DEVELOPMENT OF VALUE ADDED UNRIPE PAPAYA FLOUR FROM WASTE PRODUCT OF
FRUIT INDUSTRY

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การผลิตแป้งจากมะละกอดิบจากวัสดุที่เหลือทิ้งจากอุตสาหกรรมแปรรูปผลไม้

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        มะละกอ (*Carica papaya* L.) ผลไม้ในตระกูล Caricaceae ที่เป็นแหล่งของใยอาหารและสารต้านอนุมูลอิสระ ซึ่งในงานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาและพัฒนาแป้งมะละกอดิบจากวัสดุเหลือใช้ในอุตสาหกรรมแปรรูปผลไม้ และนำมาศึกษาคุณสมบัติทางเคมีกายภาพ คุณสมบัติเชิงหน้าที่และการนำมาทดแทนแป้งสาลีในผลิตภัณฑ์แพนเค้ก แป้งมะละกอดิบทำโดยการใช้มะละกอดิบอบแห้งถูกนำมาบดและร่อนผ่านตะแกรง 100 เมซ ซึ่งผลการทดลองพบว่าแป้งมีรูปร่างผิดปกติและมีขนาดอนุภาค 140.8±2.1 ไมครอน ค่าคุณสมบัติทางน้ำว่าแป้งมะละกอดิบสูงกว่าแป้งสาลีในขณะที่ความหนาแน่นรวมไม่แตกต่างกัน และยังพบว่าแป้งมะละกอดิบมีปริมาณสารประกอบฟีโนลิก 85.7±1.6 มิลลิกรัมเทียบเท่ากรดแกลลิคต่อ 100 กรัมแป้งและเบต้าแครอทีน 39±3.3 ไมโครกรัมต่อ 100 กรัมแป้ง นอกจากนี้แป้งมะละกอดิบยังมีฤทธิ์การต้านอนุมูลอิสระสูงแบบ ferric reducing antioxidant power (411.6±38 ไมโครโมล $FeSO_4$ ต่อ100 กรัมแป้ง) และ แบบDPPH free radical scavenging activity (37.87±3.69 มิลลิกรัมเทียบเท่าวิตามินซี ต่อ100 กรัมแป้ง) มากกว่าแป้งสาลี และยังพบว่าแป้งมะละกอดิบมีความสามารถในการจับตัวกับกรดน้ำดีและรบกวนการการรวมตัวของคลอเลสเตอรอลเข้าสู่ไมเซลล์ได้ หลังจากนั้นพบว่าแพนเค้กที่มีการทดแทนด้วยแป้งมะละกอดิบ (ไม่มีการทดแทน, 5%, 10% และ 20%) มีสีผิวหน้าเป็นสีน้ำตาลเข้มกว่าแพนเค้กสูตรมาตรฐานและเมื่อนำไปวัดค่าคุณสมบัติทางเนื้อสัมผัสพบว่าค่าความแข็งและความเคี้ยวได้เพิ่มขึ้นเมื่อมีการทดแทนด้วยแป้งมะละกอดิบเพิ่มขึ้น ในขณะเดียวกันการทดแทนแป้งมะละกอดิบในแพนเค้ก (20%) สามารถชะลอการปลดปล่อยน้ำตาลกลูโคสภายใต้การจำลองการย่อยได้ และพบว่าแพนเค้กที่ทดแทนด้วยแป้งมะละกอดิบมีค่าดัชนีน้ำตาลต่ำกว่าแพนเค้กสูตรมาตรฐานอย่างมีนัยสำคัญทางสถิติ และพบว่าค่าแป้งที่ไม่ถูกย่อยมีค่าเพิ่มขึ้นตามปริมาณการทดแทนด้วยแป้งมะละกอดิบอีกด้วย ผลการวิจัยยังแสดงให้เห็นอีกว่าแพนเค้กที่มีการทดแทนด้วยแป้งมะละกอดิบนั้นมีปริมาณสารประกอบฟีโนลิกและมีฤทธิ์การต้านอนุมูลอิสระสูงกว่าแพนเค้กสูตรมาตรฐาน ในการประเมินความชอบทางประสาทสัมผัสพบว่าที่แพนเค้กทดแทนด้วยแป้งมะละกอดิบ 20% มีคะแนนความชอบในด้านรสชาติและรสชาติตกค้างน้อยกว่าอย่างมีนัยสำคัญทางสถิติ แต่การยอมรับได้โดยรวมไม่แตกต่างกันในทุกกลุ่มแพนเค้ก จากการศึกษาทั้งหมดอาจกล่าวได้ว่าแป้งมะละกอดิบอาจนำไปใช้เป็นส่วนประกอบของผลิตภัณฑ์อาหารที่มีฤทธิ์ต้านอนุมูลอิสระและส่งผลดีต่อสุขภาพได้

สาขาวิชา	อาหารและโภชนาการ	ลายมือชื่อนิสิต ....
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Waralee Joymak : DEVELOPMENT OF VALUE ADDED UNRIPE PAPAYA FLOUR FROM WASTE PRODUCT OF FRUIT INDUSTRY. Advisor: Assoc. Prof. SIRICHAI ADISAKWATTANA, Ph.D.

Papaya fruit (*Carica papaya* L.) belonging to Caricaceae family is recognized as a good source of dietary fiber and antioxidants. The objective of this study was to develop the unripe papaya flour (UPF) from waste fruit product. The study was also investigated physicochemical, functional properties of UPF and its addition of pancake. First, the small pieces of unripe papaya were heated by hot air oven, then ground by using a high-speed universal grinder and sieved through a 100-mesh sieve. The particle size of UPF was 140.8±2.1 μm with randomly irregular shape. The hydration properties of UPF was higher than wheat flour, whereas bulk density was not significant differences. Total polyphenol and β-carotene contents of UPF were 85.7±1.6 mg gallic acid equivalent/100 g dry weight and 39±3.3 μg/100 g dry weight, respectively. In addition, UPF had the ferric reducing antioxidant power (411.6±38 μmol $FeSO_4$ per 100 g DW) and DPPH free radical scavenging activity (37.87±3.69 mg vitamin C equivalent per 100 g DW) higher than that of wheat flour. Moreover, UPF had the ability on bind bile acid and interfere the cholesterol micellization. Thereafter, the replacement of wheat flour with UPF into pancake (0%, 5%, 10%, and 20%) presented the darker shade of brown color on their surface. Hardness and cohesiveness of pancake were increased in a higher level of replacement. However, pancake with UPF (20%) could attenuate the glucose release under simulated digestion. Moreover, pancake with UPF had significantly lower predicted glycemic index when compared to the control pancake. Undigestible starch in pancake was also increased when the replacement of UPF was increased. Furthermore, pancake with UPF significantly increased total polyphenol content and antioxidant activity. In sensory evaluation, the taste and aftertaste parameters were significantly decreased in pancake with 20% UPF. However, the overall acceptance showed no significant difference among all groups. The results suggest that the unripe papaya flour can be regarded as a good ingredient for natural antioxidants for development of healthy products.

Field of Study: Food and Nutrition   Student's Signature ....

Academic Year: 2018   Advisor's Signature ....

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CHAPTER I

INTRODUCTION

1. Background and significant of the study

Metabolic disorder is a complex process and disrupts normal metabolisms, leading to chronic diseases such as hyperlipidemia and diabetes. Diabetic patients who cannot control blood glucose level to acceptable range contributes to long-term hyperglycemia causing the development of diabetic complications including blindness, kidney failure, cardiovascular disease, stroke and amputation (Forbes and Cooper 2013). World health organization reported the number of diabetic patients has been increasing from 4.7 percentage in 1980 to 8.5 percentage in 2014. (World Health Organization 2016). In addition, the Thai health report (Bureau of non communicable diseases 2017) also revealed that the death rate of Thai diabetic patients has increased for 2 times from 2012 to 2016, especially in elderly. With a high prevalence of diabetes, there is a group perceived with severe disabilities and multiple comorbidities, a poor quality of life and diminished life expectancy which become a factor influencing the increased burden of medical cost.

Carbohydrate, an essential nutrient in food, is one of contributing factors to increase blood glucose. The high consumption of carbohydrate diet elevates postprandial blood glucose level, hyperinsulinemia and insulin resistance (Ludwig

2002). Glycemic index is a measure the effects of the consumption of a food on blood glucose level.

It has reported that regular consumption of high glycemic index food can increase higher level of blood glucose and insulin level when compared to low glycemic index food (Ludwig 2002). Proper portion intake of carbohydrate enriched diet is the recommending effective method to control blood glucose (Diabetes UK 2014). The low glycemic index diet can delay carbohydrate digestion and glucose absorption into bloodstream that maintains blood glucose level to acceptable range (Shulman 2000). Nowadays, natural food sources are widely used for alternative ingredient because of their containing enriched nutrients including resistant starch, dietary fiber and phytochemical compounds. The alternative ingredients are added into food products in order to provide healthier food choices. For example, unripe banana flour and black rice flour as a partial wheat replacement in bakery product can enhance total phenolic compound and antioxidant capacity (Agama-Acevedo et al. 2012, Mau et al. 2017, Segundo et al. 2017a). Moreover, diets containing natural alternative ingredient also increase the content of dietary fiber into food products. It helps decrease the postprandial blood glucose. (Drzikova et al. 2005).

Papaya, one of tropical fruit plants, is widely cultivated and distributed in most tropical countries and islands. In Thailand, papaya is a favorite plant and generally found in many areas. Both ripe and unripe fruit of papaya have culinary use because of its nutritional value and taste acceptability. It found that papaya contains high vitamin C, β-carotene and fiber. It was reported that papaya has antioxidant and anti-inflammation properties. The study also suggested that unripe papaya contains lower glucose and high content of fiber than ripe papaya (Asmah R 2014). In food industry, papaya can be consumed as fresh fruits and processed food; for example dried papaya, candy and ice cream. (Annegowda and Bhat 2016). However, only middle part of unripe papaya is selected for dried papaya products, but by-products generated during processing including the upper and lower parts of them are discarded as a waste. Numerous environmental problems may be created globally due to large volumes of fruit wastes, innovative technologies and processing of wastes are a major challenge and the aspects to dealt with.

As mentioned above, flour production could be potential waste processing technique for unripe papaya waste. The method of flour production was investigated. In addition, the flour from this process was studied on its functional to value-added. In brief, the papaya waste was prepared for unripe papaya flour using hot air oven and grinding mill. Physicochemical and functional properties of

unripe papaya flour were also determined. Finally, replacing wheat flour by unripe

papaya flour was investigated for development of healthier nutritional products.

2. Objectives of the study

1. To study the preparation method of the unripe papaya flour from waste

   product of food industry.

2. To investigate physicochemical and functional properties of the unripe

   papaya flour

3. To develop value added bakery product from the unripe papaya flour

3. Benefits of the study

This research might provide information about physicochemical and functional

properties of unripe papaya flour and its application in food. It might be useful for

the food product development and food industry in the future.

4. Conceptual framework
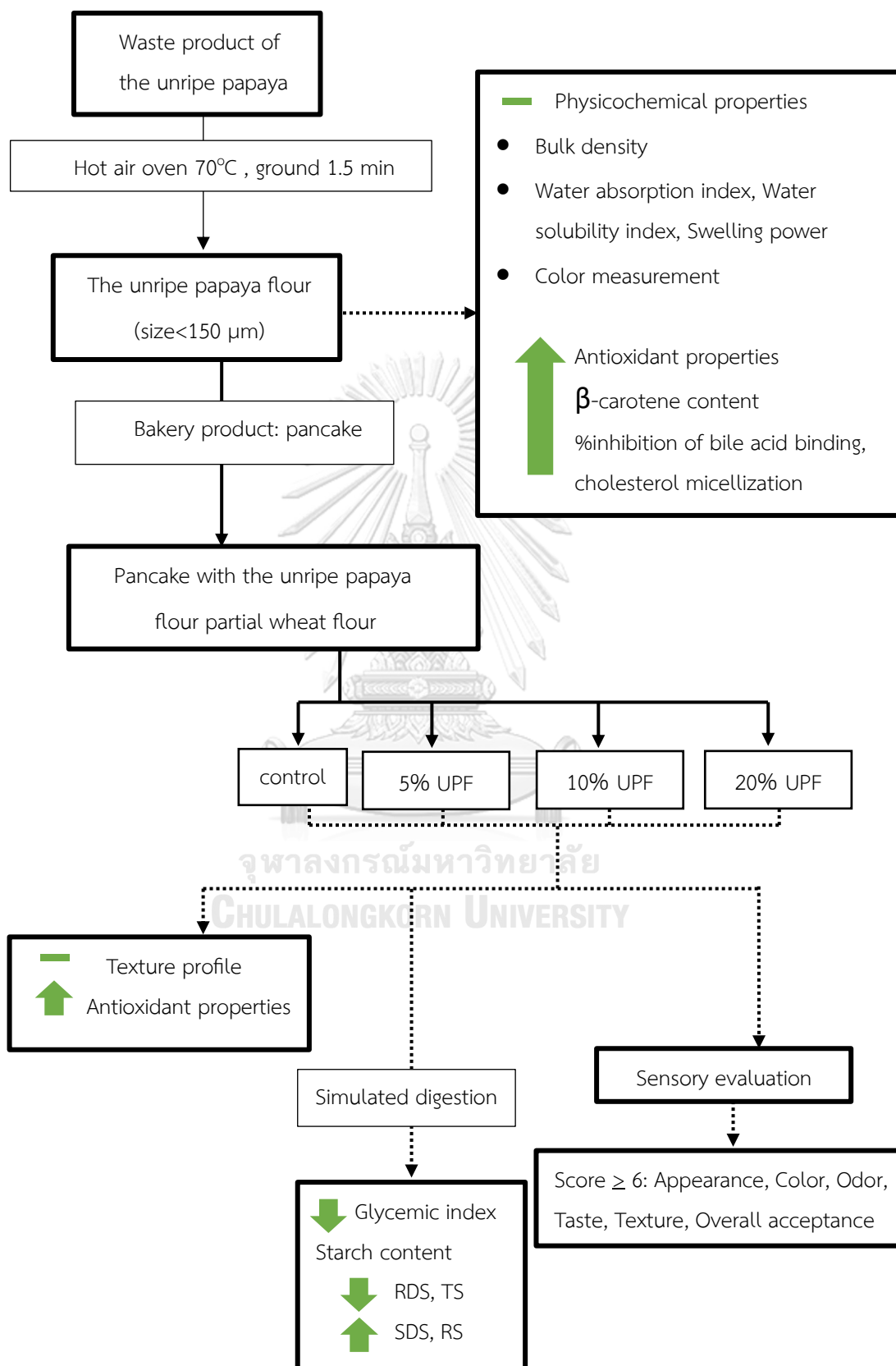
CHAPTER II

REVIEW OF LITERATURE

1. Papaya

Papaya (*Carica papaya* Linn. family *Caricaceae*), one of a tropical fruit, originated in South America. Now, it is widely cultivated in certain countries: India, Sri Lanka, Malaysia, Bangladesh, Australia, Philippines, Taiwan, Jamaica, and Thailand fruits (Annegowda and Bhat 2016).

