2 days.

### 2.3.2.3 Water Binding Capacity

According to the research of Yang, et al. (2017), most of the researchers have tried controlling the temperature and water content along with other methods to inhibit the occurrence of Gluten-free flour (rice) retrogradation, but they are still unable to stop it. Research on the effect of emulsifier on the retrogradation process of rice at low temperatures is also limited. The mechanism of how these surfactants influenced starch structure is not completely understood. Therefore, the aim of this study was to investigate the changes in the retrogradation index, textural properties, and water content at low temperatures (4 °C and − 20 °C) during the whole storage process and compare the effect of different emulsifiers on rice starch, helping to elucidate the mechanism of anti-retrogradation of emulsifiers. These results could provide some theoretical guidance for retarding the retrogradation of rice during its storage.

Al-Hajji, et al. (2016) reported that, during prolonged storage time (one to seven weeks), retrogradation mainly occurs in amylopectin. In our research, the amylose content of rice was 10.43% and amylopectin was 89.57%. Thus, retrogradation may be mainly determined by amylopectin. As GMS could adhere to the surface of amylopectin and change the water distribution, interaction with the forked chain via hydrogen bonding was altered, reducing the water-binding capacity of starch and ultimately indirectly delaying the long-term retrogradation (Wang, et al., 2015).

### 2.3.3 Retarding Retrogradation with Added Cassava Starch/ Flour

Frozen ready-to-eat food products are convenient to use since they require less time to prepare than raw food. With proper frozen storage, these products can be kept for up to one year then a variety of new frozen foods are continually being launched onto world markets. However, upon freezing, water in the food is transformed into ice and as the ice separates out, the concentration of the unfrozen phase in contact with the ice increases. Both the ice formation and the increasing concentration of the unfrozen component result in physical stress on the food matrix. When this frozen food is thawed for consumption, the moisture readily separates from the matrix and causes a change in the texture, drip loss, and often deterioration in the overall quality (Reid, et al., 1994).

Starch-based frozen food products undergo textural changes related to amylose and amylopectin retrogradation and show syneresis after thawing. These changes have been attributed to starch retrogradation (Ferrero, et al., 1994; BeMiller, 1998; Varavinit, et al., 2000) and may make such products unacceptable to consumers (Ferrero, et al., 1993).

There have been studies on using starch or flour to improve the quality of frozen starch-based food products, with examples being bread made from frozen dough (Yi, et al., 2009), frozen Chinese steamed bread (Qin, et al., 2007) and frozen fried dumplings (Hayakawa, et al., 2004). However, the explanation for the mechanism of the addition of flour or starch on product quality is not clear because these studies were carried out in real food systems. This study attempted to investigate the possibility of using cassava starch (CS) to improve the freeze-thaw stability of rice starch gel as well as explain the causes of the effects. CS is cheaper than most hydrocolloids and can be widely produced commercially in Thailand.

Considering in pasting properties and composition of rice starch, cassava starch and wheat starch, there are significantly different between amylopectin in the gel model which can order in cassava starch, wheat starch and rice starch, respectively. In this study, there is no added lipid in the gel model as same as very low lipid content in starch. Thus, setback viscosity and peak viscosity are in the same conclusion.

Therefore, CS is a root starch with specific properties such as a low paste temperature and a low gelling point and a lower amylose content and retrogradation tendency compared with cereal starches (Moore, et al., 2005).

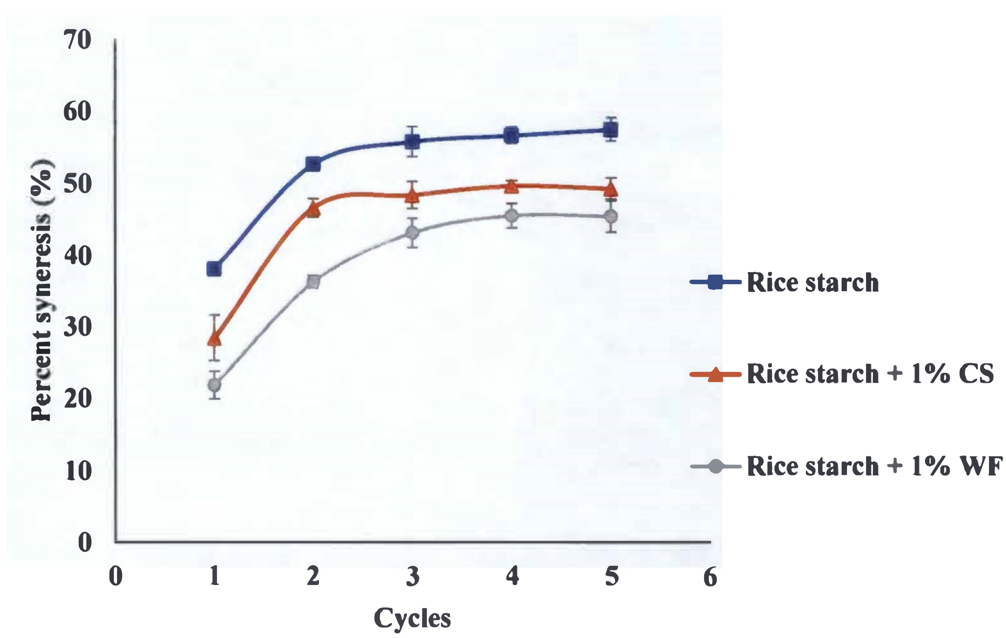



**Figure 2.7** Percent syneresis of rice starch, rice starch with 1% cassava starch and rice starch with 1% wheat flour

The percentage syneresis (% syneresis) for freeze-thawed starch was used to evaluate the ability of starch to withstand the undesirable physical changes which occur during freezing and thawing. Syneresis of a freeze-thawed gel is caused by an increase in molecular associations between starch chains in particularly of amylose retrogradation (Morris, 1990) which results in the expulsion of water from the gel structure (Saartratra, et al., 2005). Thus, the amount of water released due to syneresis is a useful indicator of the tendency of starch to retrograde (Karim, et al., 2000). However, in a starch gel containing ingredients such as hydrocolloids or sugars, which can bind to water molecules, syneresis is reduced (Arunyanart and Charoenrein, 2008; Baker and Rayas-Duarte, 1998).

The effect of CS on the % syneresis in rice starch gels is presented in Figure 2.7. Freeze–thawed rice starch gels without CS had a high syneresis value (38.1%) after the first cycle and had a significantly higher value after two freeze-thaw cycles. After that, the values change slightly through cycles 3 – 5. High syneresis in this sample resulted from a high amylose content (37.50%) in the rice starch. A previous study (Charoenrein, et al., 2008) showed that gels made from medium-amylose rice flour (17.6%) had a significantly lower % syneresis after the first freeze-thaw cycle than did gels made from high-amylose rice flour (32.5%). This result implies that amylose plays an important role in the retrogradation associated with freezing and thawing. Therefore, cassava starch can be useful for reducing the syneresis of frozen rice gel.

# CHAPTER 3 MATERIALS AND METHODS

## 3.1 Materials and Chemical Reagents

Cassava roots of two low-cyanide cultivars (Pirun 4 and Hanatee) were received from National Science and Technology Development Agency (NSTDA), Thailand. They were grown in Khonkaen province, Thailand, and harvested with 8 months after planting. After transportation to King Mongkut's University of Technology Thonburi, Bangkok, the roots were kept at -20 °C within 1 month before used in the flour preparation method. The commercial wheat flour (Pioneer, Japan) and rice flour (New grade, Thailand) were brought from a supermarket in Tokyo, Japan and Bangkok, Thailand, respectively. All chemical used in this study were of analytical reagent grade (AR grade).

## 3.2 Flour Preparation

Frozen cassava roots were thawed for 2 h. Cassava roots were washed, peeled and sliced into 3 ± 1 mm thickness. They were subsequently dried in the hot air oven (UFE600, Memmert, Germany) at 50 ± 2 °C for 10 h. The dried cassava was ground by cyclone mill machine (Foss, CT193 CyclotecTM, Suzhou, Chaina) and sieved through 80-mesh (180 µm). Cassava flour was kept in aluminum polypropylene plastic bag, place in desiccator box.

## 3.3 Chemical Composition and Pasting Properties of Flours Samples

### 3.3.1 Chemical Compositions

The chemical compositions of flour samples including moisture (method 934.01), fat (method 954.02), starch, ash (method 945.46) and protein (method 992.15) were determined by methods described in according to the Association of Official Analytical Chemists (AOAC, 2012). Moreover, the starch, amylose, and cyanide content were also determined (Brien, et al., 1991). The details for each measurement were described as follows.

#### 3.3.1.1 Moisture Content

The sample was weighed (3 g) and dried at 105 °C for 24 h. in a hot air oven (ULN, Memmert, Germany). The loss in weight was recorded as moisture.

#### 3.3.1.2 Protein Content

Nitrogen contents were determined by the Kjeldahl method. The method involved digestion of the sample at 420 °C for 1 h in sulfuric acid (99.8%) to liberate the organically-bound nitrogen in the form of ammonium sulphate. The ammonia in the digest (ammonium sulphate) was then distilled off into a boric acid receiver solution and then titrated with standard hydrochloric acid (HCl). Protein contents were calculated by a conversion factor of 6.25 from total nitrogen contents.

#### 3.3.1.3 Lipid Content

The aluminum cup for fat analysis was dried at 105 °C for 3 h. then record the constant weight ($w_1$). Flour was weighed approximately 3 ± 0.5 g ($w_2$) and covered with filter paper (No.1). The sample was put into a thimble, connected to Soxtech machine (lipid extraction) with an aluminum cup that contains 75 ml of hexane.

Conditions of fat extraction were started with boiling step (sample was dripped into boiling hexane) for 1 h, rinsing step (remove the sample from boiling hexane) for 1 h and evaporating until aluminum cup was empty. The cups were weighed again ($w_3$) and calculated fat content with the equation below (3.1).

$$\%\ \text{lipid} = \left[\frac{w_3 - w_1}{w_2}\right] \text{x}100 \qquad (3.1)$$

#### 3.3.1.4 Ash Content

This was determined by the method of AOAC, 2012. The method involved burning off moisture and all organic constituents at 600 °C in a furnace. The weight of the residue after incineration was recorded as the ash content.

#### 3.3.1.5 Starch Content

Starch content was measured by using the colorimetric method (570 nm).

### 3.3.1.5.1 Standard Curve

Fifty-grams of starch was dispersed into 50 ml distilled water in 100 ml of volumetric flask, boiling solution for 1 min and completed sample by DI water for 100 ml. Sample was filtrated by filter paper WHATMAN No. 1. Starch sample was pipetted into test tube for 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml and complete 1.0 ml solution with distilled water or DI water. Acetic acid ($CH_3COOH$) 2 N 1.25 ml, potassium iodide (KI) 10% w/v 0.25 ml and potassium iodate ($KIO_3$) 1/600 M 2.5 ml were added into test tube. UV-VIS spectrophotometer (UV-2600, Shimadzu, Japan) with 570 nm absorbance was selected to measure brown color and the distilled water was used as a blank.

### 3.3.1.5.2 Sample Analysis

Flour was prepared as same as standard curve measurement. After filtration, the sample was pipetted for 0.7 ml completed with 0.3 DI water, added reagents and measure absorbance, respectively. Starch content (percent dry basis) was calculated as followed equation (3.2).

$$\text{Starch content} = \frac{C_s(100 \times 100)}{V \times 1000 \times [50-(50 \times M)]} \tag{3.2}$$

where $C_s$ is Starch concentration (µg/ml)

$V$ is Sample volume (ml)

$M$ is Moisture content in flours sample

### 3.3.1.6 Amylose Content

Amylose content was examined by the colorimetry method described in Juliano (1971). A hundred grams of sample was mixed with 1 ml of 95% ethanol and 9 ml of 2 M NaOH. After that, the mixtures were adjusted to 100 ml with distilled water. The solutions were added with 2.0 ml of 0.2% iodine solution and the absorbance was measured at 620 nm by using a UV-spectrophotometer. The amylose content was calculated by referring to a standard curve. The standard curve was prepared by different concentration of amylose and amylopectin content standard (Table 3.1) with the same solution as amylose content measurement in flour then result of standard curve was plotted between absorbance and milligram of amylose.

