

Development of riceberry cracker supplementation with unripe papaya flour



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การพัฒนาผลิตภัณฑ์แครกเกอร์จากข้าวไรซ์เบอร์รี่เสริมแป้งมะละกอดิบ



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การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของการทดแทนแป้งสาลีบางส่วนด้วยแป้งไรซ์เบอร์รี่และแป้งมะละกอดิบต่อคุณสมบัติทางเคมีกายภาพ คุณสมบัติเชิงหน้าที่ และการประเมินทางประสาทสัมผัสของแครกเกอร์ จากผลการศึกษาพบว่า การทดแทนแป้งสาลีบางส่วนด้วยแป้งไรซ์เบอร์รี่ร้อยละ 5, 10, 15 และ 20 (5, 10, 15 และ 20%RBF) ส่งผลทำให้ค่าความแข็งและค่าสีแดงเพิ่มขึ้นอย่างมีนัยสำคัญ แต่ส่งผลให้ค่าความกรอบ ค่าความสว่าง และค่าสีเหลืองของแครกเกอร์ลดลง นอกจากนี้ยังพบว่า การทดแทนแป้งสาลีด้วยแป้งไรซ์เบอร์รี่ส่งผลให้ปริมาณสารประกอบฟีนอลิกเพิ่มขึ้นจาก 2.03 ± 0.36 (สูตรควบคุม) เป็น 8.78 ± 0.19 (20%RBF) มิลลิกรัมของกรดแกลลิกต่อ 10 กรัมแครกเกอร์ สารประกอบแอนโทไซยานินเพิ่มขึ้นจาก 153.45 ± 14.77 (5%RBF) เป็น 803.92 ± 5.29 (20%RBF) ไมโครกรัมของไซยานิดิน-3-กลูโคไซด์ต่อ 10 กรัมแครกเกอร์ ซึ่งส่งผลให้ฤทธิ์การต้านอนุมูลอิสระในแครกเกอร์ไรซ์เบอร์รี่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) นอกจากนี้ยังพบว่า แครกเกอร์ไรซ์เบอร์รี่สูตร 10, 15 และ 20% มีค่าดัชนีน้ำตาลลดลงเมื่อเทียบกับสูตรควบคุม ($P < 0.05$) เมื่อพิจารณาถึงโครงสร้างทางจุลภาคของแครกเกอร์พบว่า การทดแทนแป้งสาลีด้วยแป้งไรซ์เบอร์รี่ส่งผลให้พื้นผิวหน้าของแครกเกอร์ขรุขระ และมีโครงสร้างภายในที่อัดตัวกันแน่น จึงส่งผลให้คะแนนความชอบด้านความแข็งของ 20%RBF ลดลง แต่เป็นที่น่าสนใจอย่างยิ่งเมื่อพบว่า แครกเกอร์สูตร 20%RBF มีคะแนนความชอบโดยรวมต่อผลิตภัณฑ์สูงสุด จากผลการศึกษาข้างต้น แครกเกอร์ 20%RBF มีฤทธิ์การต้านอนุมูลอิสระและคะแนนความชอบโดยรวมสูงสุด และมีค่าดัชนีน้ำตาลต่ำที่สุด ดังนั้นแครกเกอร์สูตร 20%RBF จึงถูกเลือกมาทดแทนแป้งสาลีบางส่วนด้วยแป้งมะละกอดิบร้อยละ 3, 5 และ 7 (3, 5 และ 7%UPF) โดยพบว่า การผสมแป้งมะละกอดิบในแครกเกอร์ไรซ์เบอร์รี่ ส่งผลทำให้ค่าความกรอบ ค่าความสว่าง ค่าสีแดง และค่าสีเหลืองเพิ่มขึ้น แต่ส่งผลให้ค่าความแข็งของแครกเกอร์ลดลงอย่างมีนัยสำคัญเมื่อสัดส่วนของแป้งมะละกอดิบเพิ่มขึ้นในแครกเกอร์ การผสมแป้งมะละกอดิบในแครกเกอร์ไรซ์เบอร์รี่ส่งผลให้ปริมาณสารประกอบฟีนอลิกเพิ่มขึ้นจาก 8.61 ± 0.20 (20%RBF) เป็น 11.59 ± 0.29 (20%RBF+7%UPF) มิลลิกรัมของกรดแกลลิกต่อ 10 กรัมแครกเกอร์ ซึ่งส่งผลให้ฤทธิ์การต้านอนุมูลอิสระในแครกเกอร์ไรซ์เบอร์รี่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) นอกจากนี้ยังพบว่า การผสมแป้งมะละกอดิบในแครกเกอร์ไรซ์เบอร์รี่ส่งผลให้ค่าดัชนีน้ำตาลลดลงจาก 60.10 ± 0.34 (20%RBF) เป็น 56.26 ± 0.21 (20%RBF+7%UPF) เมื่อพิจารณาถึงโครงสร้างทางจุลภาคของแครกเกอร์ ถึงแม้ว่าการผสมแป้งมะละกอดิบในแครกเกอร์ไรซ์เบอร์รี่ส่งผลต่อการเปลี่ยนแปลงโครงสร้างทางจุลภาคโดยมีผลทำให้พื้นผิวหน้าของแครกเกอร์ขรุขระ แต่เป็นที่น่าสนใจอย่างยิ่ง เมื่อการผสมแป้งมะละกอดิบในแครกเกอร์ไรซ์เบอร์รี่ส่งผลช่วยลดโครงสร้างที่อัดแน่นภายในผลิตภัณฑ์แครกเกอร์ไรซ์เบอร์รี่ เมื่อพิจารณาคะแนนความชอบของผลิตภัณฑ์พบว่า แครกเกอร์สูตร 20%RBF+5%UPF ได้รับคะแนนความชอบโดยรวมต่อผลิตภัณฑ์สูงสุด ดังนั้นการทดแทนแป้งสาลีบางส่วนด้วยแป้งไรซ์เบอร์รี่และแป้งมะละกอดิบช่วยเพิ่มความสามารถในการต้านอนุมูลอิสระและลดค่าดัชนีน้ำตาลของผลิตภัณฑ์ได้ดีกว่าแครกเกอร์ที่มีส่วนผสมของแป้งไรซ์เบอร์รี่เพียงอย่างเดียว ดังนั้นการนำแป้งมะละกอดิบมาใช้เป็นส่วนผสมจึงเป็นการเพิ่มมูลค่าวัสดุเหลือทิ้งและเป็นการใช้ประโยชน์วัตถุดิบจากธรรมชาติในการพัฒนาผลิตภัณฑ์เพื่อให้ได้แครกเกอร์ที่มีคุณค่าทางโภชนาการสูง