Papaya or in some common names -- pawpaw, papaw, papita, and Ma-la-kor -- is a small tropical tree with 3-10 m in height. In botanical aspects, papaya has a palm-like habit with a cylindrical trunk and an umbrella shaped leaves on the top (Figure 1a). Once, the leaves are falling, it will leave the scar. Papaya also contains milky latex, which is a good source of enzymes such as papain, chymopapain, glycyl endopeptidase, and caricain. Papaya is a polygamous species including male, female and hermaphrodite. Its commonly grows in clusters under the leaf canopy, at the tip of the trunk with green color and change into yellow or orange color during ripening process (Figure 1b). Fruits have cylindrical shape and a thick wall of firm flesh, vary in size along with a seed cavity (Vij and Prashar 2015, Annegowda and Bhat 2016).
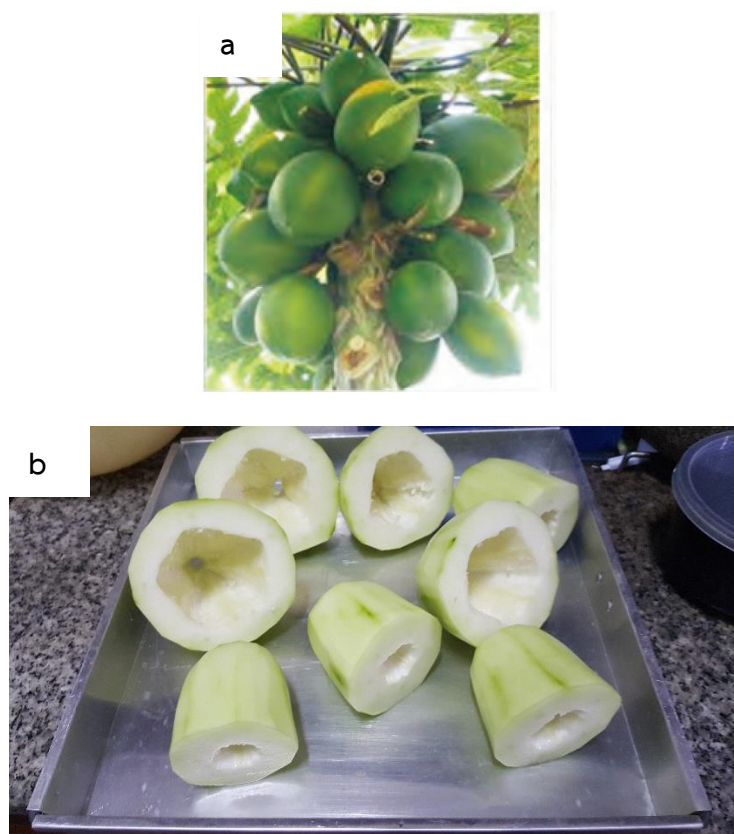
Figure 1 (a) Papaya tree, **(b)** the top and bottom part of unripe papaya

(Vij and Prashar, 2015)

In Thailand, papaya is also recognized as one of the country's important economic crops because in appropriate environmental conditions, papaya fruits can grow throughout the entire year. It has been shown that 6 million tons of papaya are produced around the world in each year and they are being exported and consumed in developing countries. It makes papaya become an international fruit, in both fresh fruit and processed fruit (Vij and Prashar 2015).

Papaya can be consumed both ripe and unripe due to its nutritional value and taste acceptability. Maisarah (2014) reported that papaya has high nutritional value shown (Table 1) and demonstrates antioxidant and anti-inflammatory activity (Asmah R 2014).

Moreover, Ganiyu et al. also discovered that papaya contains many phytochemicals such as β-carotene, vitamin C, procyanidin, gallic acid, catechin, ρ-coumaric acid, epicatechin and quercetin (Oboh et al. 2015). Furthermore, unripe papaya tends to have lower glucose and higher fiber than ripe papaya (Asmah R 2014). Due to various phytochemicals and nutritional value, papaya has been interested in its health potential effect in human (Ikram et al. 2015).

Table 1 Nutritional value of ripe papaya and unripe papaya (gram/100grams)

	Ripe papaya	Unripe papaya
Moisture	85.7 ± 0.3	92.2 ± 0.01
Protein	6.1 ± 0.02	7.9 ± 0.03
Ash	5.8 ± 0.20	6.6 ± 0.04
Total fat	0.01 ± 0.05	0.00 ± 0.09
Fiber	7.6 ± 0.34	9.8 ± 0.09
Carbohydrate	70.7 ± 0.22	64.2 ± 0.06

Papaya is commonly consumed both ripe and unripe. For example , consumption of fresh ripe fruit, using unripe fruits as an ingredient in diet , or food processing into various products such as juice, and dried fruits have been reported (Annegowda and Bhat 2016). Furthermore, papain, the major enzyme in papaya, has been widely used in pharmaceutical and cosmetic industry (Annegowda and Bhat 2016). Food corporation states that mixed fruits (Figure 2 (right)) as an ingredient in food industry are produced from unripe papaya. The production uses only the central part of the unripe papaya but discards the top and bottom. Then, they become waste around 1000 kilograms per year. For value adding of the discarded unripe papaya and reducing waste, it might have the method to apply these wastes into something more useful like flour. Moreover, there is only few researches related to unripe papaya as flour and application of its.

Figure 2 (Left) dried papaya, (Right) mixed fruit

2. Flours

Flour is a powder made from grains, roots, beans and seeds, but normally wheat. Flour production comprises many processes including cleaning, tempering, and milling or grinding. Milling is the simplest way to remove the kernel of wheat and separate the germ and bran. Milling processes can be classified into 2 groups (Finnie and A. Atwell 2016);

- Dry milling: this type of milling grinds wheat into the desirable size by using forces including compression, abrasion, shear, and impact. There are many devices using for dry milling; for example, stone, roller and hammer mills

- Wet milling: use to separate between wheat and gluten.

Nowadays, the application of fruits and vegetable waste processing is being interested extensively (Ferreira et al. 2015). Not only cereals or grains can be made as flour, but those wastes can also be made. Flour production process has been modified in different ways in order to suit the conditions of each miscellaneous types of sources. From previous studies, many fruits and vegetables were processed into flour and can be applied for food; for example, fruits and vegetable waste from juice (Ferreira et al. 2015), pineapple stem (Nakthong et al. 2017), unripe banana (Agama-Acevedo et al. 2012) ,and pomelo segment (Reshmi et al. 2017). Like fruits and vegetables, their waste products also provide high phytochemical contents and dietary fiber (Saleh et al. 2019). Therefore, the development of food

products from waste is being considered as sustainable waste management by reducing food wastes, plus the development of new functional foods.

3. Wheat flour replacement

Wheat flour replacement has been widely used as alternative food choices for a long time. First, this idea was employed for people who suffer from gluten intolerance by replacing wheat flour, a great gluten source, and replacing it with free gluten flour. With the passing of time, not only gluten intolerance people interest in wheat flour replacement, but also other consumers who need healthier food choice from food products.

Nowadays, the demand to use novel sources as wheat partial replacement has been increasing to fulfill the consumer requirement, enhance nutritional value of food, and provide more health benefits. Many cereals and fruits including their waste products from company become an interested source to manufacture the flour (Sirichokworrakit et al. 2015). The waste products are dehydrated and ground into flour for the appropriate substitution. The study shows that the processed by-product also provides high amount of phytochemicals and dietary fiber (Saleh et al. 2019). This finding makes the technique and proposed processing technologies in the food industry become more interested.

Apart from the production of flour, they still need to investigate on physical and functional properties of flour for its application. Due to the negative characteristics are commonly found in food products after the replacement including textural properties and sensory properties (taste, color or odor). Not only nutritional analysis such as proximate analysis and moisture content of flour is being tested, but other parameters have been analyzed to identify flour characteristics for its application and overall consumer acceptance.

4. Microstructural properties of flours

The microstructural properties of flour including morphology and particle size distribution could affect functional property. The specific morphology of granule surface of flour has been associated with the source of flour (Segundo et al. 2017a) which results swelling properties and solubility (Nakthong et al. 2017). The surface of normal morphology of flour is generally investigated by scanning electron microscope (SEM). Topography demonstrates two-dimension from the irradiation to sample and the emission of secondary electron from the specimen surface (JEOL). Researchers stated that starch granule differs from its botanical origins. The observed shapes are oval, round, spherical, polygonal, and irregular (Lindeboom et al. 2004). For example, green banana flour showed large particle attached with granule on the surface, indicating the component associated with banana pulp including fiber and protein (Segundo et al. 2017a). Nakthong et al. studied starch from pineapple stem waste describing that it has a different semi-angular shape

with partially rounded segments because pineapple stem starch granules are

tightly packed in parenchyma cells (Nakthong et al. 2017).
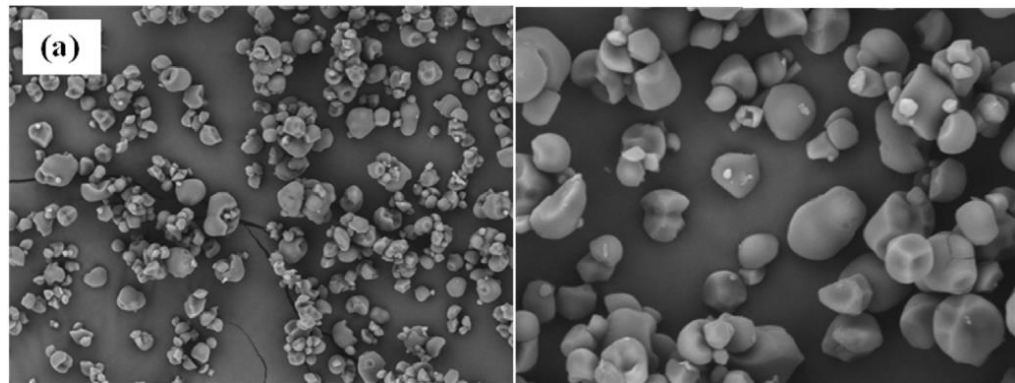
Figure 3 Scanning electron microscopy (SEM) images of (a) pineapple stem at two

magnifications of 1000x (left) and 3000x (right).

(Nakthong, Wongsagonsup and Amornsakchai. 2017)

Apart from the surface morphology of flour, particle size is one of parameters

required for investigation of structural properties (Segundo et al. 2017a). There are

several methods to determine particle size of flour. Laser light scattering is one of

the popular and easy methods because of its reliability. This technique can assess

through small granules which (<0.1 µm). Segundo et al. revealed that difference

particle size leads to the different quality of final product of cake. Small particle

size of green banana flour (<80 µm) showed better cake quality and sensory

evaluation than large particle size (156–200 µm) (Segundo et al. 2017a).

Moreover, previous study suggested that particle size could affect to quality of cookie product. The finer particle size of maize flour could increase water holding capacity and swelling volume, resulting in the increasing of dough stickiness. However, the quality of product was contrasted with other study. The finer particle size increased the hardness and lightness, while decrease the spread factor of cookies (Belorio et al. 2019). It can be observed that finer particle size could increase specific volume of bread, and result in the elevation of hardness (De la Hera et al. 2013b). This study suggested coarser particle size might appropriate for bread making. These results inform that the particle size of flour might be an important factor that need to consideration.

5. Antioxidant activity of flours

Wheat flour is the prominent ingredient of bakery product. To enhance the potential health benefits, antioxidant property is one of the concerning factors for the partial replacement of wheat flour. At the present, consumers increasingly concern about the consumption of food enriched with antioxidant, which can improve their health (Kim and Kim 2017). Many types of flour from natural sources have been studied the anitioxidant activity of flour and food products. For example, unripe banana flour contained phenolic compounds whereas replacement of cake by unripe banana flour showed low IC_{50} of DPPH free radical activity. It can indicate that the addition of unripe banana flour in cake could increase antioxidant activity and provide beneficial effect on health (Segundo et

al. 2017a). Furthermore, papaya pulp flour contained high phenolic compound and demonstrate of DPPH radical scavenging ability (Varastegani et al. 2015). In addition, increasing percentage of papaya pulp flour substitution in cookies showed higher phenolic compound and DPPH radical scavenging activity when compared with wheat cookies. These findings suggest that addition of flour with different natural sources improves antioxidant activity of the products.

6. *In vitro* digestion, starch fraction and glycemic index

In vitro digestion model is the demonstration of the structural changes and digestibility of food components under simulated gastrointestinal conditions. There are many methods of *in vitro* digestion depending on the specific food component being analyzed.

Even though a good method for food digestion investigation is an *in vivo* model, it has some limitations including difficulty, expensiveness and ethical problems. As a consequent, *in vitro* digestion has been widely used in the experiments because this model provides much shorter time and inexpensive cost. This method did not have any ethical restrictions. It showed a good correlation between *in vivo* and *in vitro* digestion on prediction of protein, total phenolic compound, and carbohydrate digestion outcome. There also reported that *in vitro* model considered as a good indicator of *in vivo* behavior for both macronutrients and micronutrients (Bohn et al. 2017). .

Starch is one of the important compositions of carbohydrate in food. Starch fraction can be classified into 3 types according to the rate of digestion in the small intestine (Zhang and Hamaker 2009);

- Rapid digestible starch: the digested starch portions within 20 min. this type of starch can rapidly digest and absorb into the small intestine, resulting in the expeditious elevation of postprandial blood glucose. This condition may lead to the rapid change and unsteadiness of blood glucose level and hypoglycemia.

- Slow digestible starch: the digested starch portions between 20 to 120 min. This type of starch fraction can be completely digested in lower rate. It can prolong rate of digestion and glucose releasing rate.

- Undigestible starch: the persisting starch portions after 120 min. This starch fraction cannot be digested in small intestine.

According to physiological function, many researchers suggested that consumption of slow digestible starch and undigestible starch provides more beneficial effects including reduction of the risk of cardiovascular and other chronic diseases (Aller et al. 2011).

Apart from starch fractions, glycemic index (GI) is the one of parameters for rating of carbohydrate by analyzing the digestion rate of carbohydrate and the elevation of postprandial blood glucose level within two hours comparing with reference food. Glycemic index can divide into three groups:

1. Low glycemic index: GI ranged 55 or less defines foods affect to the elevation of postprandial blood glucose slowly and steadily for example food containing high fiber, vegetable, cereal, apple, and grapefruit, etc.

2. Medium glycemic index: GI ranged 56-69 defines food elevating postprandial blood glucose level faster than low GI, the examples of food in this group are noodle, corn, potato, whole wheat, and brown rice, etc.

3. High glycemic index: GI ranged more than 70, white bread, white rice, ice cream, dried fruits, and sweet fruits (watermelon, mango, etc.) are the examples of diet in this group

Consumption of high GI diet results in high amount of carbohydrate that can be digested into glucose and absorbed into body promptly. After glucose absorption, blood glucose rapidly increases, and consequently decrease. It would reflect the baseline. This can raise the sense of hunger, and eventually, a rapid change in blood glucose level can cause hypoglycemia. On the other hand, if consumption of low GI food, the glucose from food will slowly affect blood glucose level and help patients control their blood glucose level more easily.

According to the this concept, food industry aims to develop novel products with low GI ingredients such as product containing wheat flour partial replacement by cereal, grain or fruits (Agama-Acevedo et al. 2012, Shumoy and Raes 2017) .

Hydrolysis index was introduced by Goni et al. (Goni et al. 1997) as a relationship between area under the hydrolysis curve (AUC) for a given sample and the AUC for a reference food such as white bread. Hydrolysis index is deployed as indicator of glycemic response and calculation for glycemic index.

7. Texture analysis

Apart from other parameters, texture analysis is one of the important parameters. Texture of food is crucial key point of food products. It can reflect the consumer preference and the quality of food product (Day and Golding 2016).

Texture profile is one of considerable factors for food. Some types of food need effective texture for itself such as potato chips and cornflake, some needs specific texture for its quality such as bread and cheese. Moreover, food processing can directly affect the textural properties. To achieve the quality characteristics of food, texture profile also needs to be considered (Bourne 2002a).

Texture of food might be related to density, viscosity, surface tension, and other physical properties depending on type of food product (Day and Golding 2016). Texture profile analysis (TPA) is the effective method for measurement and description of the textural properties of food. It can determine both solid and liquid form of food product. This method can easily access multiple parameters in one experiment, so it makes TPA become popular due to the convenience (Nishinari et al. 2013). There are many parameters for TPA, but pancake products commonly determine for:

- Hardness is the point of deepest compression. The increase of hardness results in the hard texture of product whereas the decrease results in soft texture.