**Table 3.1** Standard concentration of amylose and amylopectin in standard curve preparation.

| Amylose (mg) | Amylopectin (mg) |
|---|---|
| 2 | 8 |
| 4 | 16 |
| 6 | 24 |
| 8 | 32 |
| 10 | 40 |
| 12 | 48 |

### 3.3.1.7 Cyanide Content

Cyanide content was analyzed by method of Brien, et al. (1991). Bispyrazolone, 3-methyl-1-phenyl-5-pyrazolone, chloramine T, linamarase, linamarin (98%) and potassium cyanide with AR grade were obtained from BDH Ltd, Poole, UK. Acetone cyanohydrin (99%) was a product of Aldrich Chemical Co, Poole, UK. All other chemicals used were analytical grade.

The following stock solutions were prepared. Ethanol/acid extraction medium is 0.1 M orthophosphoric acid containing 25% v ethanol. Buffer A: pH 4.0, 6.0 and 7.0 prepared from 0.1 M $H_3PO_4$, and 0.1 M $Na_3PO_4$. Buffer B: pH 6.0 and 7.0, 0.1 M $H_3PO_4$, adjusted with 5 M NaOH. Linamarase preparation: enzyme was dissolved in buffer A (pH 6.0) to give an activity of about 5 EU $ml^{-1}$ (Cooke, 1978).

For the colorimetric procedure, chloramine T reagent (0.2 ml: 0.5% w/v) was added to 4 ml buffered extraction in a stoppered Quickfit test tube and mixed well. Tubes were placed in an ice/water bath for 5 min, then pyridine/pyrazolone reagent (0.8 ml) was added in a fume cupboard. After 90 min, the absorbance at 620 nm was determined. Duplicate analyses were performed and blanks containing extraction medium were run for each analysis.

Calibration standard and test were performed by following steps

(i) Cassava flours were homogenized in 160 ml extraction medium for 2 min. Using a further 20 ml of extraction medium the homogenate was washed on to a glass-fiber filter (Whatman, GFA) and the extract was collected under vacuum. The extract (0.1 ml) was diluted with buffer A (3.9 ml, pH 4.0) and aliquots were assayed calorimetrically.

(ii) Assay procedure (i) was calibrated using KCN standards. A stock solution of KCN (250 mg/l in 0.2 ml NaOH) was diluted with buffer A (pH 6.0) and aliquots were subjected to assay (i) so that the solutions subjected to colorimetry contained between 0.25 µg KCN (equivalent to 0.1-1.0 µg HCN).

From assays for total cyanide content, the calculation was performed by the following equations (3.3 and 3.4)

$$\text{Cyanide (mg/kg)} = \frac{10 \text{ x } V_a \text{ x } A_{620}}{A_{equiv} \text{ x } DW} \qquad (3.3)$$

$$V_a = V_e + \frac{[M_c \text{ x } FW]}{100} \qquad (3.4)$$

| | | | |
|---|---|---|---|
| where | $V_e$ | is | volume extraction medium used (ml) |
| | $M_c$ | is | the percentage moisture of sample |
| | $FW$ | is | the fresh weigh of sample (g) |
| | $V_a$ | is | the volume adjusted to include sample moisture (ml) |
| | $A_{620}$ | is | the mean absorbance recorded at 620 nm wavelength |
| | $A_{equiv}$ | is | the absorbance corresponding to 1 µg HCN derived from standard |
| | $DW$ | is | the dry weight of sample (g) |

### 3.3.2 Pasting Properties

Pasting properties of flour were measured by the method of Chotineeranat, et al. (2006). Rapid Visco Analyzer (RVA4, Newport Scientific, Australia) was used to evaluate these properties., 3 g of flour samples ( The moisture contents of flour samples were the range of 8-14%) was dispersed in 25 g of distilled water. The method was started at 50 °C 1 min, heated from 50 to 95 °C with 12 °C/min heating rate, and then held at 95 °C for 2.5 min. The paste was cooled down to 50 °C with a cooling rate of 12 °C/min and then held for 2 min (Standard program No. 1). The recorded values included pasting temperature (°C), peak viscosity (the maximum viscosity, RVU), trough viscosity (the hot paste viscosity at 95 °C, RVU), final viscosity (the cold paste viscosity at 50 °C, RVU), breakdown (the difference between peak and trough viscosity, RVU) and setback from trough (the difference between final and trough viscosity, RVU).

## 3.4 Syneresis and Textural Properties of Cassava Flour Gels During Frozen Storage

### 3.4.1 Preparation of the frozen flour gels

To investigate the starch retrogradation, the percentage of syneresis and textural changes of the freeze-thawed flour gel samples were determined. Figure 3.1 demonstrates the flow chart of the experimental steps in the study.

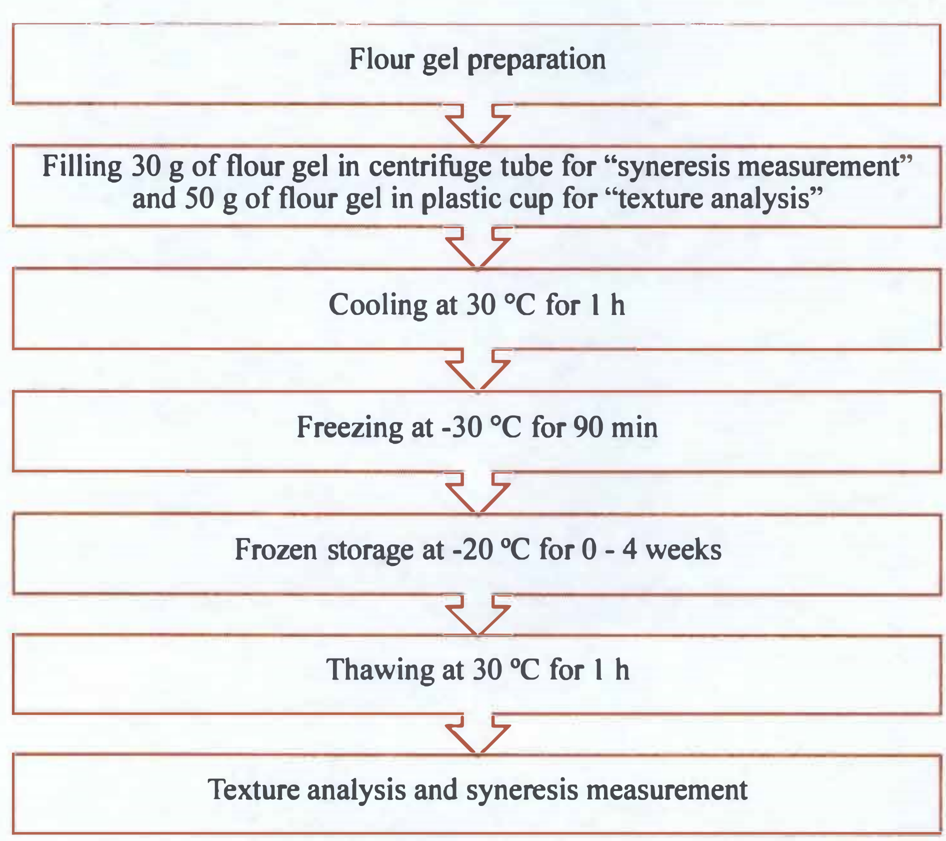



**Figure 3.1** The overall of determination properties of flour gel model system

Flour and water with 20 % suspension were continuously stirred in the water bath at 95 °C for 15 min. 30-gram of flour gel was immediately transferred to 50 ml centrifuge tubes (approximately 28.6 cm diameter and 10.6 cm height) cover cap with paraffin film for syneresis measurement. On the other hand, the other 50 g of the gel was immediately transferred to a cylinder plastic cups (5.5 cm diameter with 4.4 cm height) with a plastic cap and cover with paraffin film for the texture analysis. Both samples were placed in water bath at 30 °C for 1 h in order to cool down gel, prior to freezing at -30 °C for 90 min in an air-blast refrigerator (Test 40 machine, manufactured by MAYEKAWA MFG. Co., Ltd., Japan) with and air velocity is 2.0 m/s. In the preliminary study, it was confirmed that temperatures of flour gels in either a centrifuge tube or a cylinder plastic cup reached -30 °C after they were frozen for 90 min. After freezing, the samples were immediately transferred by place sample into icebox during moved to a refrigerator (SCR-R451G, Sanyo, Japan), and kept at -20 °C for 1, 7, 14, 21 and 28 days prior to the measurements of syneresis and texture attribute. Also, the flour gel samples without the freeze-thawing process (0 day storage) were subjected to the measurements of syneresis and texture attribute as a control. The experiments were performed in triplicate.

### 3.4.2 Syneresis Measurement

Samples in centrifuge tubes were thawed in 30 °C water bath for 1 h and then centrifuged at 5000 g and 25 °C for 15 min by using high-speed refrigerated centrifuge (Suprema 21, Tomy, Japan). The supernatant was removed from the centrifuge tube. The percentage of syneresis was calculated by the following equation (3.5).

$$\text{Syneresis percentage (\%)} = \frac{\text{Total weight - Weight after remove supernatant}}{\text{Total weight}} \times 100 \quad (3.5)$$

where Total weight is the weight of sample in centrifuge tube before freezing

### 3.4.3 Texture Analysis of Flour Gels

The sample in the plastic cup was placed in a water bath at 30°C for 1 h. Texture Profile Analysis (TPA) with a TA. XT Texture Analyzer (Stable Micro Systems, Godalming, U.K.) test was carried out using a P/40 cylindrical probe and a double compression test to compress cake to 50% of their original height. The setting of experiment was performed as follow: pre-test speed is 2 mm/s; test speed is 2 mm/s; post speed is 2 mm/s; time interval of 2 compression cycles are 15 s. The textural parameters considered were firmness and cohesiveness. Firmness (N) is the maximum force required to compress the sample. Cohesiveness is directly related to the tensile and compression strength of bread. The data was processed using Stable Micro System Software.

## 3.5 Frozen Storage of Gluten-Free Chiffon Cake Based on Cassava Flour

Overall cake preparation shows in Figure 3.2. There are 8 steps of chiffon cake starting with ingredient preparation (flour, egg yolk, egg white, sugar cream of tar tar, baking powder, oil, whipping cream, and water) until chiffon cake measurement.