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Napassorn Payantakom : Development of riceberry cracker supplementation with unripe papaya flour. Advisor: Asst. Prof. SATHAPORN NGAMUKOTE, Ph.D. Co-advisor: Asst. Prof. Porntip Pasukamonset, Ph.D.

The aim of this study was to investigate the effect of partial replacement of wheat flour with riceberry flour and unripe papaya flour on the physicochemical, functional properties and sensory evaluation of crackers. In the current study found that partial substitution of wheat flour with 5, 10, 15 and 20% riceberry flour (5, 10, 15 and 20%RBF) resulted in significantly increased in hardness and redness together with a decreased fracturability, lightness and yellowness of crackers. Researcher also found that substitution of wheat flour with riceberry flour resulted in an increase phenolic content from 2.03 ± 0.36 (control cracker) to 8.78 ± 0.19 (20%RBF) mg GAE/10g cracker and anthocyanin content from 153.45 ± 14.77 (5%RBF) to 803.92 ± 5.29 (20%RBF) $\mu\text{g C3G}/10\text{g cracker}$. Consequently, resulted in significantly increased antioxidant activity in riceberry crackers ($P < 0.05$). Moreover, riceberry crackers 10, 15 and 20% formula had a lower glycemic index compared to the control cracker ($P < 0.05$). Considering the microstructure of crackers, it was found that substitution of wheat flour with riceberry flour resulted in the surface of the crackers being rough and has a tightly packed internal structure. Therefore, resulted in a decrease in the preference score of hardness of 20%RBF. Surprisingly, cracker with 20%RBF had the highest overall preference score. In this study, it was found that cracker with 20%RBF had the highest antioxidant activity, overall acceptance score and had the lowest glycemic index. Hence, cracker with 20%RBF was chosen for partial substitution of wheat flour with 3, 5 and 7% unripe papaya flour (3, 5 and 7%UPF). The results found that the incorporation of unripe papaya flour into riceberry crackers resulted in significantly increased in fracturability, lightness, redness and yellowness together with a significantly decreased hardness when the proportion of unripe papaya flour increased in the crackers. The incorporation of unripe papaya flour into riceberry crackers resulted in an increase phenolic content from 8.61 ± 0.20 (20%RBF) to 11.59 ± 0.29 (20%RBF+7%UPF) mg GAE/10g cracker. Consequently, resulted in significantly increased antioxidant activity in riceberry crackers ($P < 0.05$). Moreover, the incorporation of unripe papaya flour into riceberry crackers resulted in a decrease in the glycemic index from 60.10 ± 0.34 (20%RBF) to 56.26 ± 0.21 (20%RBF+7%UPF). Considering the microstructure of crackers, although the incorporation of unripe papaya flour into riceberry crackers resulted in the surface of the crackers being rough. Interestingly, the incorporation of unripe papaya flour into riceberry crackers reduced the compacted structure of the internal cracker product. When considering product preference scores, cracker with 20%RBF+5%UPF had the highest overall preference score. Therefore, the partial replacement of wheat flour with riceberry flour and unripe papaya flour enhances the antioxidant capacity and reduces the glycemic index of the product better than crackers that contain only riceberry flour. Consequently, increasing the value of waste materials and using of natural ingredients for product development in order to obtain nutritious crackers.