- Cohesiveness is the two compressive cycles which include both the downstroke work and the withdrawal work. It can refer as the strength of internal bonds making up in food products. This parameter can refer to the stickiness inside the product. The increase of cohesiveness can refer to the firm characteristics inside product. On the other hand, the decrease of parameter can cause to friableness.

- Springiness is the emulation of the sensory chewing experience, and yet thoroughly chewed foods generally do not have sufficiently remaining structural integrity to spring back. It can refer to elasticity of product. The

increasing springiness can interpret the higher ability to reform structure of product.

- Chewiness is energy required to disintegrate food product to a state where it is ready to be swallowing. This parameter also correlates with hardness.

Figure 4 Texture profile analyzer instrument

Normally, bakery products such as bread and cake might be soft, tender and slightly springy characteristics. In contrast, the substitution of flour from natural sources might worsen the final quality of bakery product. Several studies investigated the textural properties of wheat flour from replacement product by various flour types such as unripe banana flour (Segundo et al. 2017a, Segundo et al. 2017b), *Eucheuma* powder (Huang and Yang 2019), black rice flour (Mau et al.

2017) and flaxseed (Kaur and Kaur 2018). Based on these studies, the replacement of wheat flour by these ingredients can increase hardness and chewiness of final product because of the loss of gluten protein. The previous statement showed that the textural properties might help increase the rate of consumption and also improve quality of food product.

8. Sensory evaluation

Sensory evaluation is a measurement of human responses to foods and minimizes biasing effects of brand identity and other information that have a big influence on consumer perception (Lawless and Heymann 2010, Choi 2014). It is the scientific method for analyzing the responses of products by perceiving through five senses including;

- Sight: by eyes perceive color, size, shape, texture, consistency and opacity

- Smell: volatility of odor is detected, but it would decrease over time called adaptation

- Taste: or gustatory input is the most influential factor and it composes of four basic tastes; sweet, salty, sour and bitter. Moreover, flavor is one of the factors that might affect significantly since it relates to taste, odor and mouthfeel.

- Sound: sound such as sizzling, crunching, popping can communicate about food.

- Touch: the impression of texture through oral sensations and skin.

It has many methods for sensory evaluation and line scaling is one of the most popular applications to determine. The scaling method is the quantifying tool of sensory experience of panelists. This application is affected by the stimulation of product and then generating a response. It reflects the intensity of person's perceive of the sensations generating by that product. It can be done in both trained and untrained panelist. Firstly, untrained panelists can answer to reflect the intensity of the change of product. For trained panelists, they can evaluate this application as descriptive analysis. The scaling application is also separated into 3 methods;

- Category scale is the oldest method which provides choices for the attribute for panelist to choose.

- Line scale is the widely used technique by making a mark on a line to indicate the intensity of the attributes.

- The magnitude estimation; this method asks panelists to assign numbers to sensory without restriction. The ratio of the numerical assignments will result the ratio of sensory perceptions (Lim 2011).

The line scaling has been used for many years. It is recognized as a popular evaluation in descriptive analysis with multiple attributes and become the logical scaling method for the acceptance scaling (Lawless and Heymann 2010).

Moreover, the line scaling can provide accurate results of product differentiation and identification of consumer segments (Svensson 2012).
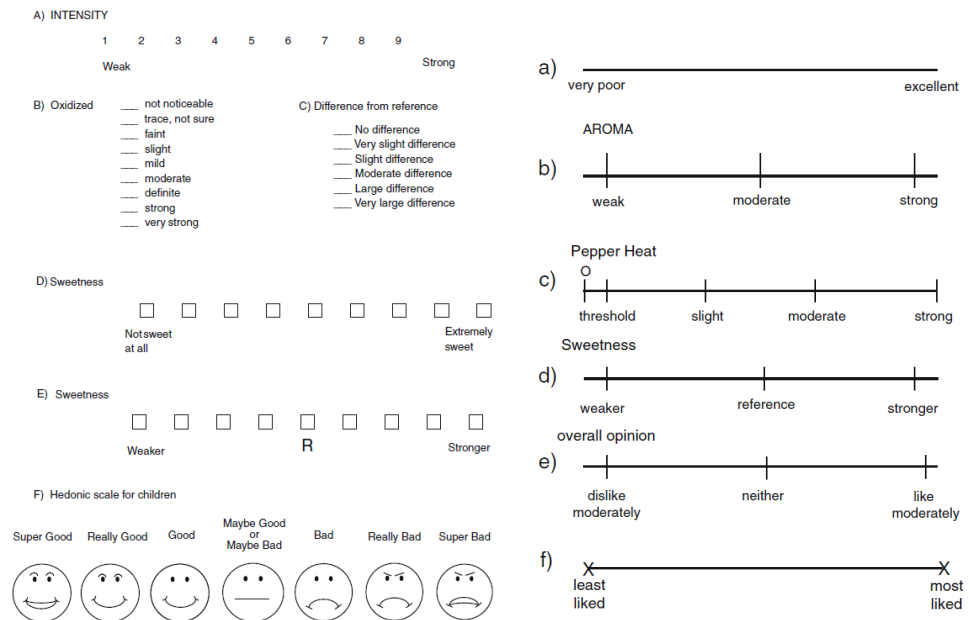
Figure 5 Category scale (left), Line scaling (right)

(Lawless and Heymann 2010)

Sensory properties relate to both physiology and psychology of human. The alteration of food product also affects the acceptance of change (Carpenter et al. 2012). There are many factors that affect the change of food product's sensory characteristics. First of all, phytochemical compounds, which have their own color and odor, may change the attribution of food products. Moreover, the heat in food processing affects phytochemical components in natural ingredients, which result in change of color, odor and taste. To ensure consumer expectations, they are met, the sensory properties evaluation is required. For example, the addition of anthocyanin-rich flour such as black rice flour (Mau et al. 2017), turmeric powder

(Lim et al. 2011) and onion powder (Gawlik-Dziki et al. 2013) can reduce the sensory test score including color, odor and taste. The reason is most of phytochemical compounds have their own taste (mostly bitter and astringent), color and smell. It can change final quality of food product. The high level of replacement can lead to decreasing of sensory properties (Eleazu et al. 2014). Besides, the addition of fiber also affects sensory properties. For instance, muffin, with flaxseed replacement (Kaur and Kaur 2018), guava pomace (Khalifa et al. 2016), whole grain and bran-rich cereal food (Heiniö et al. 2016), can decrease the sensory score. Due to fiber content, it can reduce the tenderness and crispiness of bakery products (Kulkarni and Joshi 2013). This change of textural properties of product can decrease texture score. In addition, when the physical properties and textural property differ from the original product, it could affect the acceptability of consumer (Carpenter et al. 2012). As mentioned above, sensory evaluation is one of the most important factors to produce and improve food products.

CHAPTER III

MATERIALS AND METHODS

1. Materials and equipment

Plant Materials	Company
Unripe papaya, top and bottom part	KCG corporation (Bangkok, Thailand)

Food Ingredients	Company
Wheat flour (All-purpose flour), Red Lettuce brand	Laemthong Corporation Group (Bangkok, Thailand)
Caster sugar, Lin brand	Thai roong ruang sugar group (Bangkok, Thailand)
Rice bran oil	King rice oil group (Bangkok, Thailand)
Whole milk, Meiji brand	CP-Meiji Co., Ltd. (Bangkok, Thailand)
Salt, Prung thip brand	Thai refined salt Co., Ltd. (Bangkok, Thailand)
Baking powder	McGarrett (Bangkok, Thailand)
Egg (no.2), CP brand	Charoen Pokphand Group (Bangkok, Thailand)
Unsalted butter, Allowrie brand	KCG corporation (Bangkok, Thailand)

Chemicals	Company
L-ascorbic acid	Sigma-Aldrich CO. (St. Loius, MO, USA)
Potassium hydroxide (KOH)	Ajax Finechem (Taren Point, Australia)
Butylated hydroxytoluene (BHT)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Ethanol (C_2H_5OH)	Merck (Darmstadt, Germany)
Hexane (HPLC grade; C_6H_{14})	Fisher Scientific (Leicester, UK)
Ethyl acetate ($C_4H_8O_2$)	JT.Baker (USA)
Acetonitrile (HPLC grade; C_2H_3N)	Merck (Darmstadt, Germany)
Methanol (HPLC grade; CH_3OH)	Honeywell (Michigan, USA)
Dichloromethane (HPLC grade; CH_2Cl_2)	Merck (Darmstadt, Germany)
β-carotene standard (HPLC grade)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Methanol (analytical grade; CH_3OH)	JT.Baker (USA)
Sodium carbonate anhydrous (Na_2CO_3)	Ajax Finechem (Taren Point, Australia)
Folin-Ciocalteau's phenol reagent	Sigma-Aldrich CO. (St. Loius, MO, USA)
Gallic acid	Fluka™ (Seelze, Germany)
2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Iron (II) sulfate ($FeSO_4 \cdot 7H_2O$)	Ajax Finechem (Taren Point, Australia)
Iron (III) chloride anhydrous ($FeCl_3$)	Ajax Finechem (Taren Point, Australia)

Chemicals	Company
Glacial acetic acid (CH$_3$COOH)	Merck (Darmstadt, Germany)
Hydrochloric acid (HCl)	Merck (Darmstadt, Germany)
2,2-diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich CO. (St. Loius, MO, USA)
2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Potassium persulfate (K$_2$S$_2$O$_8$)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Sodium dihydrogen phosphate (NaH$_2$PO$_4$)	Ajax Finechem (Taren Point, Australia)
Sodium hydrogen phosphate (Na$_2$HPO$_4$)	Ajax Finechem (Taren Point, Australia)
Sodium chloride (NaCl)	Ajax Finechem (Taren Point, Australia)
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Taurochloric acid	Sigma-Aldrich CO. (St. Loius, MO, USA)
Glycodeoxychloric acid	Merck (Darmstadt, Germany)
Taurodeoxychloric acid	Merck (Darmstadt, Germany)
Total bile acids assay kit	GenWay Biotech. Inc (California, USA)

Chemicals	Company
CMC (Food grade)	A specific bakery shop; Bakeryland (Bangkok, Thailand)
Oleic acid ($C_{18}H_{34}O_2$)	Sigma-Aldrich CO. (St. Loius, MO, USA)
L-α-Phosphatidylcholine	Sigma-Aldrich CO. (St. Loius, MO, USA)
Cholesterol	Sigma-Aldrich CO. (St. Loius, MO, USA)
Total cholesterol kit	HUMAN (GmbH, Germany)
Amyloglucosidase	Megazyme (Illinois, USA)
Glucose liquicolor	HUMAN (GmbH, Germany)
α-amylase	Sigma-Aldrich CO. (St. Loius, MO, USA)
Pepsin	Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India)
Sodium hydroxide (NaOH)	Ajax Finechem (Taren Point, Australia)
Pancreatin	Sigma-Aldrich CO. (St. Loius, MO, USA)

Laboratory Equipment	Company
Incubator	Wisecube Co., Ltd. (South Korea)
Spectrophotometer	Biotek (USA)
Centrifuge	Hettich zentrifugen (USA)
pH meter	Thermo Fisher Scientific Inc., USA)
Hot plate	IKA-works (Germany)
Vortex mixer	Gemmy industrial (Taiwan)
Scanning electron microscopy	JEOL (Japan)
Laser diffraction-based particle size analyzer	Malvern (UK)
High performance liquid chromatography	Shimadzu corporation (Japan)
Sonicator	GT Sonic (China)
Evaporator	Buchi (Switzerland)
Digital dry bath	Labnet International (USA)
Water bath shaker	N-biotek (Korea)
Colorimeter	HunterLab (USA)

2. Methods

2.1 Unripe papaya flour extraction

The extraction of unripe papaya flour method was done following previously described with minor modification (Rajeev et al. 2012, Yusufu and Akhigbe 2014). The top and bottom parts of unripe papaya waste was given by KCG corporation. Briefly, papaya was soaked with salt for 30 minutes, then rinsed and cut into small pieces. They were placed onto aluminum foil in the tray, then heated by hot air oven at 70° C for 12 hours. After heating, dried unripe papaya was milled by laboratory mill for 2 minutes, sieved with a mesh no.100. Unripe papaya flour (UPF) was kept in aluminum zipper bag and stored at -20° C.

2.2 Determination of physicochemical properties

2.2.1 Bulk density measurement

Samples were calculated for bulk density by mass/volume as described in a previous report (Franklin et al. 2017). Flour was loaded into a graduated cylinder to 500 mL mark and weighed. The volume was read directly from the cylinder.

2.2.2   Water absorption, solubility index and swelling power

Water absorption index, solubility index and swelling power were done according to a previous report (De la Hera et al. 2013a). Briefly, flour (50 mg) was dispersed in 1 ml of distilled water, then heated at 90°C for 10 min. After cooling, the sample was centrifuged at 12,000 rpm at 4°C for 5 min. The supernatant was transferred into microtube and was recovered by heating at 105°C until constant weight. Residue was weight for Wr and dried supernatants (Ws) were weighed and WAI or swelling capacity, solubility index and swelling power (SP) were calculated as follows:

$$\text{WAI (g/g)} = \frac{Wr}{Wi}$$

$$\text{WSI (g/100g)} = \frac{Ws}{Wi} x100$$

$$\text{SP (g/g)} = \frac{Wr}{Wi-Ws}$$

2.2.3   Color measurement

The color of flour samples was determined using a CIE Hunter Lab colorimeter. The colorimeter was standardized with black glass and white calibrated tile. Flour (500 mg) was placed in sample dish above the port insert. The results were expressed in lightness ($L*$ value), redness ($a*$ value) and yellowness ($b*$ value).

2.2.4   Microstructural properties

2.2.4.1 Morphology of UPF

The morphology and surface appearance of the UPF were observed under scanning electron microscope (SEM). The sample was coated with gold layer by an ion sputter instrument prior observation under SEM at 15 kV.

2.2.4.2 Particle size distribution

The mean particle size of UPF and particle size analyzer were determined using a laser diffraction-based Malvern particle size analyzer Mastersizer 3000. A refractive index of flour and dispersant were chosen from the reference manual of Malvern instrument (Malvern Instruments 2007); 1.33 for water and 1.53 for flour. The volume-weighted mean diameter (D[4,3]) was employed as a parameters (McClements 2014).