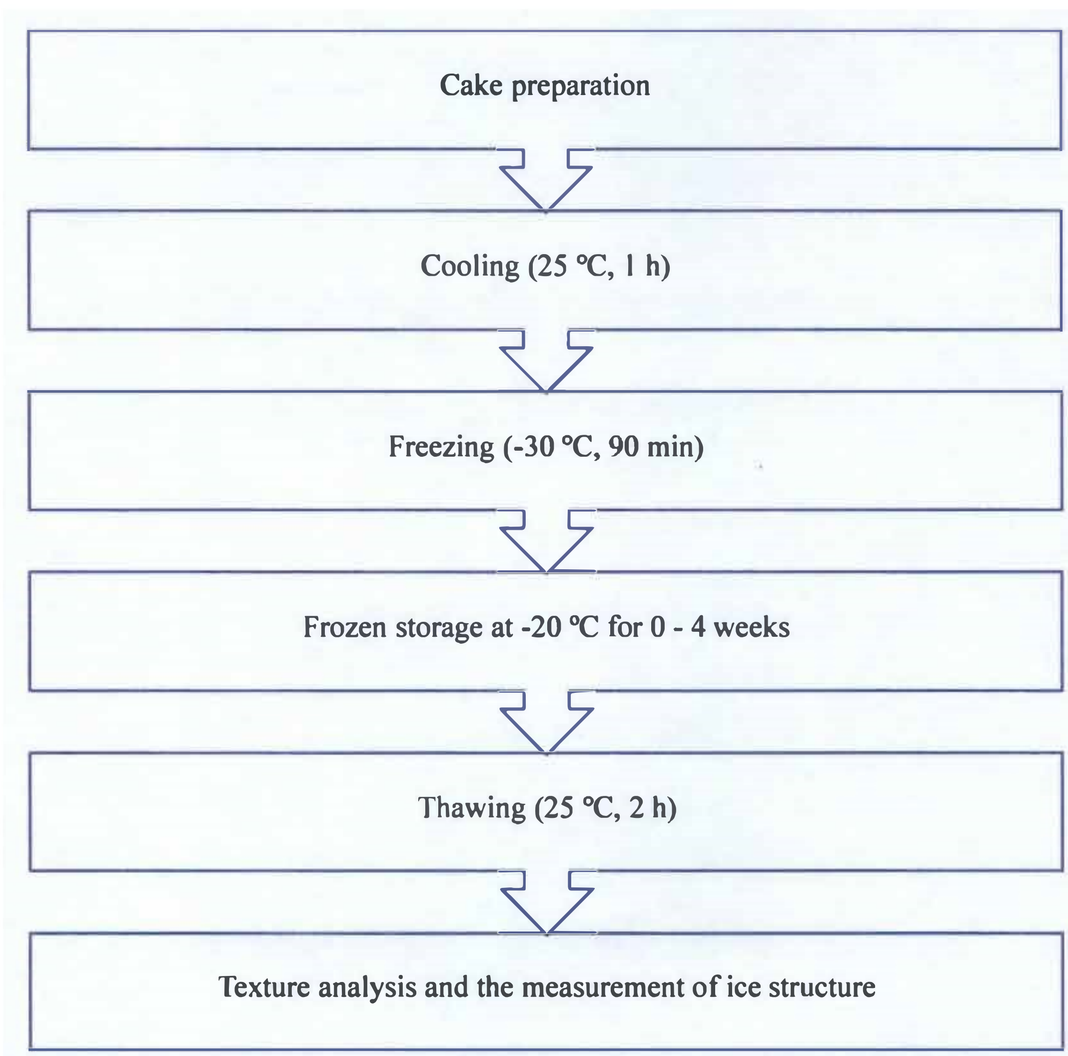



**Figure 3.2** Flow chart of the experimental steps in the study of "Frozen storage of gluten-free chiffon cake based on cassava flour"

### 3.5.1 Preparation of Frozen Gluten-Free Chiffon Cake

The ingredients for chiffon cake making were divided into 2 parts, meringue and batter. For meringue, egg white, sugar and cream of tar tar were mixed by hand mixer (250 W hand mixer, Russel hobbs, England), with middle speed for 15 min until firmed peak. Flours, sugar, baking powder, egg yolk, salt, oil, whipping cream (29% fat) and water were mix in a blow, pour meringue into blow then mixed with other ingredients by plastic spatula in from of batter. 55-gram of batter was poured in aluminum foil cup (ø 7.3 x 5.4 cm) and baked in an oven (FVC-D15A, Iris Ohyama, Japan) at 170 °C for 30 min. The cake samples without removing from the cup was cooled at room temperature for 1 h (Figure 3.3), and then the samples were frozen in an air blast freezer (Test 40 machine, MAYEKAWA MFG. Co., Ltd., Japan) at -30 °C for 90 min with the air flow of 2.0 m/s. Subsequently, the chiffon cake was immediately transferred to a refrigerator (model, company, country), and kept at -20 °C for 1, 7, 14, 21, and 28 days prior to the texture analysis and microstructure determination.

(a) (b) (c)

**Figure 3.3** Cake preparation starting with batter (a), after baking (b) and after cooling (c)

Commercial chiffon cakes (Figure 3.4) were brought from a local supermarket in, Tokyo, Japan. The commercial cake was packaged into plastic bag prior to freezing and frozen storage with the same protocol as the gluten-free samples.


**Figure 3.4** Commercial chiffon cake
(Source: Bunmeido, 2019)

### 3.5.2 Texture Analysis of Gluten-Free Chiffon Cake

Frozen chiffon cake was thawed at 25 °C for 1 h. before cutting for measurement evaluation. The textural properties of the cake were measured with a TA. XT Texture Analyzer (Stable Micro Systems, Godalming, U.K.) and the Texture Expert Exceed software for data analysis. Texture Profile Analysis (TPA) test was carried out using a P/40 cylindrical probe and a double compression test to compress cake to 50% of their original height at a speed of 2 mm/s. Measurements were carried out on two slices (20 mm thickness) taken from the center of each cake. The textural parameters considered were firmness (peak force of the first compression cycle, in N), cohesiveness (ratio of positive force area during the second compression to that during the first compression area, dimensionless), and springiness.

### 3.5.3 Microstructure Determination of The Gluten-Free Chiffon Cake by X-Ray CT

A Skyscan 1172 X-ray microcomputed tomography system (XrayCT, Bruker, Kontich, Belgium) was used to measure the microstructures of freeze-dried solids which reflected

pore size after freeze-thaw with respect to retrogradation properties of cake. Thawing cake cubes were cut into rectangular shapes 20 mm long and wide and 10 mm high.
The X-ray voltage and current were 54 kV and 100 μA, respectively. A CCD camera with 2000 x 1332 pixels was used to record the transmission of the conical X-ray beam through all samples. The distance source-object-camera was adjusted to produce images with a pixel size of 13.59 mm. Two frames averaging, a rotation step of 0.4 ° with 10 random movements and an exposure time of 1840 ms were chosen to cover a view of 180 contributed to the scan time of 60 min. Three-dimensional reconstruction of samples was created by stacking of two-dimensional tomographs from a total of 800-1000 slices with a slice spacing of 0.013 mm using the reconstruction NRecon software (Version 1.6.8.0, Bruker, Kontich, Belgium). The microstructures of cake which are pore volume, pore size, and pore count were analyzed by granulometry with CTAn software.

# CHAPTER 4 RESULTS AND DISCUSSIONS

## 4.1 Chemical Composition and Pasting Properties of Flour Samples

### 4.1.1 Chemical Compositions

Starch content in rice flour has a high amount (88.80%) followed by wheat flour Pirun 4 cassava flour and Hanatee cassava flour. Alamu, et al. (2017) reported that starch content of rice flour is between 67.1 to 82.4%. Wheat flour from Belgium in the study of Nindjin, et al. (2011) is 69.68%. Cassava flours in this study (Pirun 4 and Hanatee) have starch content is 73.65 and 68.31%. There are the differences between varieties and cultivar which affect starch content. Charoenkul, et al. (2011) and Chotineeranat, et al. (2006) reported starch content of Hanatee variety, Rayong variety and KU 50 variety are 82.41%, 81.38% and 91.10%, respectively (Table 4.1).

**Table 4.1** Chemical compositions of cassava, rice, and wheat flour

| Flours | Pirun 4 | Hanatee | Rice | Wheat |
|---|---|---|---|---|
| Starch | $73.65 \pm 2.0^{b}$ | $68.31 \pm 2.15^{c}$ | $88.80 \pm 1.69^{a}$ | $76.47 \pm 0.41^{b}$ |
| Lipid | $0.64 \pm 0.08^{a}$ | $0.41 \pm 0.03^{b}$ | $0.65 \pm 0.04^{a}$ | $0.74 \pm 0.06^{a}$ |
| Protein | $3.06 \pm 0.51^{c}$ | $3.67 \pm 0.35^{c}$ | $9.89 \pm 0.32^{b}$ | $21.84 \pm 0.56^{a}$ |
| Fiber | N/A | N/A | N/A | N/A |
| Ash | $2.92 \pm 0.06^{a}$ | $2.72 \pm 0.02^{b}$ | $0.33 \pm 0.04^{d}$ | $0.55 \pm 0.01^{c}$ |
| Amylose (g/100 g flour) | $33.45 \pm 0.54^{a}$ | $32.67 \pm 0.08^{b}$ | $28.02 \pm 0.61^{c}$ | $16.78 \pm 0.33^{d}$ |
| Cyanide content (mg/kg flour) | $23.71 \pm 2.23$ | $8.41 \pm 0.34$ | | |

Each value is the mean of three independent determination. Statistical analysis is determined in row. Values followed by the same letter in the same row are not significantly different, $p < 0.05$.

There are 3 groups of protein content is flours which are protein in wheat flour, rice flour and cassava flours (Pirun 4 and Hanatee). Wheat flour has high protein content (21.84%) because it contains gliadin and glutenin (gluten). Cardoso, et al. (2019) reported protein content in wheat flour was 13.2 ± 0.8 %. Protein content in rice flour is 9.89%. Varavinit, et al. (2003) indicated protein content from different varieties in the range of 7.61 and 13.19%. On the other hand, protein content in cassava flours is between 3 to 3.67%. There are on significantly different between Pirun 4 and Hanatee. Charoenkul, et al. (2011) and Chotineeranat, et al. (2006) reported that protein content on both studied were between 0.8 to 1.8%. Moreover, lipid content in this study which came from wheat flour rice flour and Pirun 4 cassava flour are not significantly different ($p < 0.05$) except Hanatee cassava flour. The others study (Charoenkul, et al., 2011; Chotineeranat, et al., 2006; Cardoso, et al., 2019) reported that there are 0.04-0.34% lipid in cassava flours and approximate 0.3-0.4 % lipid in rice flours while lipid in wheat flour is 0.92 ± 0.02 %, respectively. These compositions, especially for protein and lipid contents, may affect the retrogradation properties of cassava flour prior to the content in flour.

### 4.1.2 Pasting Properties

There are 2 interesting parameters which are resulted in retrogradation properties (peak viscosity and setback viscosity). Peak viscosity accounts for the water-binding capacity of flour gel. It is often related to final product quality, and also be an indicator of viscous load likely to be encountered by mixing (Alamu, et al., 2017). Peak viscosity is an important property of flour for flour or starch industries, usually focusing on high peak viscosity of starch (Sriroth and Piyachomkwan, 2007). On the other hand, the setback is the gel set of the cooked Rapid Visco Analyser (RVA) sample then recrystallization of the starch in the sample. RVA graph in RVU unit of Pirun 4 cassava flour, Hanatee cassava flour, commercial rice flour and commercial wheat flour were presented in Figure 4.1.

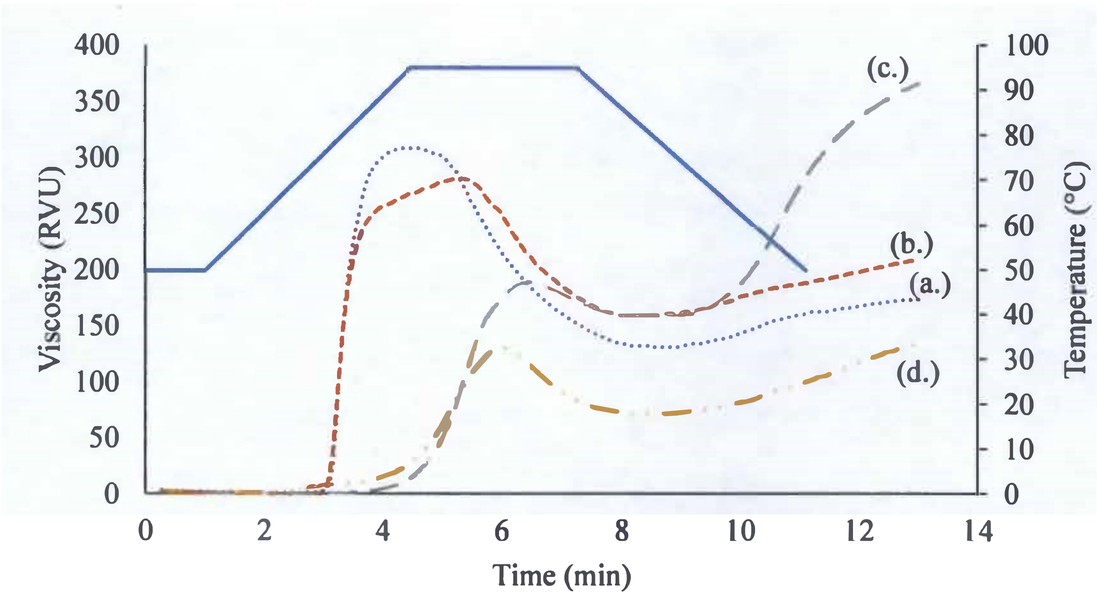



**Figure 4.1** Rapid visco amylograph of (a.) Pirun 4 cassava flour, (b.) Hanatee cassava flour, (c.) commercial rice flour and (d.) commercial wheat flour

Table 4.2 demonstrated that peak viscosity of Pirun 4, Hanatee, commercial rice and commercial wheat flours were 309.79 ± 1.36, 283.29 ± 3.24, 189.75 ± 4.95 and 135.67 ± 0.12 RVU, respectively. Peak viscosity has strongly relative with flour or starch properties in terms of water blinding capacity According to peak viscosity value, Pirun 4 was the high ability of water binding capacity followed by Hanatee cassava flour, rice flour and wheat flour. The results from Chotineeranat, et al., 2006, Dudu, et al., 2018 and Jensen, et al., 2014 indicated that Peak viscosity of cassava flour was in the range of 161.17 to 296 RVU. Moreover, there is validation between cultivar of cassava prior to chemical compositions of flour. Therefore, the difference of chemical compositions are affected by the difference of pasting viscosity.