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CHAPTER I

INTRODUCTION

1. Background of the study

The burden of noncommunicable diseases (NCDs) are a major public health problem and increasing the mortality and morbidity globally (Lenoir-Wijnkoop, Jones et al. 2013). NCDs, also known as chronic diseases, tend to have a long duration, generally slow progression and are the result of a combination of genetic, physiological, environmental and behavior factors. The main types of NCDs are cardiovascular diseases (like heart attacks and stroke), cancers, chronic respiratory diseases (such as chronic obstructive pulmonary disease and asthma) and diabetes. People of all age groups, regions and countries are affected by NCDs. Many scientific studies suggest that unhealthy diet, physical inactivity, raised blood glucose and cholesterol, excess adiposity and expose to tobacco and alcohol use, all increase the risk of NCDs (Epping-Jordan, Galea et al. 2005). Moreover all of common risk factors are likely to increase reactive oxygen species (ROS) or free radical which are generated from molecular oxygen as a result of normal cellular metabolism(Lima, Vianello et al. 2014). An overproduction of ROS produce a state of oxidative stress (OS), a disturbance in the imbalance between ROS and antioxidant defenses, may lead to produce adverse modifications to cell components, such as lipids, proteins and DNA, inhibiting their normal function (Betteridge 2000). However, there are many ways to reduce the generation of oxidative stress including lowering expose to environmental pollutants, caloric restriction and moderate physical activity (Poljsak 2011). Moreover, consumption of fruits and vegetables daily at least 400 g has been recommended by the World Health Organization Sufficient amounts of those will increase of health-enhancing phytonutrients such as vitamins, minerals, fiber and

other phenolic compounds which important to control our cell to work properly (Poljsak 2011). Among the phenolic compounds, anthocyanins are a class of pigment of the flavonoid group that occur naturally in many fruits and vegetables and responsible for purple, red and blue colors (Lima, Correa et al. 2011). There are seven types of anthocyanins include cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin (Khoo, Azlan et al. 2017).

Riceberry, a black purple rice variety (*Oryza Stiva L.*), is a newly registered rice variety from Thailand developed by the Rice Science Center, Kasetsart University, which is a good source of anthocyanins especially cyanidin-3-glucoside and peonidin-3-glucoside. Moreover, riceberry is one of the most popular rice known for health promoting properties including decrease a rise of blood glucose, a strong antioxidant effect and reduce oxidative stress by enhancing activity of antioxidant enzymes (Prangthip, Surasiang et al. 2013). Daiponmak et al. (2010) reported that the increased of total phenolic content, antioxidant activity and cyanidin-3-glucoside content in riceberry seedlings presented a protective mechanism against the cellular structures from oxidative damage (Daiponmak, Theerakulpisut et al. 2010). Jiamyangyuen and Pramai (2016) determined antioxidative properties in relation to color of pigments rice varieties. It was found that the phytochemicals compound of crude riceberry extracts including polyphenol content 148.44 mg GAE/100 g flour, flavonoid content 57.35 mg CE/100 g flour, anthocyanin content 33.95 mg C3G/100 g flour and antioxidants properties exhibited DPPH- scavenging activity $IC_{50} = 9.6$ g/ml, the FRAP value 0.40 mmol Fe(II)/100 g flour and the TEAC value 0.81 mmol TE/100 g flour (Pramai and Jiamyangyuen 2016).