2.3 Proximate analysis

Carbohydrate was determined by compendium of methods for food analysis. Moisture content, ash, protein, total fat and total dietary fiber were determined by AOAC (2012). Moisture content was determined by AOAC 925.10 method. Briefly, the sample was weighed into an aluminum dish with a cover and dried. The dish cooled to room temperature before measuring the final mass. Ash content was determined by AOAC 923.03 method using gravimetric analysis. Sample was heated at 550°C to constant weight and the ash is determined by weighing. For the content of protein was determined by Kjeldahl method. The sample was digested by H_2SO_4 and using $CuSO_4*5H_2O$ as catalyst with K_2SO_4 as boiling point elevator. Then, the distillation was done by add NaOH to release NH_3 and collect H_3BO_3 solution. The receiving solution was titrated, and the conversion factor was 6.25. Next, the total fat was determined by acid hydrolysis. The sample was homogenized, then the acid was used for hydrolysis. Diethyl ether and petroleum ether were employed for solvent extraction. Finally, the sample was dried until constant weight and weighted the residue. Total dietary fiber was employed by enzymatic-gravimetric method. In brief, the duplicate sample were weighted, dried and defatted for digestion. Thereafter, the sample was added phosphate buffer and α-amylase, then incubated at 100°C for 30 min. The protease enzyme was mixed after adjusting pH to 7.5 and incubated at 60°C for 30 min. Next,

amyloglucosidase was added at pH 4.5 and incubated at 60°C for 30 min. The residue was washed by ethanol and acetone, then filtrated, dried and weighted. One of set sample was determined for protein, using 6.25 as conversion factor. The another one was heated at 525°C and weighted to determine ash content. Total dietary fiber was calculated by the weight of residue – weight of ash and protein content. Carbohydrate content was determined by calculation follow the equation:

$$\text{Carbohydrate (\%)} = 100 - \% (\text{protein} + \text{fat} + \text{moisture} + \text{ash})$$

2.4 The identification of β-carotene

2.4.1 Sample extraction

The extraction method was performed according to a previous report (Ruttarattanamongkol et al. 2016) with minor modification. Briefly, 1 g flour and 1 g ascorbic acid were mixed in the test tube, then dissolved with 2 ml distilled water. Thereafter, 80% KOH (3 ml) and 0.1% BHT in ethanol (3 ml) were added, then stirred and heated with water bath shaker at 50°C for 30 minutes. After cooling down the test tube, the mixture of hexane and ethyl acetate (8:2) was added and left until the appeal of separation. The upper clear part was transferred and kept in amber bottle, then re-extracted the left part until the upper layer become colorless. The total supernatant was dried with nitrogen gas for storage at -20°C for further analysis.

2.4.2   HPLC analysis

The sample was dissolved with 1 ml of hexane. The HPLC system with UV-detector was employed. A reverse phase column 5 µm, 150 mm C_{18} with gradient elution was used. The HPLC condition was performed according to a previous report (Zhong et al. 2016) with minor modifications. Solvent A [80% acetonitrile, 15% methanol, and 5% dichloromethane (v/v)] and solvent B [30% acetonitrile, 20% methanol, and 50% dichloromethane (v/v)] were used and the condition was follows: 5–70% B (0–18 min), 70–5% B, (18–20 min), and 5% B, (20–25 min) with 0.8 ml/min flow rate and UV detection was performed at 458 nm wavelength. β-carotene was identified by comparing retention time with reference standard. The concentration of β-carotene was calculated from the standard curve of β-carotene.

2.5 Determination of total phenolic content and antioxidant properties

2.5.1   Sample extraction

The method of extraction was described by Maisarah et al. (Maisarah et al. 2013) with minor modifications. 5 g of sample was extracted with 80% methanol (50 ml), then incubated at room temperature with 200 rpm shaker for two hours. After that the mixture was centrifuged at 1,000 rpm for 15 min. The supernatant was kept and dried with evaporator.

2.5.2   Total phenolic content

Total phenolic content was determined using the Folin Ciocalteu method (Maisarah et al. 2013). The sample (10 mg) was dissolved with methanol. The Folin Ciocalteu reagent was diluted for 10-fold with distilled water. The sample (20 µl) was mixed with 150 µl of Folin Ciocalteu reagent, then incubated for 5 min. Next, 6% sodium bicarbonate solution (150 µl) was added and incubated for 90 min. The absorbance was read using a spectrophotometer at the wavelength of 760 nm. Gallic acid was used for the standard curve. The results were presented as Gallic acid equivalents (GAE) inw mg per 100 g sample extracts.

2.5.3   FRAP assay

FRAP assay was performed according to a previous study with minor modifications (Jorjong et al. 2015). FRAP reagent was prepared fresh daily and warmed 37°C. It was 0.3 M sodium acetate buffer, 10 mM TPTZ solution in 40 mM HCl and 20 mM of iron (III) chloride solution in ratio of 10:1:1 (v/v). Sample (20 µl) was mixed with 180 µl FRAP reagent in 96-well plate and incubate for 30 min. Spectrophotometer was used for analysis at wavelength 595 nm. Iron (II) sulfate solution was used for the standard curve. The results were expressed as millimole ferrous ion per gram dry weight (mmol Fe (II)/g DW).

    2.5.4   DPPH assay

        DPPH free radical scavenging method was performed according to a

previous study with minor modifications (Jariyapamornkoon et al. 2013). 40

mg/ml UPF extract (100 µl) was added with 100 µl of 0.2 mM DPPH solution in

methanol, then incubated for 30 min in dark at room temperature. After

incubation, the absorbance was measured at wavelength 515 nm. The results

were expressed as vitamin C equivalent in mg per 100 g sample extract.

    2.2.5   TEAC assay

        Trolox equivalent antioxidant capacity assay (TEAC) was performed

according to a previous study (Suantawee et al. 2015). The ABTS·$^+$ was

generated by the mixing of 7 mM ABTS in 0.1 M phosphate buffer saline (pH

7.4) with 2.45 mM $K_2S_2O_4$ in distilled water, then incubated at room temperature

for at least 16 hours in darkness. The working solution was diluted until the

absorbance at wavelength 734 nm about 0.700-0.800. The extract (5mg/ml) (10

µl) was mixed with 90 µl of working solution and incubated for 6 min. The

decrease in absorbance was measured at 734 nm. The TEAC value was

determined using the standard curve of Trolox. The results were expressed

micromole of Trolox equivalents per 100 g of extract.

  2.3   Bile acid binding

    The bile acid binding assay was performed according to previous study

(Adisakwattana et al. 2012). Briefly, the sample (final concentration 2 mg/ml) was

incubated with each bile acid including taurocholic acid, glycodeoxycholic acid and taurodeoxycholic acid (2mM) in 0.1 M phosphate buffered saline (PBS), pH=7, at 37 °C for 90 min. After that, the mixtures were separated bound form of bile acids by filter through 0.2 μm nylon filter. The free bile acids were determined by using bile acid analysis kit (Genway biotech. Inc, USA). The absorbance was recorded at 540 nm. Carboxymethyl cellulose (CMC) was used as positive control in this study.

2.4 Cholesterol micellization

The method of cholesterol micellization was described by Adisakwattana et al (Adisakwattana et al. 2012). Artificial micelles were imitated the natural mixed micelle by containing sodium taurocholate, egg lecithins, cholesterol, and oleic acid. First, 2 mM cholesterol, 1 mM oleic acid, and 2.4 mM phosphatidylcholine were dissolved in methanol and dried under nitrogen. The mixture was added 15 mM PBS containing 6.6 mM taurocholate salt, at pH=7.4 and sonicated twice for 30 min. The micelle solution was incubated at 37°C for 16 hr. The sample (final concentration 10 mg/ml) was incubated with the mixed micelle at 37°C for 2 hr. The mixture was centrifuged 10,000 rpm for 30 min, then collect the supernatant for determination of cholesterol by total cholesterol test kit. The absorbance was recorded at 500 nm. Gallic acid was used as a positive control.

2.5   Product development of unripe papaya flour

    2.5.5   Determination of appropriate ratio for partial wheat replacement

        in pancake

Pancake formula was made as follow commercial formula, and wheat

flour was replaced by unripe papaya flour for 5%, 10% and 20%.

Table 2 Formula of control pancake and UPF pancake (unit gram)

	wheat flour	sugar	Vegetable oil	milk	salt	baking powder	egg	UPF
control	112	40	21.5	220	1	21.5	56.5	0
5% UPF	106	40	21.5	220	1	21.5	56.5	6
10% UPF	100	40	21.5	220	1	21.5	56.5	12
20% UPF	89	40	21.5	220	1	21.5	56.5	23

The previous ingredients were mixed, and the batter was left for 15 min

at room temperature. Then, the frying pan was heated with medium heat and

pour 1 tablespoon of batter and brown on both sides for 1 min.

2.5.6   Texture analysis of pancake

The texture of pancake was determined by a texture analyzer (TA. XT plus texture analyzer, Stable Micro Systems, UK). Hardness, cohesiveness, springiness and chewiness were determined. A double bite compression test was performed and equipped with cylinder probe (SMPS/100). Pancake was placed on a flat base and compressed to a fixed height 5 mm and recorded using condition from previous study with some modifications (Yemmireddy et al. 2013). The condition was pretest speed: 1.0 mm/s, test speed: 1.0 mm/s, posttest speed: 1.0 m/s, compression distance: 25% and trigger force: 0.098N.

2.5.7   Color measurement of pancake

The surface color of pancakes was performed by colorimeter. The colorimeter was standardized with black glass and white calibrated tile. After that the pancake was place over the port insert and recorded the color of surface pancake represented as L^*, a^* and b^* value.

2.5.8   Determination of total starch

Total starch content was determined followed a previous report with some modifications (Goni et al. 1997). Pancake samples were weighted 50 mg in 50 ml screw-cap tube and mixed with 6 ml of 2 M KOH. Then, the mixture was incubated at room temperature for 1 hr. After incubation, 3 ml of 0.4 M sodium acetate buffer was added and adjust pH to 4.75. Next, amyloglucosidase (3,260 U/ml) was added for 60 µl and incubated at 60°C,

shaking 100 rpm for 45 min. The mixture was aliquoted and heated at 100°C in heat block for 10 min, then centrifuged at 13,000 rpm for 5 min. The concentration of glucose was determined by glucose oxidase kit. The glucose concentration was converted into starch by multiplying 0.9. The total starch (TS) was calculated and presented in gram/100gram sample.

   2.5.9   *In vitro* starch digestion of pancake

   The procedure of *in vitro* digestion was performed according to a previous study with some modifications (Yousif et al. 2012).The sample was weighted 500 mg in a 50 ml screw-cap plastic tube. 1 ml of Porcine α-amylase (250 U/ml; α-Amylase from porcine pancreas Type VI-B, ≥5 units/mg) solid was added. Then, 5 ml of pepsin solution (4500 U/ml; Pepsin 1:3000 ex. Porcine stomach mucosa, 0.8 Anson U/mg) was added, and the mixture was incubated at 37°C in water bath shaking 100 rpm for 1 hr. The mixture was neutralized by adding 5 ml of 0.02M NaOH, and 25 ml of 0.2M sodium acetate buffer was added afterward. Finally, 5 ml of the mixture of pancreatin (2mg/ml) and Amyloglucosidase (28 U/ml; Amyloglucosidase (*Aspergillus niger*) ~3,260 U/ml) was added and incubated for 180 min. The aliquot was kept at the difference time point for 0, 20, 30, 60, 90, 120 and 180 min, then heated at 100°C in heat block for 10 minutes and centrifuged at 13,000 rpm for 10 min. The aliquots were kept in -20°C for further analysis.

The concentration of glucose was determined using glucose oxidase kit. In brief, the sample (5 µl) was mixed with 500 µl reagent and incubated for 10 min. The absorbance was determined at the wavelength of 500 nm. The area under the curve (AUC) was calculated by glucose concentration curve using the trapezoidal rule. Glycemic index was calculated from equation with the use of glucose as the reference food, whereas hydrolysis index (HI) was calculated as AUC of sample as percentage of AUC of the reference food.

$$GI = 39.71 + 0.549HI$$

For starch content, the glucose concentration was converted into starch by multiplying 0.9. The rapidly digestible starch (RDS), slowly digestible starch (SDS) and undigestible starch were calculated from described equation (Englyst et al. 1992, Zhang and Hamaker 2012).

$$RDS\ (\%) = (G_{20} - FG)/TS*100$$

$$SDS\ (\%) = (G_{120} - G_{20})/TS*100$$

$$Undigestible\ starch\ (\%) = (TS - (RDS+SDS))/TS*100$$

2.5.10 Determination of total phenolic content and antioxidant

properties

2.5.10.1 Sample extraction

The extraction was described as the abovementioned method.

2.5.10.2 Total phenolic content

The content of total phenolics was described as the

abovementioned method.

2.5.10.3 FRAP assay

FRAP assay was performed according to the abovementioned

method.

2.5.11 Sensory evaluation

Sensory evaluation of pancake was performed with untrained 50

volunteers the inclusion and exclusion criteria given below:

Inclusion criteria

- Participants with all gender aged 18-50 years old

- Healthy participants

- Voluntary participation in research project

Exclusion criteria

- Gluten allergy

- Lactose intolerance

- Participants with eating and swallowing difficulty

- Participants with sensory problem for example color blindness, cough, sore throat.

- Pregnant or breast-feeding woman

The study was exempted for ethic review in compliance with the office for Human Research Protection (OHRP Exempt categories) by the office of Ethics Review Committee for Research Involving Human Research Subjects, Human Science Group, Chulalongkorn University (COA No.133/2019). Participants were given a sensory evaluation form and pancake in circle shape with 2.5 cm diameter and 10 g weight in difference ratio of unripe papaya flour replacement for 4 formulations (0%, 5%, 10% and 20%). In each formulation was coded with the difference number. This process was performed at 2nd floor of Chulaphat 14 building in the sensory evaluation room for 20 minutes. Participants were participated for 1 time by eating sample, spitting out and drinking water to rinse their mouth between sample then giving score in sensory evaluation form. Seven parameters were employed on appearance, color, odor, taste, aftertaste, texture, and overall acceptance by lining scale.

2.6 Statistical analysis

All experiments were performed at least triplicate and data was expressed as mean ±SEM. Difference was considered statistically significant at $P < 0.05$. Data was analyzed using independent sample t-test for comparing mean between UPF and wheat flour for bulk density, water absorption, solubility index, swelling power and antioxidant activities. One-way ANOVA followed by Duncan's post hoc test was employed for the significant differences among group of pancake conditions for texture analysis, color measurement, starch content, glycemic index and antioxidant activities. Sensory evaluation was performed the Kolmogorov-Smirnov test for normality, then using Kruskal-Wallis Test for significant difference test. All statistical analysis was conducted using SPSS version 22.0.