**Table 4.2** Pasting properties of cassava, rice, and wheat flour

| Flour types | Peak viscosity (RVU) | Trough viscosity (RVU) | Breakdown (RVU) | Final Viscosity (RVU) | Setback (RVU) | Pasting Temperature (°C) |
|---|---|---|---|---|---|---|
| Cassava flour | | | | | | |
| Pirun 4 | $309.79 \pm 1.36^{a}$ | $131.13 \pm 0.42^{b}$ | $178.67 \pm 0.94^{a}$ | $174.58 \pm 2.47^{c}$ | $43.46 \pm 2.06^{c}$ | $73.60 \pm 0.49^{c}$ |
| Hanatee | $283.29 \pm 3.24^{b}$ | $159.08 \pm 10.61^{a}$ | $124.21 \pm 7.37^{b}$ | $209.17 \pm 7.42^{b}$ | $50.08 \pm 3.18^{c}$ | $73.98 \pm 1.17^{b}$ |
| Commercial rice flour | $189.75 \pm 4.95^{c}$ | $158.46 \pm 2.83^{a}$ | $31.29 \pm 1.12^{c}$ | $365.50 \pm 0.95^{a}$ | $207.05 \pm 4.77^{a}$ | $89.08 \pm 1.10^{a}$ |
| Commercial wheat flour | $135.67 \pm 0.12^{d}$ | $101.96 \pm 0.30^{c}$ | $33.71 \pm 0.41^{c}$ | $187.30 \pm 1.59^{c}$ | $85.34 \pm 1.89^{b}$ | $87.13 \pm 1.24^{a}$ |

Each value is the mean of two independent determination. Statistical analysis is determined in row. Values followed by the same letter in the same column are not significantly different, $p < 0.05$

Setback viscosity value was calculated from the difference between the final and peak viscosity. The high setback has been associated with a high degree of starch restructuring or retrogradation. Table 4.2 shows that the highest setback viscosity was rice flour (207.05 RVU), wheat flour (85.34 RVU), Hanatee cassava flour (50.08 RVU) and Pirun 4 cassava flour (43.46 RVU), respectively. The high amount of setback viscosity implies high retrogradation thus rice flour has a high degree of retrogradation and cassava flours (Pirun 4 and Hanatee) has a low degree of retrogradation. Chotineeranat, et al. (2006), Dudu, et al. (2018) and Jensen, et al. (2014) reported setback viscosity of cassava flour from different varieties between 19 – 44 RVU. This was because of the cultivar and chemical composition of cassava. According to Kim and Seib (1993), flour which has high amylose also tends to has high viscosity after cooling (setback viscosity). However, rice flour has low amylose content and present high setback viscosity. This because some lipids in rice flour react with amylose then form an amylose-lipid complex that promoted high viscosity after cooling. Therefore, variation in the paste characteristics of flour might affect the quality of resulting products.

## 4.2 Syneresis and Textural Properties of Cassava Flour Gels During Frozen Storage

Flour gel samples (the initial concentration of flour suspension was 20% w/w flour on wet basis) were subjected to the texture and syneresis measurements. This concentration of flour gel was governed in accordance with the percentage of flour used in the gluten-free chiffon cake samples (approximately to 17%); even though, 8–11% w/w starch/flour was generally used as the samples in the previous studies (Charoenrein and Preechathammawong, 2012; Katekhong and Charoenrein, 2012; Phimolsiripol, et al., 2011; Muadklay and Charoenrein, 2008). In this study, the gel model was prepared with a 20% suspension and kept in frozen storage (-20 °C). There are 3 parameters with respect to retrogradation properties, firmness, cohesiveness and percent syneresis.

### 4.2.1 Syneresis Measurement

Syneresis is an overview of the flour gel model system to study the retrogradation properties. This occurs in part of weeping out of water because of recrystallization in a starch chain (Morris, 1990). Moreover, syneresis parameter can be described in terms of the ability of starch with physical changes during freezing and thawing.

Syneresis was determined for unfrozen and frozen part. Pirun 4 cassava flour gel has the lowest percent syneresis (11.44 ± 0.23) in unfrozen storage followed by commercial wheat flour gel, Hanatee cassava flour gel and rice flour gel. In terms of frozen storage, Pirun 4 and Hanatee cassava flour gel have a tendency to stop retrogradation in 14 days while commercial rice flour and commercial wheat flour presented in the increasing of retrogradation. These results were comparable to setback viscosity which were rice flour was the lower ability of water holding capacity while Pirun 4 cassava flour provided high ability.

Percentage of syneresis in terms of cassava flours indicated in different results. Hanatee cassava flour has a higher syneresis value (21.33 ± 3.36) than Pirun 4 cassava flour. This was because of the variation of starch content; starch in Pirun 4 and Hanatee cassava flours are 73.65 ± 2.0 and 68.31 ± 2.15, respectively (Chotineeranat, et al., 2006). However, the result of statistical analysis indicated that there are no significantly different in syneresis during frozen storage in 14 to 28 days ($p < 0.05$) for all treatment. In addition, percent syneresis of all treatment was comparable to peak viscosity result-cassava flour present high ability of water holding capacity.

To summarize, Pirun 4 cassava flour resulted in lower syneresis which implied for high water-holding ability when comparing to those flours. This was because of less change during frozen storage while Hanatee cassava flour has higher syneresis value in unfrozen storage but remain constant during frozen storage.

**Table 4.3** The percentage syneresis of frozen flour gels (20% w/w) after a storage at -20 °C for 1 – 28 days, comparing with unfrozen flour gel

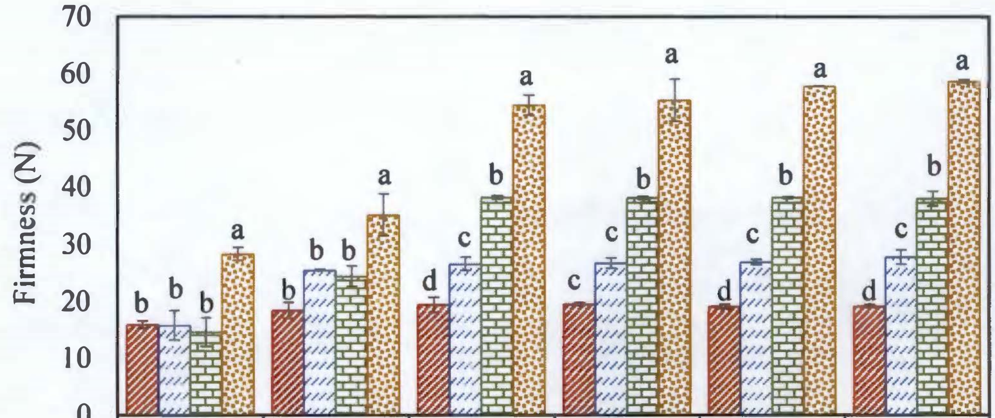
| Samples | Syneresis (%) | | | | | |
|---|---|---|---|---|---|---|
| | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Cassava flour | | | | | | |
| Pirun 4 | $11.44 \pm 0.23^{c}$ | $25.72 \pm 0.48^{b}$ | $31.78 \pm 0.63^{b}$ | $34.30 \pm 0.66^{a}$ | $36.05 \pm 3.63^{a}$ | $37.31 \pm 0.66^{a}$ |
| Hanatee | $21.33 \pm 3.36^{ab}$ | $32.07 \pm 0.48^{a}$ | $34.42 \pm 0.39^{ab}$ | $34.43 \pm 0.21^{a}$ | $34.57 \pm 0.32^{a}$ | $35.03 \pm 1.44^{a}$ |
| Commercial rice flour | $27.04 \pm 1.44^{a}$ | $27.42 \pm 0.70^{b}$ | $38.08 \pm 1.54^{a}$ | $38.35 \pm 2.65^{a}$ | $39.89 \pm 2.36^{a}$ | $39.82 \pm 3.00^{a}$ |
| Commercial wheat flour | $17.00 \pm 1.09^{bc}$ | $30.07 \pm 0.62^{a}$ | $30.94 \pm 1.30^{b}$ | $33.97 \pm 0.17^{a}$ | $37.05 \pm 1.58^{a}$ | $39.33 \pm 0.22^{a}$ |

Each value is the mean of three independent determination. Statistical analysis is determined in column ($p < 0.05$).

### 4.2.2 Texture Analysis of Flour Gels

Textural property parameters in this study which related to retrogradation properties are firmness (N) and cohesiveness. Firmness value represents in first force that presses to gel. Cohesiveness implies for Cohesion of the food or the internal structure of food structure. Retrogradation present in gel model the firmness increased because of restructuring of the starch chain while cohesiveness decreased.

The textural properties in terms of firmness (N) in unfrozen and frozen storage were conducted, and the results were shown in Figure 4.2. For unfrozen storage, Pirun 4 cassava flour, Hanatee cassava flour firmness were comparable which are 15.90 ± 0.62 N and 15.73 ± 2.57 N, respectively while commercial rice flour provided lower firmness than those cassava flours (14.60 ± 2.50 N). On the other hand, firmness value of wheat flour was the highest firmness which was 20.30 N. In case of frozen storage, result of Pirun 4 cassava flour gel firmness value presented in slightly increased in 1 day storage and remain constant from 7 days (19.33 ± 1.32 N) until 28 days (19.09 ± 0.29 N). Hanatee cassava flour and rice flour firmness value increased in 1 day while the result in 7 days until 28 days was comparable to Pirun 4 cassava flour. In addition, wheat flour firmness value was dramatically increased in 7 days frozen storage (from 35.16 ± 3.64 N to 54.44 ± 1.74 N) and provide increased trend until 28 days. According to statistical analysis, cassava flour from different varieties effect to firmness during frozen storage.

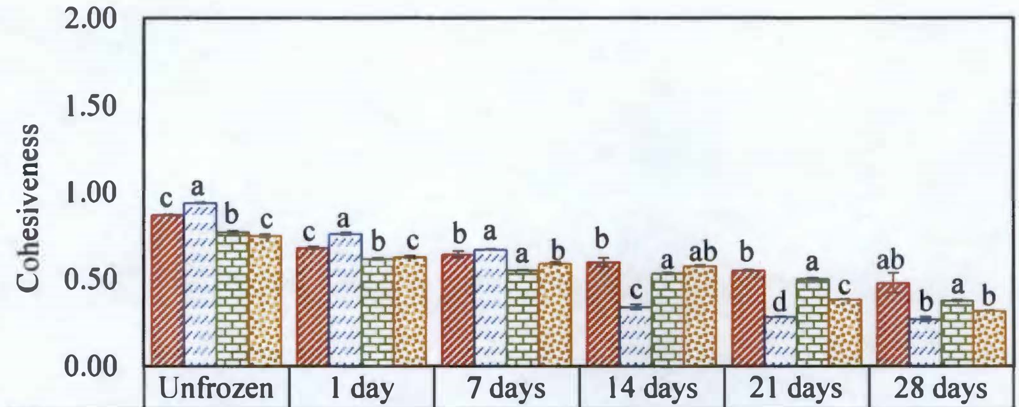



| | Unfrozen | 1 day | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|---|---|
| Pirun 4 | 15.90 ± 0.62 | 18.35 ± 1.37 | 19.33 ± 1.32 | 19.42 ± 0.28 | 19.02 ± 0.36 | 19.09 ± 0.29 |
| Hanatee | 15.73 ± 2.57 | 25.42 ± 0.13 | 26.52 ± 1.18 | 26.67 ± 0.90 | 26.85 ± 0.46 | 27.76 ± 1.29 |
| Rice | 14.60 ± 2.50 | 24.33 ± 1.82 | 38.41 ± 0.27 | 38.09 ± 0.22 | 38.12 ± 0.13 | 38.00 ± 1.27 |
| Wheat | 28.30 ± 1.15 | 35.16 ± 3.64 | 54.44 ± 1.74 | 55.32 ± 3.69 | 57.75 ± 0.05 | 58.62 ± 0.30 |

**Figure 4.2** Firmness of Pirun 4 cassava flour gel, Hanatee cassava flour gel, commercial rice flour gel and commercial wheat flour gel with unfrozen storage and frozen storage (-20 °C) for 28 days (Statistical analysis is determined in column, $p < 0.05$)

Cohesiveness presents the texture of food structure ability to holding in structure. Figure 4.3 represents the cohesiveness of the flours gel system. Hanatee cassava gel cohesiveness value was the highest during unfrozen storage (0.94 ± 0.00) followed by Pirun 4 cassava gel (0.87 ± 0.00), rice gel (0.77 ± 0.01) and wheat gel (0.75 ± 0.01), respectively. In frozen storage, Hanatee rice flour cohesiveness value resulted in higher cohesiveness in 1 day and rapidly decreased during frozen storage while Pirun 4 cassava flour slightly decreased during frozen storage. Last day of frozen storage (28 days), the trend of cohesiveness value of flour gels from high to low order were Pirun 4 cassava gel, rice gel, wheat gel and Hanatee cassava gel.

Cohesiveness can be implied for the recrystallization of starch structure. In frozen storage, there are some water loss and present in term of retrogradation thus amylose and amylopectin play role to hold water in their structure especially for amylopectin (Yang, et al., 2017). Therefore, gel structure shrinkage which was comparable to setback viscosity-Pirun 4 cassava flour has the lower degree of recrystallization.




| | Unfrozen | 1 day | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|---|---|
| Pirun 4 | 0.87 ± 0.00 | 0.68 ± 0.01 | 0.64 ± 0.02 | 0.60 ± 0.03 | 0.55 ± 0.00 | 0.48 ± 0.06 |
| Hanatee | 0.94 ± 0.00 | 0.76 ± 0.01 | 0.67 ± 0.00 | 0.34 ± 0.02 | 0.28 ± 0.00 | 0.27 ± 0.01 |
| Rice | 0.77 ± 0.01 | 0.62 ± 0.01 | 0.55 ± 0.00 | 0.53 ± 0.01 | 0.50 ± 0.01 | 0.38 ± 0.00 |
| Wheat | 0.75 ± 0.01 | 0.63 ± 0.01 | 0.59 ± 0.01 | 0.58 ± 0.00 | 0.38 ± 0.00 | 0.32 ± 0.00 |

**Figure 4.3** Cohesiveness of Pirun 4 cassava flour gel, Hanatee cassava flour gel, commercial rice flour gel and commercial wheat flour gel with unfrozen storage and frozen storage (-20 °C) for 28 days (Statistical analysis is determined in column, $p < 0.05$)

In the flour gel model system, retrogradation properties faster in linear amylose and slow in amylopectin. However, linear amylose is easy to interact with lipid and form amylose-lipid complex thus recrystallization of amylose to present less of starch retrograde than amylopectin chain. According to the study of Katekhong and Charoenrein, (2012), an increase in amylose formation due to flour help to retard retrogradation during the storage of rice gel. High amylose concentration in the regions facilitates the starch chains to associate forming thick filaments, whereas water molecules aggregate into ice crystals forming a separate phase. Therefore, cassava flours present low retrogradation during frozen storage this may mean that retrogradation stop after 7 days storage.

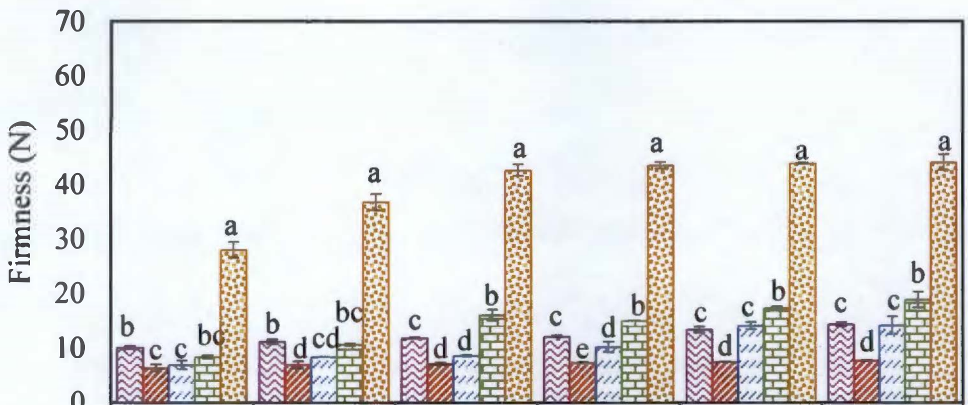
In the study of syneresis and textural properties of cassava flour gels during frozen storage with commercial flours (rice flour and wheat flour), the result shows that cassava flour which is Pirun 4 has a trend to retard retrogradation according to low syneresis percentage, low change of firmness and high cohesiveness.

## 4.3 Frozen Storage of Gluten-Free Chiffon Cake Based on Cassava Flour

All previous results account for flour properties and gel modeling to evaluate the trending of retrogradation retarding. In this study, gluten-free chiffon cake was selected as a commercial product to confirm the result that cassava flours (Pirun 4 and Hanatee) can be used as a wheat flour replacement. Parameters in this section were determined from x-ray CT scan and textural properties analysis. Cakes from 2-type of cassava flours, commercial rice flour and commercial wheat flour were baked, kept in frozen storage (-20 °C) for 28 days and were assayed with mentioned methods. Finally, all of the result was compared with commercial cake for both unfrozen and frozen storage.

### 4.3.1 Texture Analysis of Gluten-Free Chiffon Cake

Firmness is the one parameter that accounts for the loss of moisture in the cake layer or call cake staling (retrogradation in cake). Figure 4.4 represents the firmness of cakes in unfrozen and frozen storage. Firmness of commercial chiffon cake in unfrozen storage was 10.17 ± 0.28 N while wheat chiffon cake was 28.04 ± 1.47 N. Cassava chiffon cake from Pirun 4 and Hanatee cassava flours were 6.39 ± 0.67 N and 6.95 ± 0.82 N thus, cassava chiffon cake texture might softer than commercial cake. However, rice chiffon cake firmness value was comparable to commercial cake than other flours. In part of frozen storage chiffon cakes, Pirun 4 cassava chiffon cake indicated a few changes of firmness value (between 6.39 ± 0.67 N to 7.75 ± 0.08 N). In statistical analysis, cassava flour from different varieties effect to firmness during frozen storage.

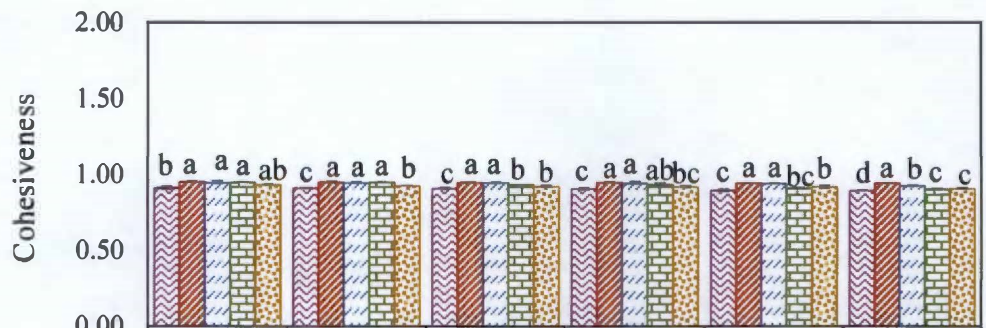



| | Unfrozen | 1 day | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|---|---|
| Commercial | 10.17 ± 0.28 | 11.22 ± 0.44 | 11.93 ± 0.16 | 12.22 ± 0.21 | 13.43 ± 0.58 | 14.41 ± 0.36 |
| Pirun4 | 6.39 ± 0.67 | 6.98 ± 0.64 | 7.15 ± 0.25 | 7.39 ± 0.13 | 7.51 ± 0.06 | 7.75 ± 0.08 |
| Hanatee | 6.95 ± 0.82 | 8.42 ± 0.03 | 8.67 ± 0.11 | 10.29 ± 0.95 | 14.18 ± 0.66 | 14.22 ± 1.61 |
| Rice | 8.43 ± 0.29 | 10.67 ± 0.20 | 16.08 ± 1.03 | 15.09 ± 0.03 | 17.34 ± 0.32 | 18.91 ± 1.44 |
| Wheat | 28.04 ± 1.47 | 36.80 ± 1.53 | 42.70 ± 1.05 | 43.56 ± 0.59 | 43.90 ± 0.07 | 44.10 ± 1.44 |

**Figure 4.4** Firmness of commercial chiffon cake, Pirun 4 cassava chiffon cake, Hanatee cassava chiffon cake, rice chiffon cake and wheat chiffon cake with unfrozen storage and frozen storage (-20 °C) for 28 days (Statistical analysis is determined in column, $p<0.05$)

Cohesiveness is used to describe the texture that indicates the cohesion of the food or the internal structure of the food structure. Pirun 4 cassava cake, Hanatee cassava cake and rice cake provided high cohesiveness in unfrozen storage (0.95 ± 0.01) except wheat cake (0.93 ± 0.00) and commercial cake (0.91 ± 0.01). Cohesiveness value of Hanatee cassava cake did not change during frozen storage. Since Pirun 4 cassava cake has high cohesiveness and remain constant during frozen storage (0.91 ± 0.01 to 0.89 ± 0.00) while those cakes resulted in slightly decreased especially for rice cake (0.95 ± 0.00 to 0.90 ± 0.00).

In the study of Gomez, freezing more affected to sponge cakes. This indicating that the effect of processing on volume produced greater changes in texture. In sponge cakes, firmness and cohesiveness were the greater parameters to present result of retrogradation. From the study of Witczak, et al. (2019), these changes are mainly caused by the migration of water from crumb to crust. The firmness of cake relates to starch retrogradation which is caused by water retention or starch recrystallization.




| | Unfrozen | 1 day | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|---|---|
| Commercial | 0.91 ± 0.01 | 0.91 ± 0.00 | 0.91 ± 0.00 | 0.90 ± 0.01 | 0.90 ± 0.01 | 0.89 ± 0.00 |
| Pirun 4 | 0.95 ± 0.00 | 0.95 ± 0.00 | 0.95 ± 0.00 | 0.95 ± 0.00 | 0.94 ± 0.00 | 0.94 ± 0.00 |
| Hanatee | 0.95 ± 0.01 | 0.94 ± 0.00 | 0.94 ± 0.00 | 0.94 ± 0.01 | 0.94 ± 0.00 | 0.94 ± 0.00 |
| Rice | 0.95 ± 0.00 | 0.95 ± 0.00 | 0.93 ± 0.00 | 0.93 ± 0.01 | 0.91 ± 0.00 | 0.90 ± 0.00 |
| Wheat | 0.93 ± 0.00 | 0.92 ± 0.00 | 0.92 ± 0.01 | 0.92 ± 0.00 | 0.92 ± 0.01 | 0.91 ± 0.00 |

**Figure 4.5** Cohesiveness of commercial chiffon cake, Pirun 4 cassava chiffon cake, Hanatee cassava chiffon cake, rice chiffon cake and wheat chiffon cake with unfrozen storage and frozen storage (-20 °C) for 28 days (Statistical analysis is determined in column, $p<0.05$)

According to the textural attribute of chiffon cake, Pirun 4 cassava chiffon cake present low retrogradation during frozen storage or this might summarize that Pirun 4 cassava flour can help to retard retrogradation.