CHAPTER IV

RESULTS

1. Physicochemical and functional properties of unripe papaya flour

1.1 Physicochemical properties of flours

1.1.1   Morphology and particle size distribution of unripe papaya flour

The particle size distribution was employed by a laser diffraction-based Malvern particle size analyzer. The mean particle size was 140.8±2.1 μm. The observed the particle size was a bimodial distribution (Figure 6). The first peak was presented at <100 μm and the second was observed at >200 μm. As shown in Figure 7, the irregular shape of flour was observed.
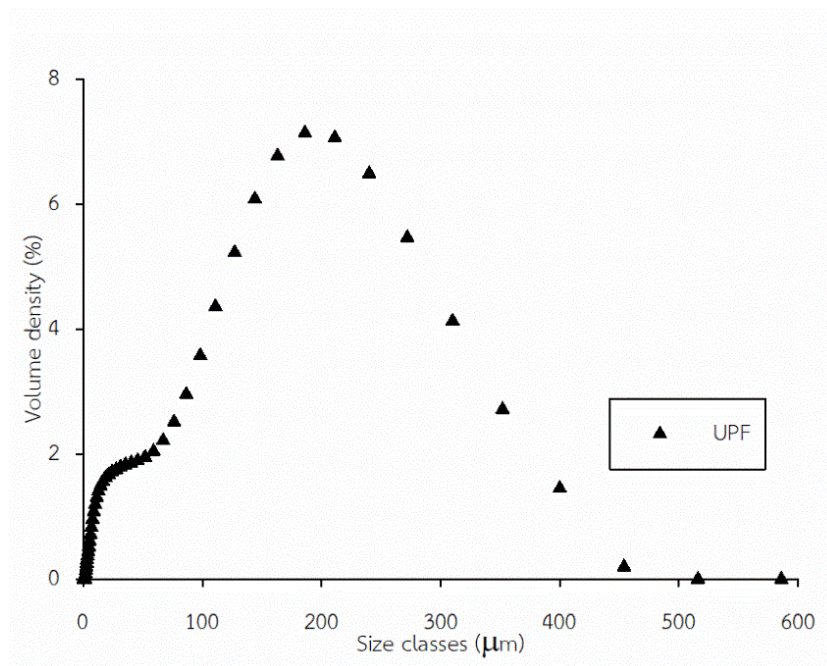
Figure 6 Particle distribution by volume density of unripe papaya flour
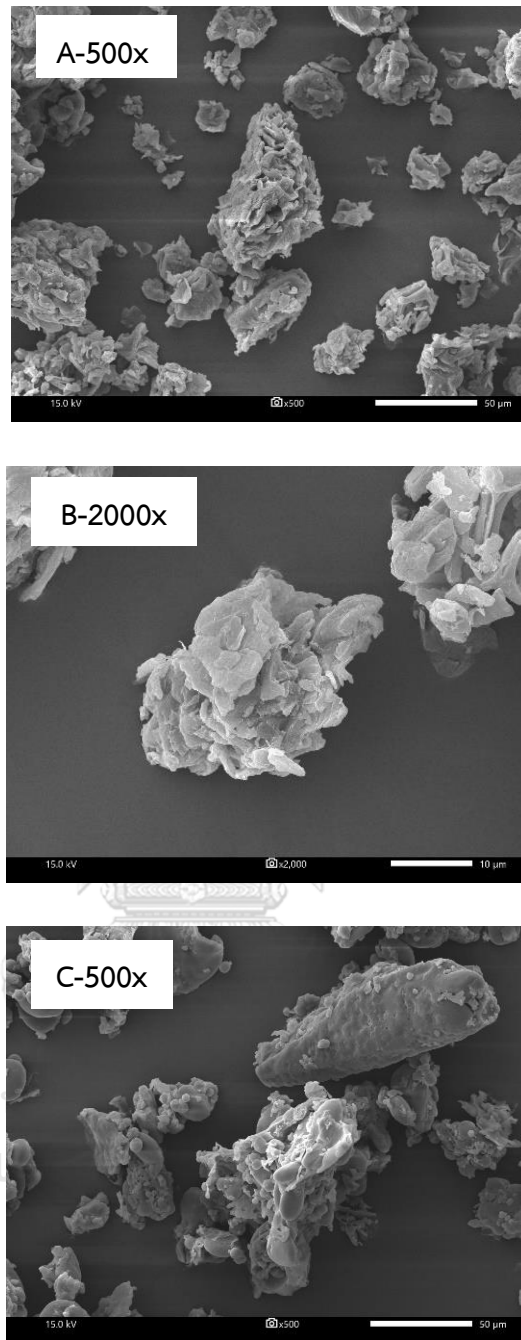
Figure 7 Scanning electron micrograph of flours. Unripe papaya flour (A) magnified 500x, (B) 2000x and wheat flour (C) magnified 500x

1.1.2   Gel hydration properties and bulk density of flours

Gel hydration properties indicated from water absorption index, water solubility index and swelling power, are shown in Table 3, UPF had significantly higher hydration properties than wheat flour. It could be observed that UPF had WAI and SP two times, and WSI 5 times higher than WF. On the other hand, bulk density of UPF was not difference when compared to WF.

1.1.3   The appearance of flours

The results of color measurement are presented in Table 3. The lightness of UPF was presented lower than WF, whereas the redness and yellowness was higher than WF. The color parameters correlated with the appearance of flour as shown in Figure 8. UPF exhibited the light-yellow color with coarser particle, while wheat flour showed the white color with fine particle.

Table 3 Gel hydration properties, bulk density and color parameters of flours

Sample	WAI (g/g)	WSI (g/100g)	SP (g/g)	Bulk density (g/ml)	Color parameters		
					$L*$	$a*$	$b*$
WF	7.51 ± 0.01^a	2.93 ± 0.53^a	7.74 ± 0.03^a	0.53 ± 0.02^a	90.11 ± 0.01^a	0.42 ± 0.01^a	1.02 ± 0.01^a
UPF	13.53 ± 0.11^b	11.40 ± 0.18^b	15.28 ± 0.1^b	0.58 ± 0.01^a	84.32 ± 0.01^b	0.85 ± 0.00^b	14.89 ± 0.01^b

WF: wheat flour; UPF: Unripe papaya flour.

WAI: water absorption index; WSI: water solubility index; SP: swelling powder.

$L*$, lightness, $a*$, redness and $b*$, yellowness.

Data are expressed as mean ± SEM, n = 3.

Values with different letters in each column are considered significant differences (P<0.05).
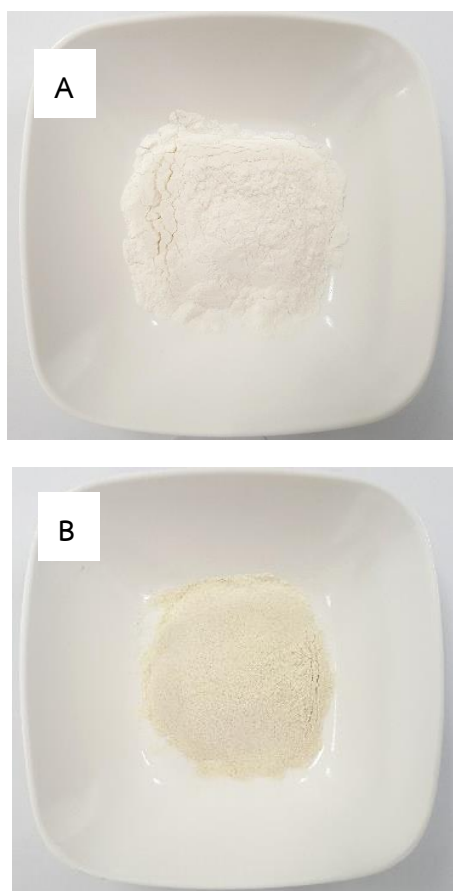
Figure 8 The appearance of wheat flour (A) and unripe papaya flour (B)

1.2 Proximate analysis of unripe papaya flour

The proximate composition of unripe papaya flour is shown in Table 4. Total dietary fiber was the abundant component of flour for 56.14% and followed by available carbohydrate 21.02%. The other major components were followed by moisture (11.61%), ash (5.22%), protein (4.65%), and total fat was the least components (1.36%).

Table 4 The proximate analysis of unripe papaya flour

Parameters	Results (g/100g sample)
Moisture	11.61
Ash	5.22
Protein (N x 6.25)	4.65
Total fat	1.36
Carbohydrate	
— Available carbohydrate	21.02
— Total dietary fiber	56.14

1.3 Identification of β -carotene

Unripe papaya flour was identified for β-carotene content by high performance liquid chromatography (HPLC). The UPF extract was mixed with the standard of commercial β-carotene to confirm the peak of β-carotene. The elevation of peak area in the same peak can be affirmed as the β-carotene peak. The β-carotene was identified at peak 1 with retention time of 19.833 min (Figure 10). The content of β-carotene in unripe papaya flour was 39±3.3 μg/100 g flour. The chromatogram of β-carotene standard (0.25 μg/ml) is presented in Figure 9.
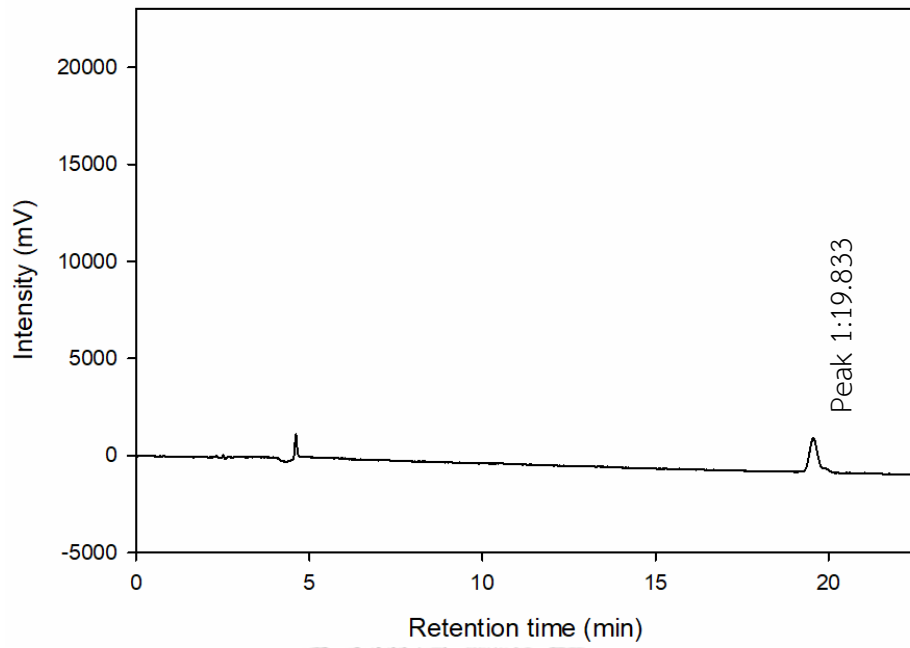
Figure 9 Chromatogram of 0.25 μg/ml β-carotene standard
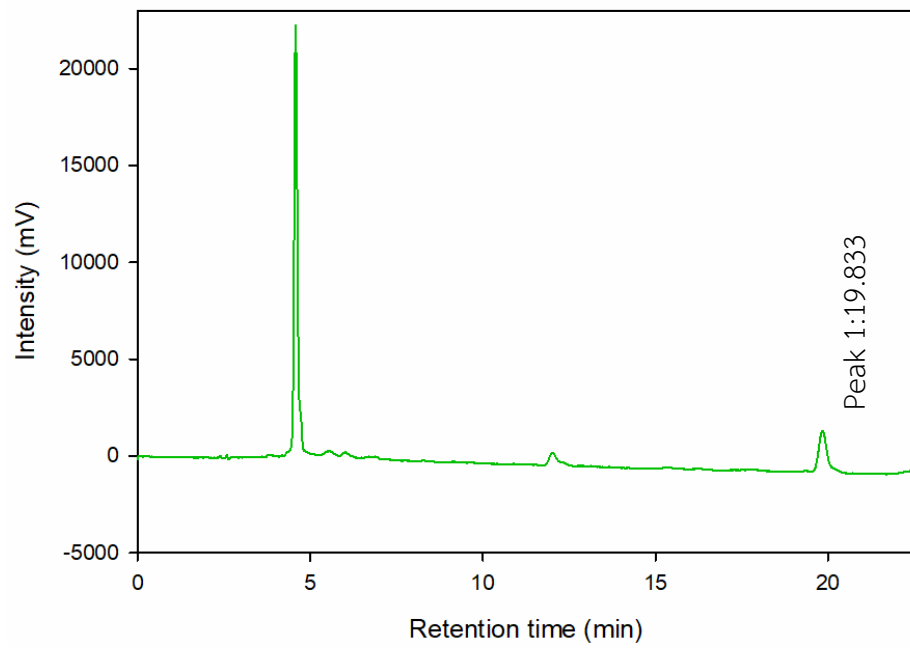
Figure 10 Chromatogram of unripe papaya flour

1.4 Total phenolic content and antioxidant properties of flours

The phytochemical content was determined by Folin–Ciocalteu and the antioxidant properties were determined by FRAP assay, TEAC assay, and DPPH assay. UPF showed significantly higher antioxidant activity including total phenolic content, FRAP, and DPPH as presented in Table 5. It could be observed that UPF had 2-time higher total phenolic content than WF. Moreover, the DPPH radical scavenging activity and FRAP value of UPF was 10 times, and 50 times higher than that of WF. Nevertheless, TEAC value was not significantly different from WF. The calibration curve of total phenolic content, FRAP assay, DPPH assay and TEAC assay are shown in Figure 11-14.

The calibration curve of gallic acid

$y = 2.8079x + 0.0151$

$R^2 = 0.9985$
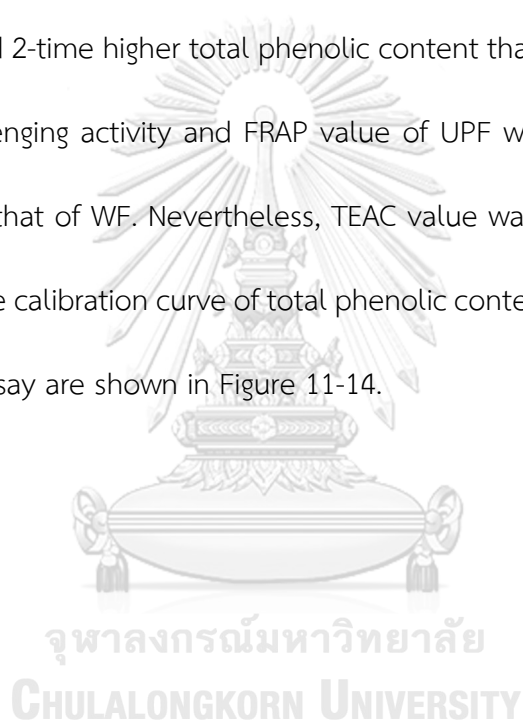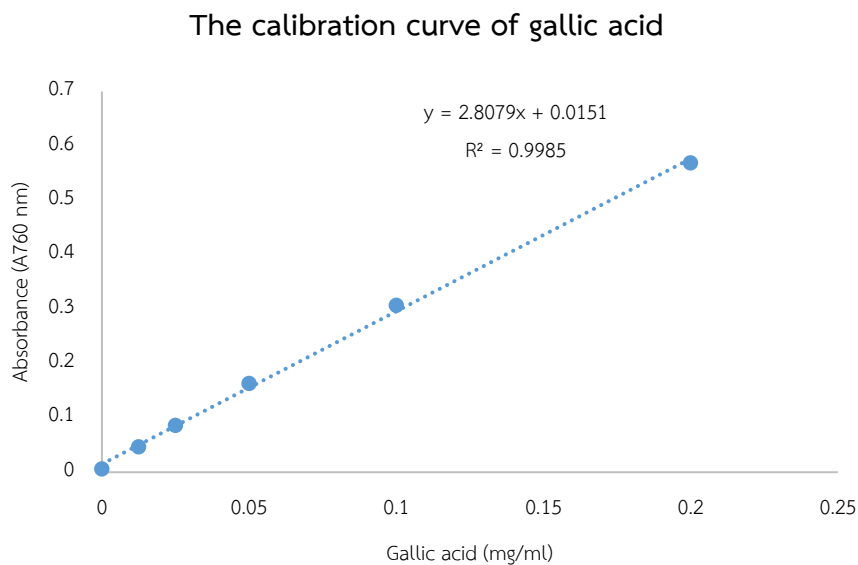
Figure 11 The calibration curve of gallic acid (0 − 0.2 mg/ml).

The linear regression was employed for calibration curve, and $R^2 = 0.99$.

The calibration curve of $FeSO_4$

$y = 0.0013x + 0.0889$

$R^2 = 0.9997$
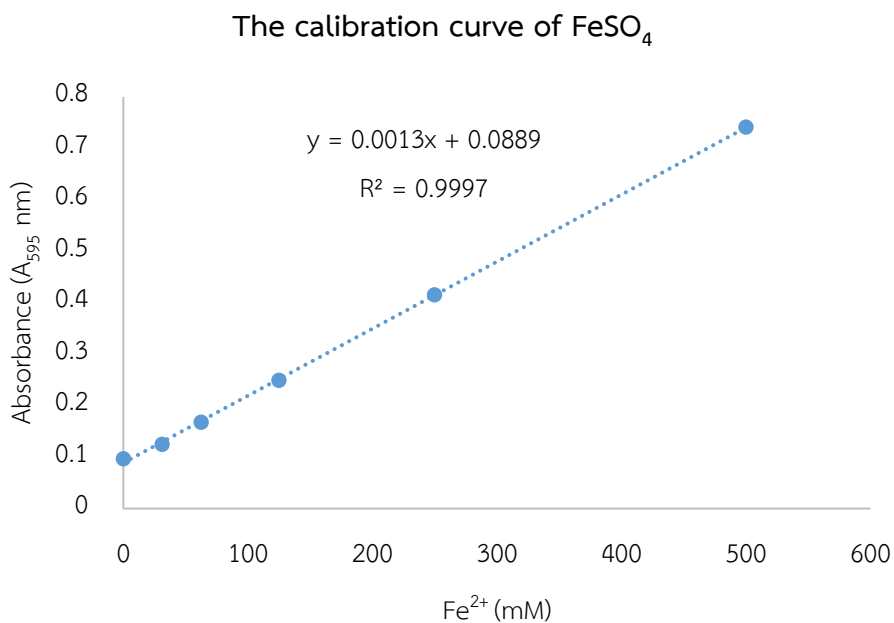
Figure 12 The calibration curve of $FeSO_4$ (0 − 500 µM).