Textural properties result indicated in terms of mouthfeel for the customer. Another parameter is appearance which is conducted by X-ray CT scan method.

### 4.3.2 Microstructure Determination of The Gluten-Free Chiffon Cake

In frozen storage of cake, there is ice crystal which is slightly grown in a pore of cake and damage the cake layers in frozen storage. After thawing, the layer of cake is destroyed. The pore of cake collapses and sticks together. To confirm this reason, x-ray CT scan measuring machine was used to evaluate pore count and cake volume.

**Table 4.4** Two directional image of commercial chiffon cake, Pirun 4 cassava chiffon cake, Hanatee cassava chiffon cake, rice chiffon cake and wheat chiffon cake in unfrozen and frozen storage

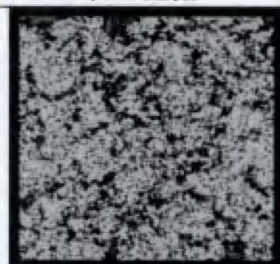
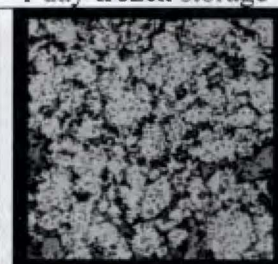
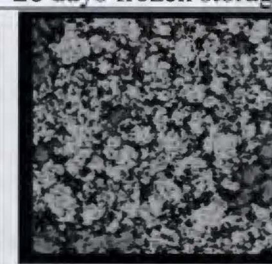
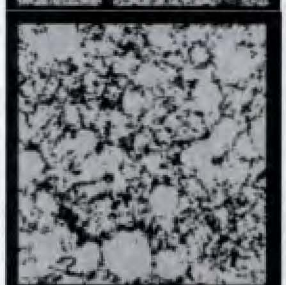
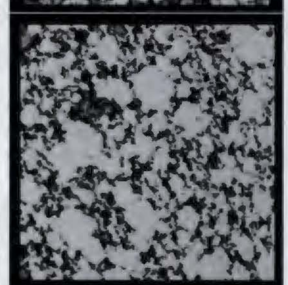
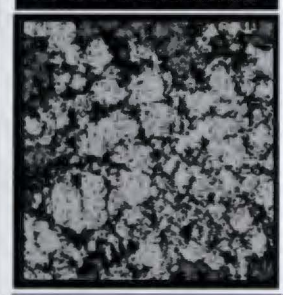
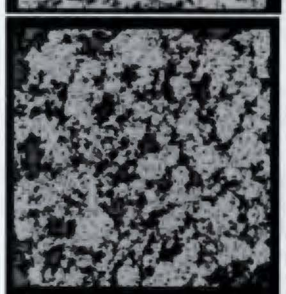
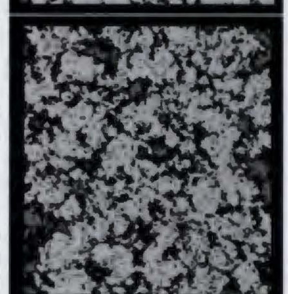
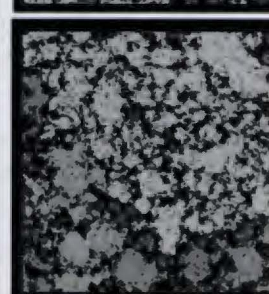
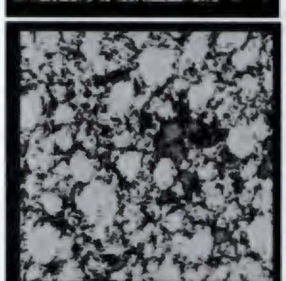
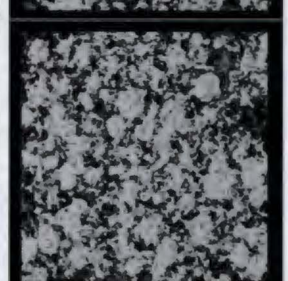
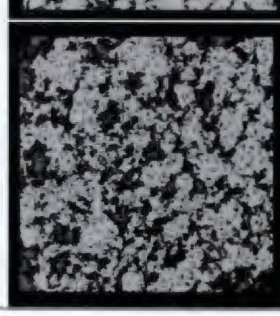
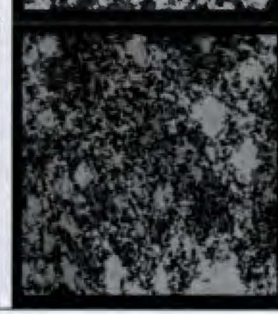
| Chiffon cake | Unfrozen | 1 day frozen storage | 28 days frozen storage |
|---|---|---|---|
| Commercial |  |  |  |
| Pirun 4 cassava |  |  |  |
| Hanatee cassava |  |  |  |
| Rice |  |  |  |
| Wheat |  |  |  |

Table 4.4 shows the 2-D image of chiffon cake from a commercial product, Pirun 4 cassava flour, Hanatee cassava flour, rice flour and wheat flour in unfrozen, 1 day frozen storage and 28 days frozen storage. White or light grey colors represent pore or air cell of cake, black or dark gray colors represents a layer of cake. The result indicated that Pirun 4 cassava cake image presented a larger size of air cell than other cakes in unfrozen storage. In 28-day frozen storage, wheat flour present high area of dark color thus, wheat cake layer collapsed during frozen storage.

The number of pores (Table 4.5) from a commercial cake (52.95 ± 1.57) was highest followed by Hanatee cassava cake (44.68 ± 3.73), wheat cake (44.13 ± 4.71), Pirun 4 cassava cake (29.73 ± 1.00) and rice cake (20.56 ± 0.72) for unfrozen storage. After 1 day frozen storage, number of pores from commercial cake, Hanatee cassava cake and wheat cake rapidly decreased to 33.57 ± 2.04, 22.00 ± 1.76 and 34.16 ± 3.94, respectively. Pirun 4 cassava cake pores decreased in 1 day to 14 days and remain constant among other cakes provide the trend to decrease during frozen storage. Since the number of pores decreased, it affected a layer of cake. All of the cake volume in this study presented in decreasing trend except Pirun 4 cassava cake. According to statistical analysis, pores of commercial was significantly different during frozen storage. In the last 7 days of frozen storage, frozen storage did not affect to pores in most of treatment except commercial cake.

There is a relation between pore count and cake volume (Table 4.6) in frozen storage. In this study, the pore can be the space of ice crystal growth. According to Zhao and Takhanr (2017), a reduction in the number of pore count is also observed due to the merger of smaller crystals to larger crystals in long time frozen storage. The effect of the study variables on cake was reported in the study of Gómez, et al (2011). Freezing led to a decrease in the volume and height of both layer and sponge cakes thus cake shape was flatter. Despite the appearance of cake changed during storage, results did not significantly affect textural properties.

Finally, Pirun 4 cassava cake present less change of firmness, cohesiveness, pore count and cake volume than those cakes thus retrogradation in this cake was low. Moreover, retrogradation in Pirun 4 cassava cake show the trend to stop during frozen storage when comparing to commercial cake.

**Table 4.5** Pore count of commercial chiffon cake, Pirun 4 cassava chiffon cake, Hanatee cassava chiffon cake, rice chiffon cake and wheat chiffon cake in unfrozen and frozen storage (-20 °C)

| Cake | Unfrozen | 1 day | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|---|---|
| Commercial | 52.95 ± 1.57$^{a}$ | 33.57 ± 2.04$^{a}$ | 30.57 ± 0.68$^{a}$ | 28.29 ± 0.80$^{a}$ | 25.87 ± 1.16$^{a}$ | 23.12 ± 1.31$^{a}$ |
| Pirun 4 cassava | 29.73 ± 1.00$^{b}$ | 23.56 ± 0.07$^{b}$ | 17.04 ± 3.30$^{b}$ | 8.41 ± 1.84$^{c}$ | 8.17 ± 0.77$^{b}$ | 6.56 ± 1.25$^{b}$ |
| Hanatee cassava | 44.68 ± 3.73$^{a}$ | 22.00 ± 1.76$^{b}$ | 18.26 ± 0.09$^{b}$ | 9.27 ± 0.24$^{b}$ | 9.03 ± 1.91$^{b}$ | 7.02 ± 0.59$^{b}$ |
| Rice | 20.56 ± 0.72$^{b}$ | 17.95 ± 2.50$^{b}$ | 10.68 ± 0.44$^{c}$ | 9.27 ± 0.24$^{c}$ | 7.03 ± 0.29$^{b}$ | 4.71 ± 0.32$^{b}$ |
| Wheat | 44.13 ± 4.71$^{a}$ | 34.16 ± 3.94$^{a}$ | 31.20 ± 0.77$^{a}$ | 28.85 ± 0.06$^{a}$ | 7.62 ± 0.39$^{b}$ | 5.71 ± 1.39$^{b}$ |

Each value is the mean of two independent determination. Pore count and cake volume are independent determination. Statistical analysis is determined in column, $p < 0.05$.

**Table 4.6** Cake volume of commercial chiffon cake, Pirun 4 cassava chiffon cake, Hanatee cassava chiffon cake, rice chiffon cake and wheat chiffon cake in unfrozen and frozen storage (-20 °C)

| Cake | Unfrozen | 1 day | 7 days | 14 days | 21 days | 28 days |
|---|---|---|---|---|---|---|
| Commercial | $2.03 \pm 0.00^{a}$ | $2.03 \pm 0.00^{a}$ | $2.03 \pm 0.00^{a}$ | $1.89 \pm 0.02^{a}$ | $1.82 \pm 0.03^{a}$ | $1.73 \pm 0.05^{a}$ |
| Pirun 4 cassava | $1.79 \pm 0.18^{a}$ | $1.61 \pm 0.00^{b}$ | $1.59 \pm 0.01^{c}$ | $1.56 \pm 0.02^{b}$ | $1.47 \pm 0.02^{bc}$ | $1.30 \pm 0.07^{b}$ |
| Hanatee cassava | $1.79 \pm 0.01^{a}$ | $1.67 \pm 0.15^{b}$ | $1.54 \pm 0.03^{d}$ | $1.47 \pm 0.02^{b}$ | $1.32 \pm 0.10^{cd}$ | $1.20 \pm 0.01^{bc}$ |
| Rice | $1.78 \pm 0.04^{a}$ | $1.70 \pm 0.01^{b}$ | $1.67 \pm 0.02^{b}$ | $1.57 \pm 0.01^{b}$ | $1.49 \pm 0.04^{b}$ | $1.24 \pm 0.14^{bc}$ |
| Wheat | $1.75 \pm 0.08^{a}$ | $1.67 \pm 0.03^{b}$ | $1.41 \pm 0.00^{e}$ | $1.31 \pm 0.05^{c}$ | $1.19 \pm 0.06^{c}$ | $0.92 \pm 1.44^{c}$ |

Each value is the mean of two independent determination. Pore count and cake volume are independent determination. Statistical analysis is determined in column, $p < 0.05$.

# CHAPTER 5 CONCLUSION AND SUGGESTION

## 5.1 Conclusion

Firstly, there is a variety of flour which affected to flours composition and pasting properties. Pirun 4 cassava flour resulted in specific composition (amylose and amylopectin content) and pasting properties (high peak viscosity and low setback) that could retard retrogradation properties.