The linear regression was employed for calibration curve, and $R^2 = 0.99$.
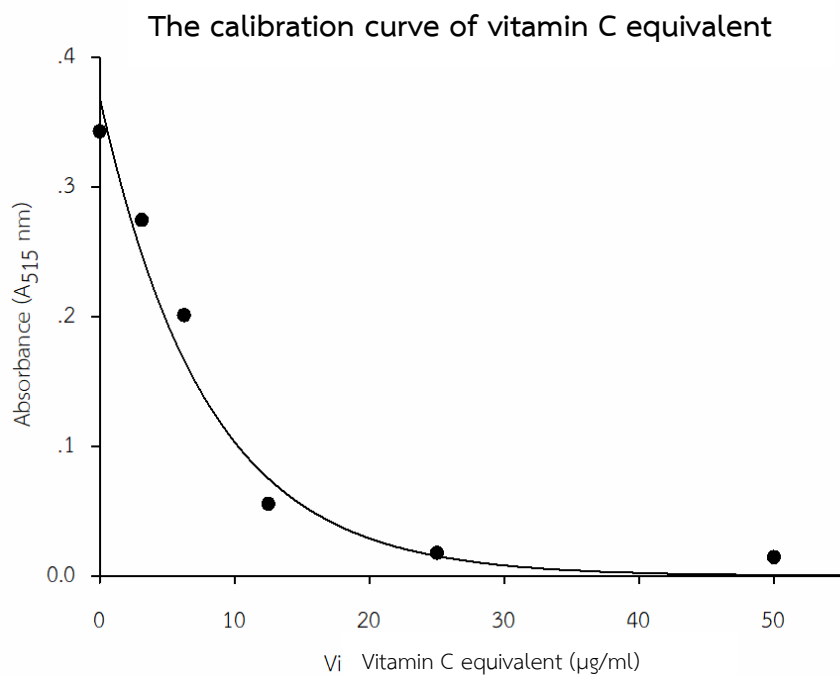
Figure 13 The calibration curve of Vitamin C equivalent (0 – 50 µg/ml).
The exponential decay was employed for calibration curve, and R^2 = 0.95.
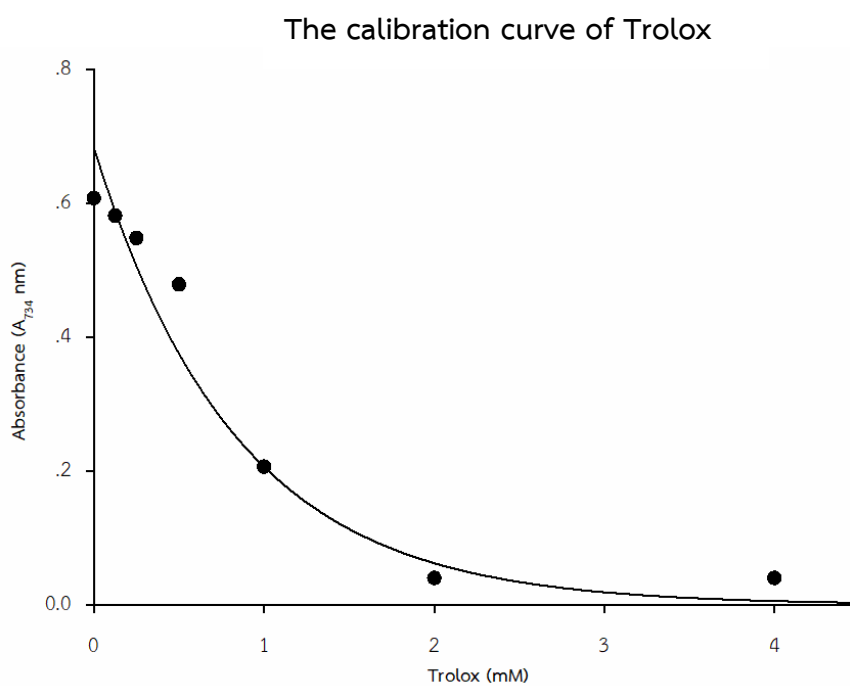
Figure 14 The calibration curve of Trolox (0 – 4 mM).
The exponential decay was employed for calibration curve, and R^2 = 0.96.

Table 5 Comparison of total phenolic content and antioxidant activity of wheat flour and unripe papaya flour

Type (per 100 g DW)	TPC (mg GAE)	FRAP (μmol Fe (II))	TEAC (mmol TE)	DPPH (mg vitamin C equivalent)
WF	36.09 ± 1.27^a	7.19 ± 0.08^a	0.69 ± 0.06^a	3.25 ± 0.28^a
UPF	85.67 ± 1.62^b	411.58 ± 38.00^b	0.77 ± 0.06^a	37.87 ± 3.69^b

Total phenolic content (TPC) was expressed as milligram gallic acid equivalents (GAE)/100 g DW. FRAP was expressed as micromole ferrous/100 g DW. TEAC was expressed as millimole Trolox equivalent/100 g DW. DPPH radical scavenging activity was expressed as the milligram vitamin c equivalent/100 g DW

Data are expressed as mean ± SEM, n = 3.

Values with different letters in each column are considered significant differences ($P < 0.05$).

1.5 Bile acid binding

Taurocholic acid, glycodeoxycholic acid, and taurodeoxycholic acid were used as bile acids in this experiment. It was found that UPF had the ability to bind both primary bile acid (taurocholic acid) and secondary bind acids (glycodeoxycholic acid and taurodeoxycholic acid). However, ability of UPF, glycodeoxycholic acid and taurodeoxycholic, to bind bile acid was 2 times less than CMC. On the other hand, it showed no significant difference on binding ability of taurocholic acid.

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Table 6 The percentage bile acid binding of UPF

Sample	Bile acid binding (%)		
	Taurocholic acid	Glycodeoxycholic acid	Taurodeoxycholic acid
UPF (2mg/ml)	8.35 ± 0.72^{a}	8.46 ± 0.19^{a}	11.89 ± 0.35^{a}
CMC (2mg/ml)	8.74 ± 1.13^{a}	18.70 ± 0.71^{b}	21.30 ± 1.38^{b}

CMC (Sodium Carboxymethyl Cellulose) was employed for positive control, UPF; Unripe papaya flour.

Data are expressed as mean ± SEM, n = 3.

Values with different letters in each column are considered significant differences (P<0.05).

1.6 Cholesterol micellization

As shown in Figure 15, UPF (10mg/ml) had the ability to interfere the formation of cholesterol micellization. It was found that UPF reduced cholesterol micellization about 35.00±2.22%, which was higher than CMC (22.9±1.84%). Nevertheless, gallic acid presented the highest ability on interfering cholesterol micellization (92.03±0.63%).
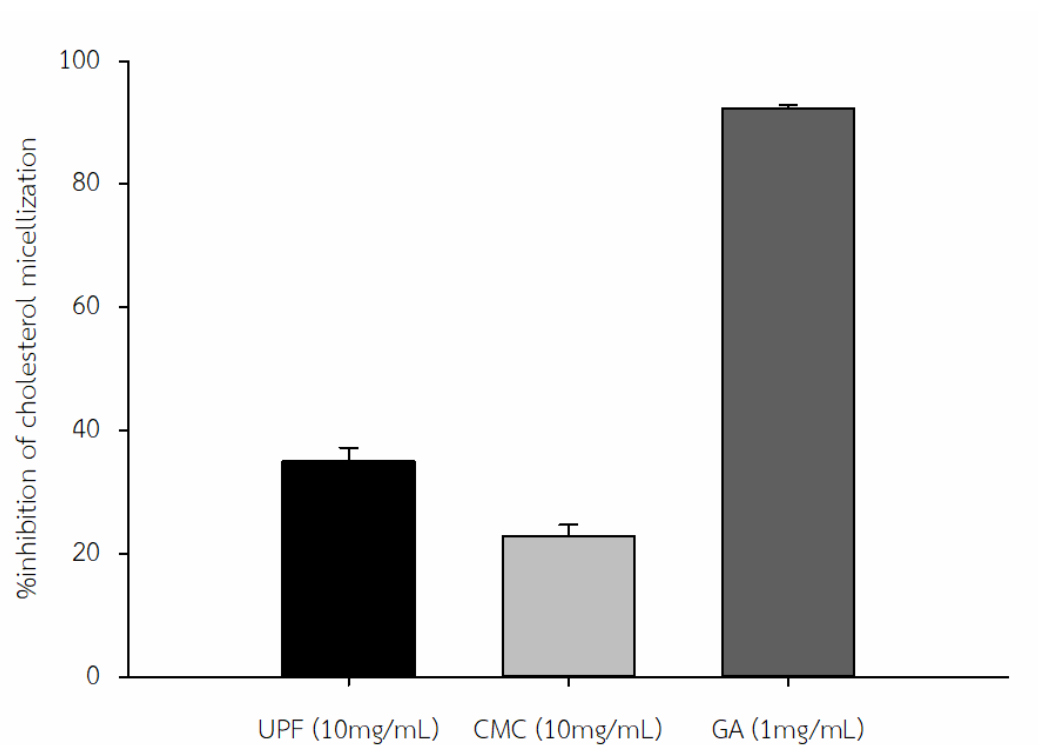
Figure 15 The percentage cholesterol micellization inhibition of unripe papaya flour,

CMC and gallic acid.

UPF; Unripe papaya flour, CMC; Sodium Carboxymethyl Cellulose, GA; Gallic acid

Data are expressed as mean ± SEM, n = 4. Values with different letters in each column

are considered significant differences (P<0.05).

2. **Functional properties of unripe papaya flour as a wheat partial replacement in pancake**

2.1. The formulation of pancake replacement with unripe papaya flour

The pancake formulation was produced for 4 formulations including the control pancake (no UPF replacement), 5%UPF, 10%UPF and 20%. The appearance of control and UPF pancake was presented in Figure 16 (top view) and Figure 17 (front view). The shape and size of pancake were control by the circle mold. It was observed that the substitution of wheat flour with UPF caused a decrease in aeration of pancake, resulting in decreased pancake height, observed by the appearance of pancake front view (Figure 17).

The difference, that could be observed directly, was the color of pancake surface (Figure 16). Pancake replacement with a higher level of UPF had darker shade of brown color on surface than control pancake.

Control   5%UPF   10%UPF   20%UPF
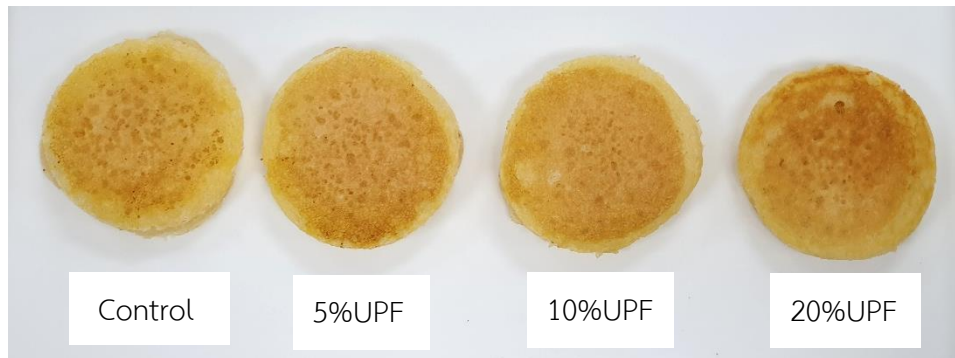
Figure 16 The appearance of control and unripe papaya pancake (top view).

Control   5%UPF   10%UPF   20%UPF
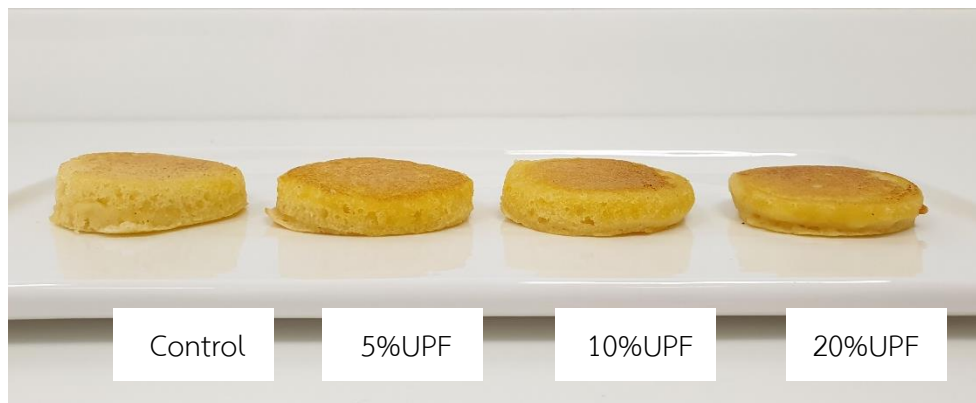
Figure 17 The appearance of control and unripe papaya pancake (front view).

Control   5%UPF   10%UPF   20%UPF
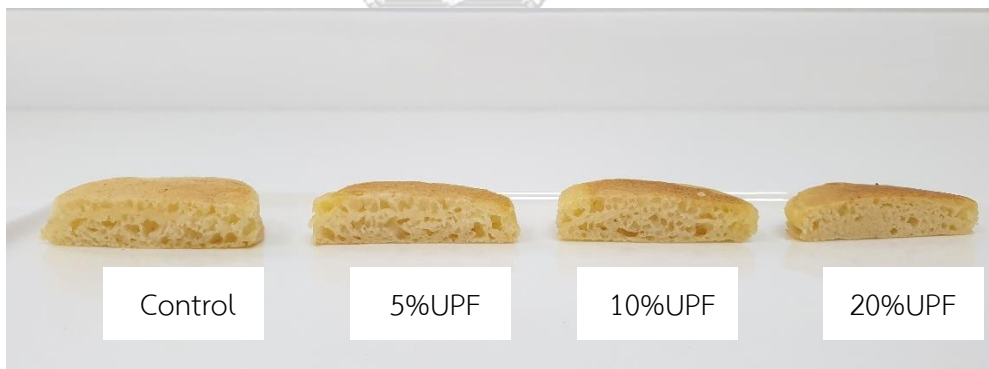
Figure 18 The cross-sectional of control and unripe papaya pancake (front view).
Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF;

pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

2.2. Texture profile analysis of pancake with unripe papaya flour (UPF)

The results of textural profile of pancake with UPF are presented in Table 7. The replacement of wheat flour with UPF greatly affected the texture profile of pancake. The hardness and chewiness of pancakes increased with a higher ratio of UPF replacement. It could be observed that pancake with 10% of UPF replacement had significantly higher than the control pancake. UPF replacement of at least 10% increased the hardness of pancake when compared to the control pancake. On the other hand, springiness and cohesiveness of pancake with UPF were not different from the control pancake.
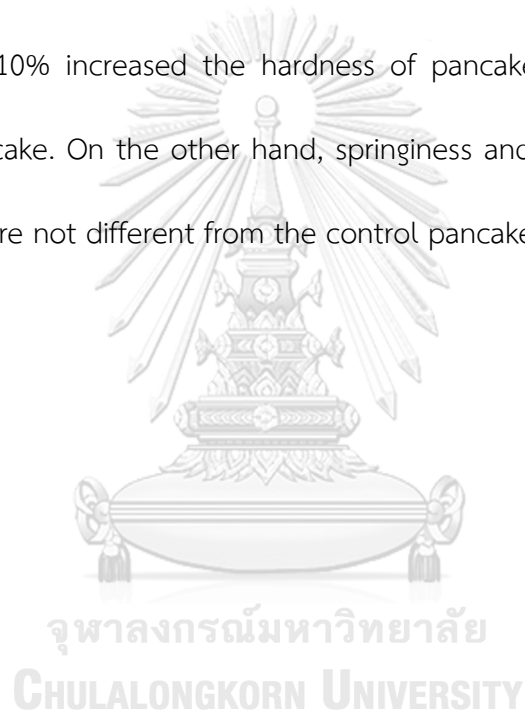
Table 7 Texture profile of pancake with UPF as wheat partial replacement

Type of pancake	Hardness (g-force)	Springiness (mm)	Cohesiveness	Chewiness (g-force.mm)
control	220.74±26.11[a]	0.97±0.05[a]	0.88±0.01[a]	192.92±33.01[a]
5% UPF	293.63±37.10[a]	0.92±0.03[a]	0.86±0.01[a]	242.05±37.53[a,b]
10% UPF	491.46±32.2[b,c]	0.93±0.03[a]	0.86±0.00[a]	398.32±37.24[b,c]
20% UPF	568.54±37.90[b,c]	0.93±0.02[a]	0.85±0.00[a]	453.36±44.09[c,d]

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF; pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

Data are expressed as mean ± SEM, n = 4. Values with different letters in each column are considered significant differences (P<0.05).

2.3. Color measurement of pancake surface

The results of surface color are shown in Table 8. As expected, the addition of UPF affected the color of pancake. Lightness ($L*$) of pancake was decreased by increasing of UPF. In contrast, redness (a*) and yellowness ($b*$) were significantly increased when the level of replacement increased. The significant difference could be observed when at least 5% UPF replacement was added. The result showed that 20% UPF replacement had the highest redness and yellowness. Nevertheless, 20% UPF pancake was the lowest lightness value among of all pancakes.

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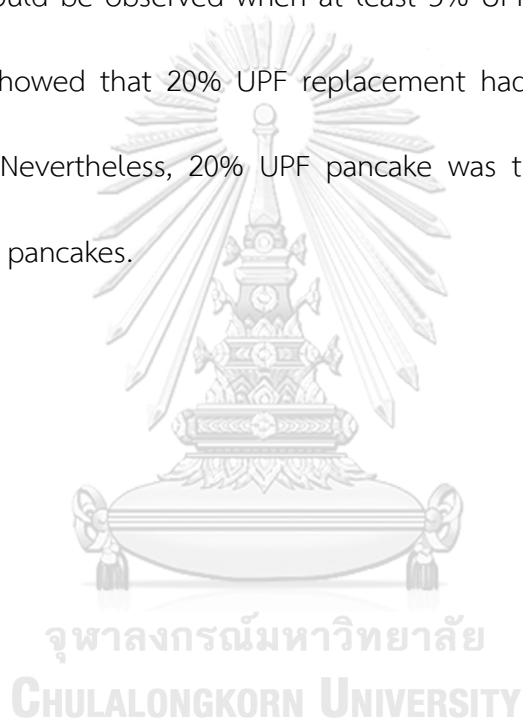
Table 8 The surface color of control pancake and unripe papaya pancake

Sample	Color parameters		
	L^*	a^*	b^*
Control	68.45±0.55[a]	7.69±0.23[a]	45.41±0.31[a]
5%UPF	66.82±0.50[a]	9.43±0.42[b]	42.5±0.39[b]
10%UPF	64.13±0.25[b]	10.63±0.62[b]	42.11±0.35[b]
20%UPF	62.87±0.72[b]	14.04±0.33[c]	40.12±0.55[c]

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF;

pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

L^*; lightness, a^*; redness and b^*; yellowness. Data are expressed as mean ± SEM, n = 3.

Values with different letters in each column are considered significant differences (P<0.05).

2.4. *In vitro* digestion of pancake

Four formulations of pancake were employed for *in vitro* starch digestion. Pancakes with UPF and the control pancake released the glucose within 20 min. At the individual time point, pancake with UPF presented slower release of glucose concentration after 30 min, as present in Figure 19. It could be observed that pancake with the addition of UPF can significantly attenuate the glucose release after 60 min. As shown in Figure 20, the UPF replacement pancake demonstrated 0.75-fold reduction of iAUCs for glucose. The hydrolysis index of pancake had a significant difference among the groups as shown in Table 9. An increase in UPF replacement in pancake led to decrease hydrolysis index. They also affected predicted glycemic index. The range of predicted glycemic index of pancake was between 50.11±0.07 to 53.50±0.35. To summary, the control pancake had the highest pGI, whereas the increase of UPF higher level could decrease the pGI value.
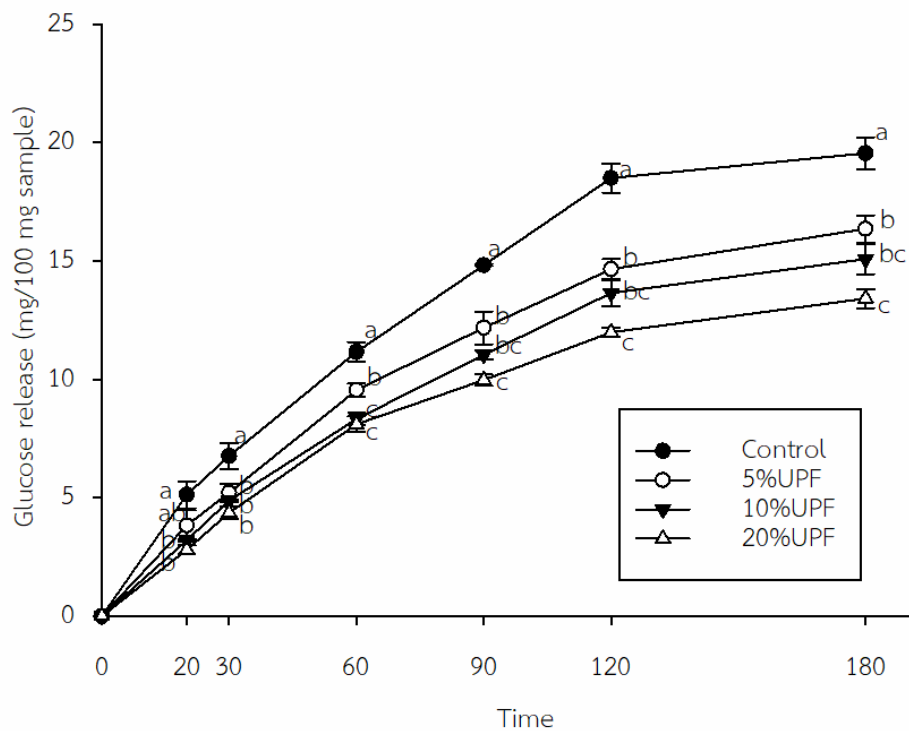
Figure 19 The effects of unripe papaya flour replacement in pancake on glucose releasing.

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF; pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

Data are expressed as mean ± SEM, n = 3. Values with different letters in each column are considered significant differences (P<0.05).

Figure 20 the area under the curves (AUCs) for glucose of four different pancakes

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF;

pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

Data are expressed as mean ± SEM, n = 3. Values with different letters in each column are

considered significant differences (P<0.05).

Table 9 The total starch content and predicted glycemic index of pancake

	TS (g/100g)	HI (%)	pGI
control	20.96±0.52[a]	25.12±0.63[a]	53.50±0.35[a]
5%	18.81±0.42[a]	21.88±0.27[b]	51.72±0.15[b]
10%	18.31±0.44[ab]	20.69±0.27[b]	51.07±0.15[b]
20%	16.49±0.25[b]	18.93±0.12[c]	50.11±0.07[c]

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF; pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

TS; total starch content, HI; hydrolysis index, pGI; predicted glycemic index

Data are expressed as mean ± SEM, n = 3.

Values with different letters in each column are considered significant differences (P<0.05).

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2.5. Starch content of pancake

Pancakes were determined for rapid digestible starch (RDS), slow digestible starch (SDS) and undigestible starch. and total starch (TS). The total starch content of pancake ranged between 16.49±0.25 to 20.96±0.52 g/100g sample. The control pancake was the highest total starch content (20.96±0.52 g/100g sample) and follow by pancake with reduced substitution of UPF. Consequently, RDS and SDS were found to be the highest content in control pancake (22.00±2.45% and 57.42±1.42 %starch content respectively), while the pancake with 20% UPF replacement had the lowest content (15.27±1.03 and 50.12±1.92 %starch content). However, it showed no significant difference between the control and UPF pancake. On the other hand, 20% UPF pancake significantly increased undigestible starch when compared to the control pancake.
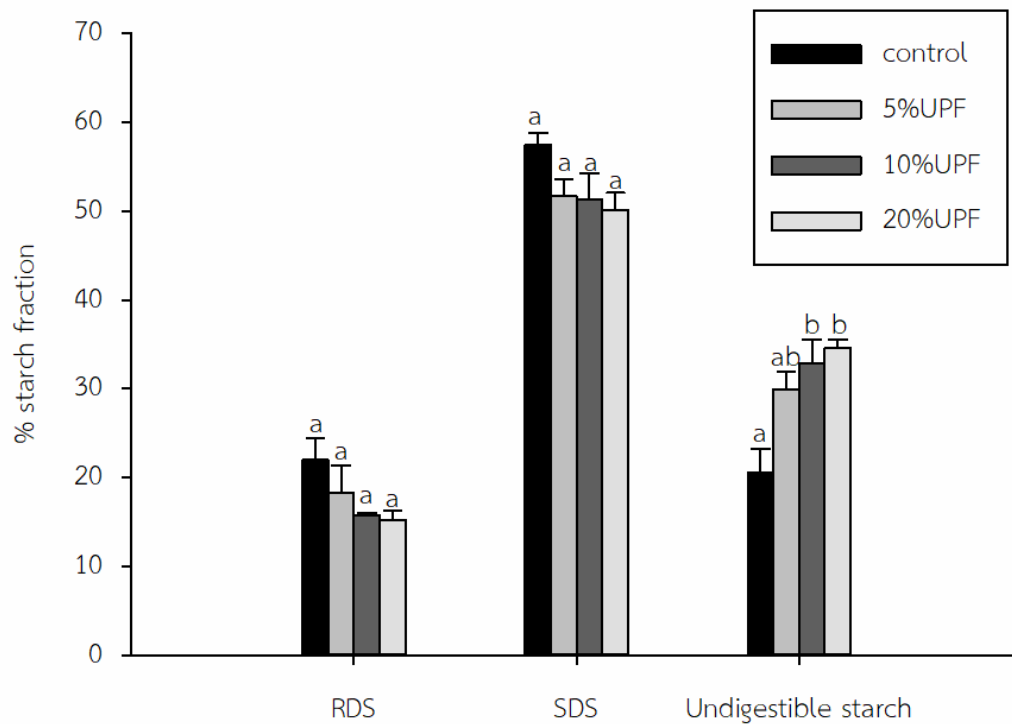
Figure 21 The effects of unripe papaya flour replacement in pancake on percentage

of starch fraction including rapidly digestible starch (RDS), slowly digestible starch

(SDS) and undigestible starch

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF;

pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

Data are expressed as mean ± SEM, n = 3. Values with different letters in each column are

considered significant differences (P<0.05).

2.6. **Total phenolic content and antioxidant property of pancake**

The total phenolic content and antioxidant activity (FRAP assay) were determined in pancake 4 formulations. The results showed that UPF affected the level of total phenolic content and antioxidant property, as presented in Table 10. The total phenolics were significantly elevated in pancake with UPF, especially in 10-20% of UPF replacement formulations. Moreover, it was observed that FRAP value was two time higher in 20% UPF replacement when compared to the control pancake.

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Table 10 The effects of UPF replacement in pancake on total phenolic content and

antioxidant activity (FRAP assay)

Sample (per 100g DW)	TPC (mg GAE)	FRAP (mM Fe (II))
Control	14.13 ± 0.46^a	31.99 ± 1.71^a
5%UPF	14.58 ± 0.61^a	$34.50\pm2.32^{a,b}$
10%UPF	17.37 ± 0.55^b	$44.27\pm2.67^{b,c}$
20%UPF	18.65 ± 0.69^b	55.07 ± 3.81^c

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF;

pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

Total phenolic content (TPC) was expressed as milligram gallic acid equivalents

(GAE)/100 g DW. FRAP was expressed as milligram ferrous/100 g DW.

Data are expressed as mean ± SEM, n = 3.

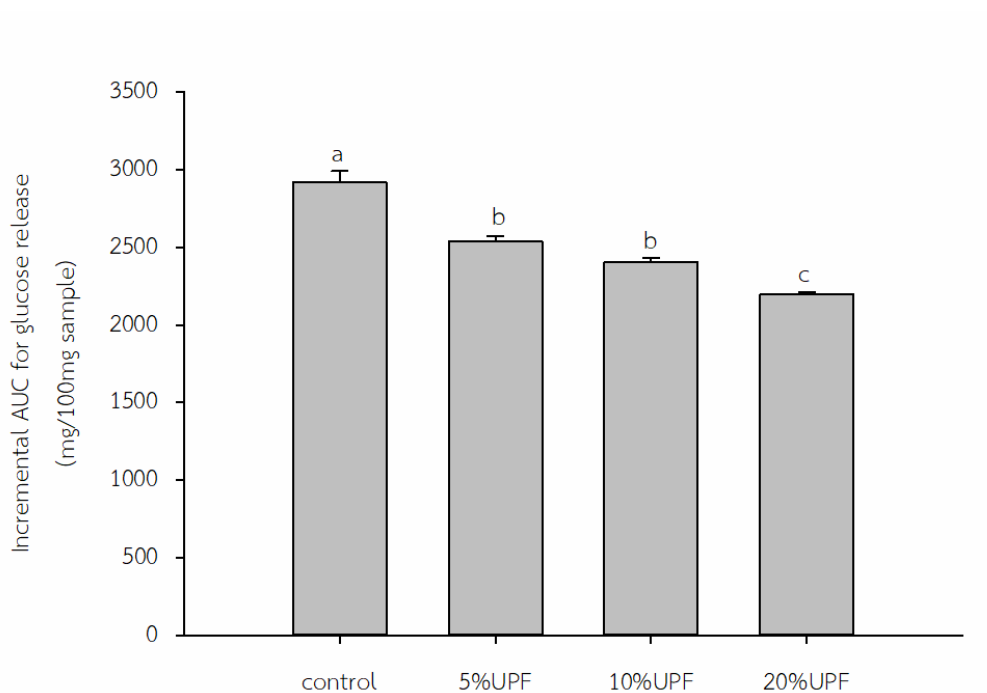Values with different letters in each column are considered significant differences (P<0.05).

2.7. Sensory evaluation

In this experiment, 4 formula of pancake; 3 UPF replacement with different ratio and 1 control pancake were deployed. The line scaling was used with the ranging score of 0-10. The seven parameters were determined for acceptance of participants including appearance, color, odor, taste, texture, aftertaste and overall acceptance. The result showed no significant difference among group of all parameters, except taste and aftertaste, as presented in Table 11. The result indicated that pancake with the highest ratio of UPF replacement significantly decreased the score of taste and aftertaste. It could be observed that participants prefer the control pancake rather than UPF; however, there was no significant difference among groups statistically.