Secondly, Pirun 4 cassava flour presented low syneresis which implied high water-holding ability while Hanatee cassava flour presented high syneresis in unfrozen storage but still constant during frozen storage. In the study, textural properties of cassava flour gels during frozen storage result showed that cassava flour which was Pirun 4 has the trend to retard retrogradation according to low change of firmness and high cohesiveness in the final date.

Thirdly, Pirun 4 cassava cake presented less change of firmness, cohesiveness, pore count and cake volume which accounted for ability to retard retrogradation in bakery product.

Finally, Pirun 4 cassava flour cake resulted in high ability to retard retrogradation and could be used as a wheat flour replacement in bakery product.

## 5.2 Suggestion

In order to, confirm the effect of cassava flour replacement, the lipid, protein and amylose content should be varied to determine their effect on gel and bakery product during frozen storage.

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## APPENDIX A

Chemical Composition and Pasting Properties of Flour Samples

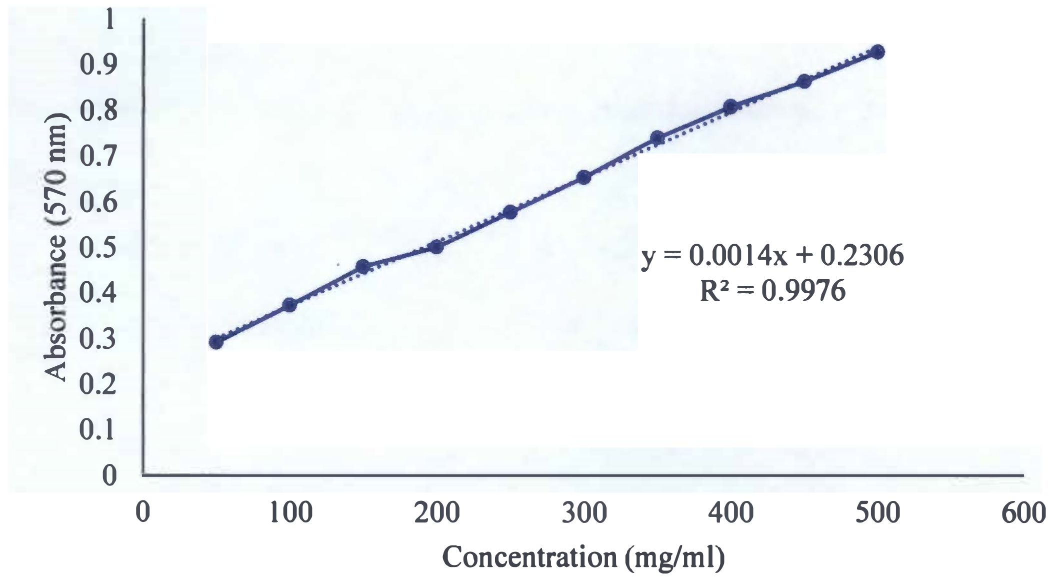


**Figure A.1** Calibration curve of cassava flour

**Table A.1** Starch content (dry basis) in Pirun 4 cassava flour and Hanatee cassava flour

| Flour | Replication | Absorbance | Concentration (mg/ml) | Starch content |
|---|---|---|---|---|
| | 1 | 0.551 | 228.86 | 71.35 |
| Pirun 4 | 2 | 0.567 | 240.29 | 74.92 |
| | 3 | 0.566 | 239.57 | 74.69 |
| | 1 | 0.517 | 204.57 | 68.15 |
| Hanatee | 2 | 0.509 | 198.86 | 66.25 |
| | 3 | 0.527 | 211.71 | 70.53 |

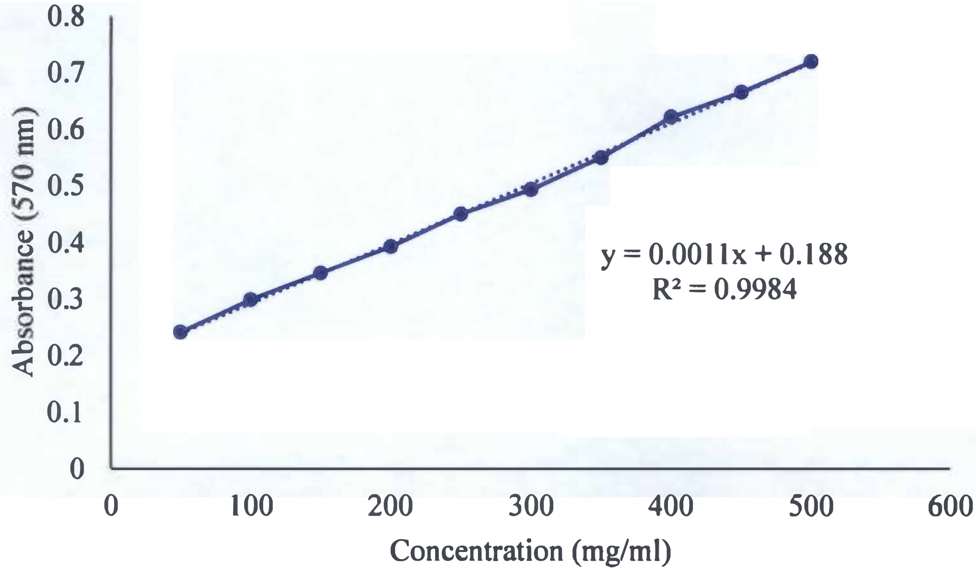


**Figure A.2** Calibration curve of rice flour

**Table A.2** Starch content (dry basis) in rice flour

| Flour | Replication | Absorbance | Concentration (mg/ml) | Starch content |
|---|---|---|---|---|
| Rice | 1 | 0.498 | 281.82 | 90.76 |
| | 2 | 0.488 | 272.73 | 87.83 |
| | 3 | 0.488 | 272.73 | 87.83 |

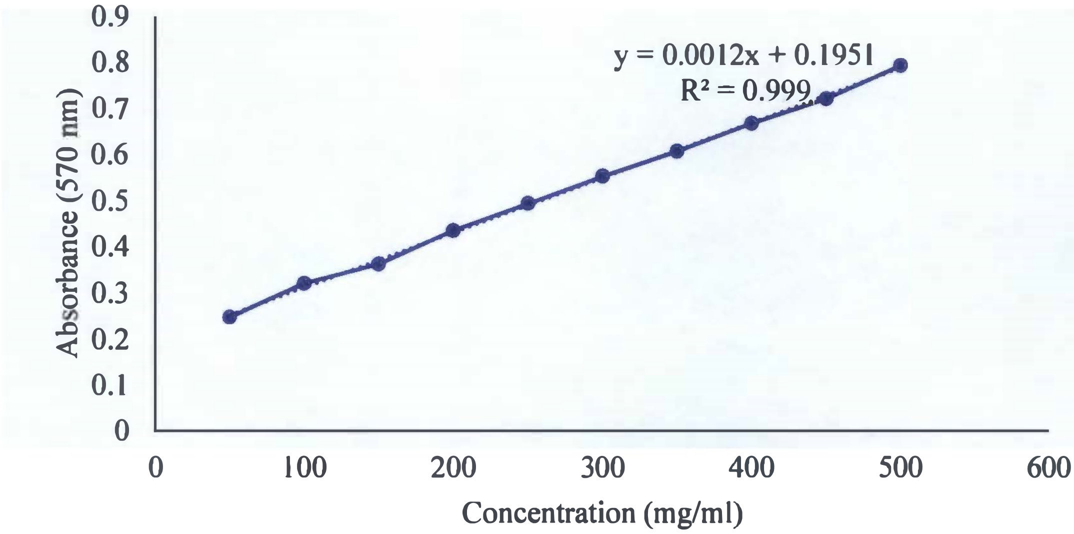


**Figure A.3** Calibration curve of wheat flour

**Table A.3** Starch content (dry basis) in wheat flour

| Flour | Replication | Absorbance | Concentration (mg/ml) | Starch content |
|---|---|---|---|---|
| | 1 | 0.450 | 237.42 | 76.39 |
| Wheat | 2 | 0.449 | 236.58 | 76.12 |
| | 3 | 0.452 | 239.08 | 76.93 |

**Table A.4** Lipid content (dry basis) of Pirun 4 cassava flour, Hanatee cassava flour, rice flour and wheat flour

| | | Before extract | | After extract | | |
|---|---|---|---|---|---|---|
| Flour | Replication | Empty can (g) | Sample (g) | Sample (g) | Can (g) | Lipid |
| Pirun 4 | 1 | 25.21 | 3.17 | 2.91 | 25.23 | 0.71 |
| | 2 | 25.75 | 3.15 | 2.89 | 25.77 | 0.68 |
| | 3 | 24.80 | 3.40 | 3.11 | 24.82 | 0.55 |
| Hanatee | 1 | 24.00 | 3.17 | 2.72 | 24.01 | 0.41 |
| | 2 | 23.40 | 3.05 | 2.62 | 23.41 | 0.44 |
| | 3 | 24.69 | 3.13 | 2.69 | 24.70 | 0.37 |
| Rice | 1 | 24.82 | 3.02 | 2.75 | 24.86 | 1.46 |
| | 2 | 25.18 | 3.05 | 2.78 | 25.22 | 1.44 |
| | 3 | 25.63 | 3.11 | 2.83 | 25.67 | 1.41 |
| Wheat | 1 | 25.22 | 3.13 | 2.78 | 25.24 | 0.69 |
| | 2 | 25.76 | 3.18 | 2.83 | 25.78 | 0.64 |
| | 3 | 24.81 | 3.04 | 2.69 | 24.83 | 0.62 |

**Table A.5** Protein content (dry basis) of Pirun 4 cassava flour, Hanatee cassava flour, rice flour and wheat flour

| Flour | Replication | HCl (ml) | Nitrogen (%) | Protein |
|---|---|---|---|---|
| | 1 | 1.1 | 0.45 | 2.78 |
| Pirun 4 | 2 | 1.1 | 0.53 | 3.28 |
| | 3 | 1.15 | 0.40 | 2.51 |
| | 1 | 1.2 | 0.64 | 4.02 |
| Hanatee | 2 | 1.3 | 0.58 | 3.63 |
| | 3 | 1.3 | 0.61 | 3.83 |
| | 1 | 3.1 | 1.55 | 9.68 |
| Rice | 2 | 3 | 1.53 | 9.58 |
| | 3 | 2.6 | 1.61 | 10.05 |
| | 1 | 4.2 | 3.46 | 21.61 |
| Wheat | 2 | 4.25 | 3.60 | 22.48 |
| | 3 | 4.25 | 3.43 | 21.44 |

**Table A.6** Ash content (dry basis) of Pirun 4 cassava flour, Hanatee cassava flour, rice flour and wheat flour

| Flour | Replication | Crucible before burned (g) | Crucible after burned (g) | Ash |
|---|---|---|---|---|
| | 1 | 36.11 | 36.19 | 2.84 |
| Pirun 4 | 2 | 31.80 | 31.88 | 2.95 |
| | 3 | 37.59 | 37.67 | 2.96 |
| | 1 | 37.03 | 37.10 | 2.72 |
| Hanatee | 2 | 31.35 | 31.42 | 2.74 |
| | 3 | 39.24 | 39.31 | 2.70 |
| | 1 | 42.12 | 42.13 | 0.32 |
| Rice | 2 | 38.15 | 38.16 | 0.30 |
| | 3 | 37.86 | 37.87 | 0.38 |
| | 1 | 47.08 | 47.10 | 0.57 |
| Wheat | 2 | 49.97 | 49.98 | 0.54 |
| | 3 | 45.66 | 45.67 | 0.55 |