Table 11 Sensory evaluation score of pancakes with unripe papaya flour replacement

Sample	Appearance	Color	Odor	Taste	Texture	Aftertaste	Overall acceptance
Control	6.83±0.28[a]	6.57±0.32[a]	6.24±0.36[a]	6.45±0.27[a]	6.65±0.29[a]	6.52±0.28[a]	6.63±0.29[a]
5%UPF	6.06±0.31[a]	6.18±0.33[a]	6.97±0.28[a]	6.63±0.32[a]	6.28±0.37[a]	6.22±0.31[a]	6.24±0.35[a]
10%UPF	6.29±0.27[a]	6.10±0.32[a]	6.87±0.28[a]	6.23±0.38[a,b]	6.59±0.32[a]	5.5±0.34[a]	6.16±0.35[a]
20%UPF	6.93±0.28[a]	6.95±0.28[a]	6.20±0.32[a]	5.27±0.35[b]	5.78±0.34[a]	5.31±0.33[b]	5.90±0.32[a]

Control; control pancake, 5%UPF; pancake with 5% UPF replacement, 10%UPF; pancake with 10% UPF replacement, 20%UPF; pancake with 20% UPF replacement.

Data are expressed as mean ± SEM. n=50

Values with different letters in each column are considered significant differences (P<0.05).

CHAPTER V

DISCUSSIONS

In the current study, researchers aimed to study the preparation method of flour from the top and bottom part of waste unripe papaya from fruit processing industry. The convection drying, followed by milling is the most prominent process. The unripe papaya flour was made using a hot air oven and a high-speed universal grinder, then sieved through 100-mesh for particle size.

UPF showed the average particle size of papaya flour (<150 μm). It was observed that UPF had the different range of particle size of other flours from waste products including pineapple stem (9.96 μm), unripe banana (80-156 μm) and passion fruits (<400 μm) (López-Vargas et al. 2013, Nakthong et al. 2017, Segundo et al. 2017a). For wheat flour has average particle size around 200 μm (Codex alimentarius 1995). The different particle size of flour made from fruit waste depend on species and the method of preparation (Yu et al. 2018). The morphology of unripe papaya flour showed irregular shape which may be due to the milling method. Morphology of flour granule plays an important role in application. The irregular shape can be presented in dry-milling method. The disruption of structure could affect the hydration properties by increase water absorption and swelling property (Horstmann et al. 2017). However, it shows beneficial effect on nutritional value that it still restores substances of granule

including dietary fiber indicating by the compact and aggregate structure (Pelissari et al. 2012, Leewatchararongjaroen and Anuntagool 2016, Yu et al. 2018).

The color of flour could be one of the important factors for the consumer's selection (Franklin et al. 2017, Klunklin and Savage 2018). The results indicate that lightness (L^* value) of UF had lower than WF, whereas redness (a^* value) and yellowness ($*b$ value) of UPF had higher than WF. It suggests that light-yellow color of unripe papaya flour might be partly attributed to the color of β-carotene. The other factors that might contribute to the light-yellow color of flour is browning reaction. It was reported that the unripe papaya contains polyphenol oxidase, the contributing factor of enzymatic browning reaction (Cano et al. 1996). The polyphenol oxidase enzymes can oxidize the phenolic component, resulting in the production of brown color. However, it might play only little part, because in this study, unripe papaya was dried at 70°C. The previous study suggested that the process of enzymatic browning can be terminated by temperature around 60-105°C (Marta Corzo-Martínez 2012).

The results of proximate analysis indicated that total dietary fiber is the abundant components of UPF. It presented the higher content of total dietary fiber (56.14g/100g) than other by-product processing including wheat and rice bran (27-45g/100 g), peach and orange concentrate (30-37 g/100g) (Elleuch et al. 2011). Moisture content of UPF showed higher content than other flour such as kiwifruit (9.8-10.5 g/100g) (Li and Zhu 2017), pumpkin (8.77-9.16 g/100g) (Aziah and Komathi 2009) and

green banana (6.55-6.88 g/100g) (Nimsung et al. 2007). This might be affected from the high initial moisture content of fruit, leading to increase moisture of flour (de Moraes Crizel et al. 2016). However, the moisture content of flour was still less than 15%. This is assured the quality of flour indicating. If the moisture content is higher than 15%, it affects the storage quality and increase the proliferation of microorganism, insect infestation and agglomeration (Aziah and Komathi 2009, Leão et al. 2017). In the current study, UPF had ash content 5.22 g/100g sample. It showed higher ash content than previous results reported by de Moraes Crizel et al. (2016) pineapple (4.58 g/100g), Blueberry (4.14 g/100g) and by Selani et al. (2016) for mango (2.43 g/100g). The content of ash could refer to mineral in flour (Varastegani et al. 2015). The previous study suggested that unripe papaya is an essential source of calcium, magnesium, sodium and potassium (Chukwuka et al. 2013). Moreover, UPF in this study, exhibited the low amount of protein content. Normally, wheat flour contains gluten which is a major protein source in flour and it seems to be absent in fruits and vegetable (Varastegani et al. 2015). According to the proximate composition of UPF, it might affect functional properties of foods, but it can provide beneficial physiological effects for example the potential to protect against cardiovascular risk factor, blood glucose and gastrointestinal disease (Mann and Cummings 2009).

The findings indicate that hydration properties of UPF exhibited higher than wheat flour. WAI relates to the hydrophilic group of components and the gel formation (Gomez and Aguilera 1983, Silva et al. 2009). It also indirectly indicates the degree of

starch gelatinization component (Julianti et al. 2017). WSI correlates with the solubility of organic components which are able to excess of water, for example; the presence of starch and protein content (Ahmed et al. 2016, Sharma et al. 2017). It was suggested that dietary fiber content in papaya might play a role in these properties (Oloyede 2005). This finding might correlate with the proximate analysis of UPF because it presented the high amount of fiber content.

Furthermore, the total polyphenol, FRAP and DPPH of UPF were significantly higher than that of wheat. It has been shown that flour made from papaya contains phytochemical components such as β-carotene, vitamin C, procyanidin, gallic acid, catechin, ρ-coumaric acid, epicatechin and quercetin (Oboh et al. 2015). These components demonstrated antioxidant activity by acting as a reductant in a redox-linked reaction, wherein Fe^{3+} is reduced to Fe^{2+} at low pH (Pulido et al. 2000). Moreover, they show potential on free radical scavenging activity by donate hydrogen atom to the DPPH radical (Kedare and Singh 2011). In addition, β-carotene has the ability to quench singlet oxygen and scavenge free radicals against lipid peroxidation (Krinsky and Johnson 2005). It is suggested that phytochemical compounds are partly responsible for the antioxidant activity of UPF.

According to the proximate analysis, UPF presented a high content of total dietary fiber, which may provide beneficial effects on cholesterol absorption especially soluble fiber, which is around 40% of total dietary fiber in unripe papaya (Chang et al. 1998). The binding of bile acid and interuption of the micelle formation are the important step of the reduction of cholesterol absorption (Jesch and Carr 2017). According to the results, UPF had the ability to bind bile acid which might relate to its fiber content. It has been studied that dietary fiber can bind primary bile acids in the small intestine, resulting in increasing in bile acid loss from fecal. This effect leads to the demand of new primary bile salt, which is synthesized by cholesterol as a precursor, and results in the reduction of plasma cholesterol level (Brownlee 2011). Apart from primary bile acid, UPF also had a potential of binding the secondary bile acid. It is stated that the circulating high level of secondary bile acid pool can cause the cell membrane damaging and inflammation (Ajouz et al. 2014). Due to highly ability on binding of secondary bile acid of UPF, it might reduce the risk of cell damaging and inflammation.

Furthermore, UPF exhibited a potential on inhibition of cholesterol micellization. It might relate to fiber component can bind to micelle and protect against the absorption of cholesterol. However, the mechanism still inconclusive. Gunness and Gidley mentioned 3 hypotheses about the dietary fiber and micelle. The first hypothesis is dietary fiber may attenuate the barrier properties of the enterocyte, result in the protection against micelle absorption. Secondly, the dietary fiber may

interfere the molecular interaction in micelle. The last hypothesis is that dietary fiber may entrap the micelle leading to prevent the cholesterol absorption (Gunness and Gidley 2010). Consistent with the previous study also suggested that dietary fiber may bind the bile acid during the micelle formation, lead to the disruption of micelle formation (Jesch and Carr 2017). The other possible reason is the bioactive compound in UPF. It was reported that gallic acid, catechin and epicatechin at the concentration 0.2 mg/mL have the ability on interfere cholesterol micellization by reduction the solubility of cholesterol micelle (Ngamukote et al. 2011). As mentioned above, gallic acid, catechin and epicatechin are one of the bioactive compounds in unripe papaya (Oboh et al. 2015). Moreover, from the previous result that UPF has total phenolic content around 0.08 mg GAE/mg DW. So, it might possible that the total phenolic content might contribute to the effect on inhibition of cholesterol micellization.

In current study, UPF was employed as wheat partial replacement in pancake product. The formulation of pancake was produced with 4 ratios of replacement including 0% (no replacement), 5%UPF replacement, 10%UPF replacement and the highest ratio 20%UPF replacement. For texture profile analysis, hardness is the force that enable to break the sample during first bite (Bourne 2002a). The pancake with a higher ratio of replacement presented significantly higher the hardness value. It might be related to fiber content of UPF. The previous study suggested that fiber can increase water absorption and attribute to the dilution of gluten, resulting in the increasing of hard texture (Kaur and Kaur 2018). Moreover, the particle size and morphology are one

of important factors for alteration of textural properties. In the previous study, the particle size on textural properties of final product resulted in the contact surface through physical and chemical interactions (Noort et al. 2010). The increasing particle size leads to the limit contact surface, which restricts chemical reaction and consequently, the interaction of gluten network and formation is decreased. (Noort et al. 2010, Bressiani et al. 2017). Furthermore, it was described that the disruption of granule as irregular shape increased the water absorption (Horstmann et al. 2017). It was also led to the dilution of gluten network. On the other hand, the reduction of wheat flour by UPF might play an important role on the formation of gluten network of pancakes. The consequence effect on hardness caused by the lack of gluten network in food (Lim et al. 2011). Incidentally, chewiness is represented for the force needed to disintegrate a food to swallow. This value can be calculated from hardness multiply by cohesiveness and springiness (Yemmireddy et al. 2013). So, the elevation of hardness might correlate with increasing of chewiness.

The color surface of pancake was analyzed by colorimeter. It presented three parameters including lightness (L^*), redness (a^*) and yellowness (b^*). The pancake surface color showed the brown color in the UPF replacement pancake. This is due to yellow color of UPF corporated with the panfrying, resulting in the darker shade of brown color in pancake. The color of UPF pancake might correlated with the pigment of its flour. As result mentioned above, UPF had the β-carotene content which is corresponding to yellow-orange color. It could carry out to final products. The similar

result was observed in other study indicating that the incorporation of flour color might attribute to color of final product, which is important to consumer attraction (Franklin et al. 2017). For example, chiffon cake with black rice powder showed darker color, because of its color of black rice powder (Mau et al. 2017). Cookies with addition of rosehip pomace presented higher redness than control cookies (Tanska et al. 2016). Furthermore, the brown shade color on surface pancake might be attributed from the non-enzymatic browning reaction called Maillard reaction. Maillard reaction is the reaction between the carbonyl group of reducing sugar and amino acid, resulting in the production of brown polymer; melanoidins (Palav 2016). In current study, the ingredients of pancake include sugar, milk and egg contain both protein and sugar which causes the formation of Maillard reaction and consequently increases brown color.

In the current study, pancake with higher level of replacement exhibited the lower starch content. Pancake with 20% of UPF showed the lowest RDS and SDS, whereas it had the highest undigestible starch. The reason of explanation is that UPF had higher amount of fiber content which might reduce the proportion of starch in wheat replacement. Moreover, the fiber content in UPF might affect starch digestion as presented in the release of glucose. Pancake with UPF can slowly release the concentration of glucose when compared to the control pancake. Dietary fiber has protective potential on the digestion and absorption of carbohydrate. The previous study purposed that the viscosity behavior of dietary fiber can improve the glycemic

control (Brennan 2005). It was stated that the increase of viscosity in food matrix with dietary fiber can interfere interactions of digestive enzymes, result in the delay of digestion and absorption glucose (Lambeau and McRorie 2017, McRorie and McKeown 2017). The previous study presented that glycemic index of commercial pancakes were categorized into low and medium GI group, ranging between 51-67 (Murakami et al. 2006, Yang et al. 2006). It was the same range of the control pancake in this study. After UPF was substituted, glycemic index of pancakes was decreased. This effect might come from the fiber content of UPF together with the reduction of wheat proportion of pancake formulation.

After the production of pancakes, they were determined for total phenolic content and FRAP assay for antioxidant activity. It could obviously see that pancake with UPF substitution had higher both total phenolic content and antioxidant activity than the control pancake. The reason is the incorporation of the phenolic content and β-carotene content in its flour as previously results of UPF above. These might increase of total phenolic content and antioxidant property in pancake as well. These results are similar to other previous researches. For example, the bread substitution with 4% turmeric powder provides higher content of curcumin, polyphenol and antioxidant properties (Lim et al. 2011). Moreover, replacement of the ripe banana flour in layer cake and sponge cake enhance the antioxidant activity and total phenolic compound. It can increase total phenolic content and decrease IC_{50} of DPPH radical scavenging activity (Segundo et al. 2017b). On the other hand, the phenolic content of pancake

with UPF seems to lower than unripe papaya flour. Because phenolic compounds and β-carotene are heat sensitive (Boon et al. 2010, Hogervorst Cvejić et al. 2017). They might be degraded during the heating of pancake, result in a decrease in the content of phytochemical compounds. The finding suggests that UPF provides a good source of phenolic content and β-carotene content and the substitution of wheat flour in the pancake with UPF can be alternative method for development of pancake with additional health benefits.

Sensory analysis is one of the important for the development of new food product (Bertin et al. 2017). The acceptance parameters were scored by panelists for 7 parameters as follows appearance, color, odor, taste, texture, aftertaste and overall acceptance, and line scaling was deployed for questionnaire. In the current study, the sensory textural score of pancake was consistent with the textural analysis . The results suggest that the increase of hardness of UPF pancake seems to decrease the texture score of sensory evaluation. Because of the limitation of human sense (Bourne 2002b), it showed no significant difference of sensory score when compared to the control pancake. The taste and aftertaste were significantly decreased in 20% UPF replacement. This explanation is why UPF leads to the alteration of taste and aftertaste parameters. UPF has specific characteristics with bitter taste which provides mouthfeel from itself after intake of pancake. Furthermore, it has been reported that phenolic compounds are responsible for astringency and bitterness (Drewnowski and Gomez-

Carneros 2000). As previous results, pancake with UPF exhibited higher polyphenol content, it might enhance the bitterness to pancake with UPF. However, overall acceptance of control pancake had the highest score, and also demonstrated a good acceptability of consumer.

CHAPTER VI

CONCLUSION

In conclusion, the unripe papaya by-products were made into flour by heating, high-speed grinding and sieving. The particle size of unripe papaya flour (UPF) was <150 µm with yellow color and an irregular shape. UPF had higher hydration properties than wheat flour. Moreover, UPF contained higher fiber content, β-carotene content and total phenolic content with antioxidant activity. UPF also had the potential on cholesterol absorption by the binding of bile acid and interruption micelle formation. The higher ratio of UPF replacement pancake exhibited the increasing of hardness, chewiness and darker shade of brown color. However, it provided lower starch content and could attenuate glucose release level. In addition, it significantly decreased glycemic index when compared to control pancake. Furthermore, the pancake with UPF demonstrated a good sensory evaluation. Therefore, the unripe papaya flour can be regarded as a good source of natural antioxidants and nutritional properties for development of healthy products.

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