**Table A.7** Amylose content (g/100 g flour) of Pirun 4 cassava flour, Hanatee cassava flour, rice flour and wheat flour

| Flour | Replication | Amylose content |
|---|---|---|
| Pirun 4 | 1 | 33.61 |
| | 2 | 34.16 |
| | 3 | 32.97 |
| | 4 | 33.07 |
| Hanatee | 1 | 32.57 |
| | 2 | 32.67 |
| | 3 | 32.77 |
| | 4 | 32.67 |
| Rice | 1 | 28.52 |
| | 2 | 28.22 |
| | 3 | 27.13 |
| | 4 | 28.22 |
| Wheat | 1 | 17.23 |
| | 2 | 16.44 |
| | 3 | 16.73 |
| | 4 | 16.73 |

**Table A.8** Pasting properties of Pirun 4 cassava flour, Hanatee cassava flour, rice flour and wheat flour

| Flour types | Replication | Peak viscosity (RVU) | Trough viscosity (RVU) | Breakdown (RVU) | Final Viscosity (RVU) | Setback (RVU) | Pasting Temperature (°C) |
|---|---|---|---|---|---|---|---|
| Pirun 4 | 1 | 310.75 | 131.42 | 179.33 | 176.33 | 44.92 | 73.25 |
| | 2 | 308.83 | 130.83 | 178.00 | 172.83 | 42.00 | 73.95 |
| Hanatee | 1 | 281.00 | 151.58 | 129.42 | 203.92 | 52.33 | 73.15 |
| | 2 | 285.58 | 166.58 | 119.00 | 214.42 | 47.83 | 74.80 |
| Rice | 1 | 186.25 | 155.75 | 30.50 | 366.17 | 210.42 | 88.30 |
| | 2 | 193.25 | 161.17 | 32.08 | 364.83 | 203.67 | 89.85 |
| Wheat | 1 | 135.75 | 101.75 | 34.00 | 188.42 | 86.67 | 86.25 |
| | 2 | 135.58 | 102.17 | 33.42 | 186.17 | 84.00 | 88.00 |

## APPENDIX B

Syneresis and Textural Properties of Cassava Flour Gels During Frozen Storage

**Table B.1** Percent syneresis of Pirun4 cassava flour gel, Hanatee cassava flour gel, rice flour gel and wheat flour gel

| Flour types | Replication | Syneresis (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Pirun 4 cassava | 1 | 11.60 | 25.38 | 31.33 | 34.77 | 33.48 | 37.78 |
| | 2 | 11.28 | 26.06 | 32.22 | 33.83 | 38.61 | 36.84 |
| Hanatee cassava | 1 | 23.71 | 31.73 | 34.14 | 34.58 | 34.34 | 36.05 |
| | 2 | 18.95 | 32.40 | 34.70 | 34.29 | 34.80 | 34.01 |
| Rice | 1 | 28.06 | 26.93 | 36.99 | 40.23 | 41.56 | 37.70 |
| | 2 | 26.02 | 27.91 | 39.17 | 36.48 | 38.23 | 41.95 |
| Wheat | 1 | 16.23 | 30.50 | 31.86 | 33.85 | 35.94 | 39.18 |
| | 2 | 17.77 | 29.63 | 30.02 | 34.09 | 38.17 | 39.49 |

**Table B.2** Firmness of Pirun4 cassava flour gel, Hanatee cassava flour gel, rice flour gel and wheat flour gel

| Flour types | Replication | Firmness (N) | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Pirun 4 cassava | 1 | 15.46 | 19.32 | 18.40 | 19.23 | 18.77 | 18.88 |
| | 2 | 16.34 | 17.38 | 20.26 | 19.62 | 19.28 | 19.30 |
| Hanatee cassava | 1 | 13.91 | 25.51 | 25.68 | 26.03 | 26.52 | 26.84 |
| | 2 | 17.54 | 25.33 | 27.35 | 27.30 | 27.17 | 28.67 |
| Rice | 1 | 16.37 | 25.62 | 37.95 | 37.93 | 38.21 | 38.89 |
| | 2 | 12.83 | 23.05 | 38.34 | 38.24 | 38.02 | 37.10 |
| Wheat | 1 | 27.49 | 32.59 | 53.21 | 52.71 | 57.71 | 58.83 |
| | 2 | 29.12 | 37.74 | 55.67 | 57.93 | 57.78 | 58.41 |

**Table B.3** Cohesiveness of Pirun4 cassava flour gel, Hanatee cassava flour gel, rice flour gel and wheat flour gel

| Flour types | Replication | Cohesiveness | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Pirun 4 cassava | 1 | 0.87 | 0.69 | 0.66 | 0.62 | 0.55 | 0.44 |
| | 2 | 0.87 | 0.68 | 0.63 | 0.58 | 0.55 | 0.52 |
| Hanatee cassava | 1 | 0.94 | 0.76 | 0.67 | 0.35 | 0.28 | 0.26 |
| | 2 | 0.94 | 0.77 | 0.67 | 0.33 | 0.28 | 0.28 |
| Rice | 1 | 0.78 | 0.62 | 0.55 | 0.54 | 0.50 | 0.38 |
| | 2 | 0.77 | 0.61 | 0.55 | 0.53 | 0.51 | 0.38 |
| Wheat | 1 | 0.74 | 0.62 | 0.58 | 0.57 | 0.38 | 0.32 |
| | 2 | 0.75 | 0.63 | 0.59 | 0.58 | 0.38 | 0.32 |

## APPENDIX C

Frozen Storage of Gluten-Free Chiffon Cake Based on Cassava Flour

**Table C.1** Number of pores of commercial chiffon cake, Pirun4 cassava chiffon cake, Hanatee cassava chiffon cake, rice flour chiffon cake and wheat flour chiffon cake

| Cake | Replication | Number of pore (x $10^5$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Commercial | 1 | 54.06 | 31.66 | 31.27 | 29.20 | 27.20 | 24.46 |
| | 2 | 51.84 | 35.73 | 30.52 | 28.00 | 25.41 | 23.06 |
| | 3 | N/A | 33.34 | 29.92 | 27.69 | 25.01 | 21.84 |
| Pirun 4 cassava | 1 | 30.43 | 23.61 | 19.37 | 9.71 | 8.71 | 7.45 |
| | 2 | 29.02 | 23.52 | 14.70 | 7.11 | 7.63 | 5.68 |
| Hanatee cassava | 1 | 42.05 | 23.25 | 18.32 | 17.08 | 10.38 | 7.43 |
| | 2 | 47.32 | 20.76 | 18.20 | 15.73 | 7.68 | 6.60 |
| Rice | 1 | 21.07 | 19.72 | 10.99 | 9.44 | 7.23 | 4.94 |
| | 2 | 20.05 | 16.18 | 10.38 | 9.09 | 6.82 | 4.48 |
| Wheat | 1 | 47.46 | 36.94 | 31.75 | 28.90 | 7.89 | 6.70 |
| | 2 | 40.80 | 31.37 | 30.66 | 28.81 | 7.34 | 4.73 |

**Table C.2** Cake volume of commercial chiffon cake, Pirun4 cassava chiffon cake, Hanatee cassava chiffon cake, rice flour chiffon cake and wheat flour chiffon cake

| Cake | Replication | Cake volume ($cm^3$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Commercial | 1 | 2.03 | 2.03 | 2.03 | 1.91 | 1.85 | 1.77 |
| | 2 | 2.03 | 2.03 | 2.03 | 1.90 | 1.83 | 1.74 |
| | 3 | N/A | 2.03 | 2.03 | 1.87 | 1.79 | 1.67 |
| Pirun 4 cassava | 1 | 1.92 | 1.61 | 1.60 | 1.57 | 1.46 | 1.35 |
| | 2 | 1.67 | 1.61 | 1.59 | 1.54 | 1.39 | 1.25 |
| Hanatee cassava | 1 | 1.79 | 1.78 | 1.56 | 1.49 | 1.39 | 1.21 |
| | 2 | 1.78 | 1.56 | 1.52 | 1.45 | 1.25 | 1.20 |
| Rice | 1 | 1.81 | 1.71 | 1.68 | 1.57 | 1.52 | 1.34 |
| | 2 | 1.76 | 1.70 | 1.65 | 1.56 | 1.47 | 1.14 |
| Wheat | 1 | 1.80 | 1.69 | 1.42 | 1.35 | 1.23 | 1.02 |
| | 2 | 1.69 | 1.64 | 1.41 | 1.27 | 1.15 | 0.82 |

**Table C.3** Firmness of commercial chiffon cake, Pirun4 cassava chiffon cake, Hanatee cassava chiffon cake, rice flour chiffon cake and wheat flour chiffon cake

| Cake | Replication | Firmness (N) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Commercial | 1 | 10.19 | 10.81 | 12.04 | 12.33 | 13.81 | 14.73 |
| | 2 | 10.43 | 11.17 | 12.00 | 12.35 | 13.72 | 14.02 |
| | 3 | 9.87 | 11.68 | 11.75 | 11.98 | 12.76 | 14.48 |
| Pirun 4 cassava | 1 | 6.86 | 6.52 | 7.32 | 7.30 | 7.55 | 7.69 |
| | 2 | 5.92 | 7.44 | 6.97 | 7.49 | 7.47 | 7.81 |
| Hanatee cassava | 1 | 6.37 | 8.40 | 8.59 | 10.96 | 14.65 | 13.08 |
| | 2 | 7.53 | 8.44 | 8.74 | 9.62 | 13.72 | 15.36 |
| Rice | 1 | 8.64 | 10.82 | 16.81 | 15.11 | 17.11 | 19.14 |
| | 2 | 8.23 | 10.53 | 15.35 | 15.08 | 17.57 | 18.68 |
| Wheat | 1 | 26.99 | 37.88 | 43.44 | 43.14 | 43.85 | 45.12 |
| | 2 | 29.08 | 35.72 | 41.96 | 43.98 | 43.95 | 43.09 |

**Table C.4** Cohesiveness of commercial chiffon cake, Pirun4 cassava chiffon cake, Hanatee cassava chiffon cake, rice flour chiffon cake and wheat flour chiffon cake

| Cake | Replication | Cohesiveness | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unfrozen | 1-day storage | 7-day storage | 14-day storage | 21-day storage | 28-day storage |
| Commercial | 1 | 0.90 | 0.91 | 0.90 | 0.91 | 0.89 | 0.89 |
| | 2 | 0.91 | 0.91 | 0.91 | 0.90 | 0.90 | 0.89 |
| | 3 | 0.92 | 0.91 | 0.91 | 0.91 | 0.89 | 0.89 |
| Pirun 4 cassava | 1 | 0.95 | 0.94 | 0.95 | 0.95 | 0.94 | 0.94 |
| | 2 | 0.95 | 0.95 | 0.95 | 0.95 | 0.94 | 0.94 |
| Hanatee cassava | 1 | 0.94 | 0.95 | 0.95 | 0.95 | 0.94 | 0.93 |
| | 2 | 0.95 | 0.94 | 0.94 | 0.94 | 0.94 | 0.92 |
| Rice | 1 | 0.95 | 0.95 | 0.93 | 0.94 | 0.91 | 0.90 |
| | 2 | 0.95 | 0.95 | 0.93 | 0.93 | 0.90 | 0.90 |
| Wheat | 1 | 0.93 | 0.92 | 0.92 | 0.92 | 0.91 | 0.90 |
| | 2 | 0.93 | 0.92 | 0.92 | 0.92 | 0.92 | 0.91 |

# CURRICULUM VITAE



| | |
|---|---|
| **NAME** | Miss Manapaporn Chaiwanichaya |
| **DATE OF BIRTH** | 28 November 1994 |



**EDUCATIONAL RECORD**

| | |
|---|---|
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| BACHELOR'S DEGREE | Bachelor of Engineering (Chemical Engineering)<br>King Mongkut's University of Technology Thonburi, 2016 |
| MASTER'S DEGREE | Master of Engineer (Food Engineering)<br>King Mongkut's University of Technology Thonburi, 2018 |

| | |
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| **SCHOLARSHIP/ RESEARCH GRANT** | Food Engineering Practice School (FEPS) Scholarship, 2017 – 2018 |

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Signature........................Witness
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Signature........................Witness
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