Lifestyle and pattern of daily diet changing may result to the food intake and nutritional requirement; for this reason, a healthy diet becomes crucial for human. Functional foods are any foods that fortified, enriched or enhanced foods or dietary

component and provide a potential health and physiological benefit (Hasler 1998). Demirci and his colleagues have recently suggested that the application of the prebiotic *L. casei* 431 strain with rice bran in yoghurt formulations showed significantly increased of *S. thermophiles* which is a good bacteria and improved DPPH scavenging activities of yoghurt samples (Demirci, Aktaş et al. 2017). In a study by Nagao et al., investigating the effect of continuous ingestion of a catechin-rich beverage in patients with type 2 diabetes showed that a catechin-rich beverage might be useful in the prevention of obesity and vascular disorder by reducing cholesterol, blood pressure and visceral fat (Nagao, Meguro et al. 2009). Therefore, functional foods proposed opportunity to improve quality of food products and responded consumer demand of nutritious foods. The rising consumer demand of healthy and convenient foods in recent year, this has led to develop the snack foods in the area of bakery products which is still underdeveloped (Sedej, Sakač et al. 2011, Ahmed and Abozed 2015). Among snack foods; crackers are popular snack products belong to a group of bakery products which is widely consumed by populations around the world (Sedej, Sakač et al. 2011, Ahmed and Abozed 2015). Crackers are described as thin and crisp wafer or biscuits, normally made of unsweetened and unleavened dough (Han, Janz et al. 2010). The previous study by Sedej et al. demonstrated acceptability, sensory evaluation, chemical and antioxidants contents of gluten-free crackers made from buckwheat flour. The results illustrated that buckwheat crackers have significantly higher total phenolic, tocopherol and flavonoids content than wheat crackers. In addition, buckwheat crackers also have scavenging activity (Sedej, Sakač et al. 2011). However, commercial cracker products focus on the flavour or taste, but did not focus on nutritional point such as minerals, dietary fiber, phytochemical compound and antioxidant activity.

Papaya (*Carica papaya* L.) is economical fruit that widely cultivated in the world, especially in tropical regions (Devitt, Fanning et al. 2009). Papaya pulp is generally consumed as fresh. Moreover, the processed papaya has been used in a variety of food products including dried fruits, drinking milk, jams, chocolate and fruit juice. In mixed fruit processing industry, only the top and bottom of unripe papaya were used to produce the mixed fruit products. Therefore, it generates huge amounts of waste product which count for 3.6 – 7.2 ton per year. Many scientific investigations have been suggested that the major phytochemicals of papaya including polyphenols, carotenoids, vitamin C and E. Maisarah et al (2013) analyzed unripe papaya and found that phenolic content 339.91 mg GAE/100 g of dry weight, flavonoids content 53.44 mg RE/100 g of dry weight and the unripe papaya exhibited maximum DPPH-scavenging activity (EC₅₀ = 4.3 mg/ml) (Maisarah, Nurul Amira et al. 2013). Additionally, the medicinal properties of papaya include anti-hypertensive, anti-bacteria, anti-tumor, wound-healing and free radical scavenging properties (Milind and Gurditta 2011). The previous study has been studied the substitution of wheat flour with papaya flour on nutritional properties, physical characteristics and organoleptic attribute of butter cookies. The finding indicated that the substitution of wheat flour with papaya flour can improve the nutritional value of cookies. The papaya cookies contained 17.16% dietary fiber, 8.55 mg/g polyphenol, 1.05% resistant starch and 15.48% DPPH oxidation (Varastegani, Zzaman et al. 2015). Consequently, the use of agro-industry residues to make zero waste production by turning the unripe papaya waste product into value added products as a material for the production of crackers, it might rise the valuable of waste papaya and considered as an alternative (Calvache, Cueto et al. 2016).

Thus, the aims of this study are to investigate the effect of partially substituted wheat flour with riceberry flour on physicochemical, functional

properties, and sensory evaluation of crackers and investigate the effect of riceberry cracker supplementation with unripe papaya flour on physicochemical, functional properties, and sensory evaluation. The new food product development would be good functional food choices which present a good source of bioactive compounds.



2. Objectives of the study

- 2.1 To investigate the physicochemical and functional properties of wheat flour and riceberry flour
- 2.2 To investigate the effect of partially substituted wheat flour with riceberry flour on physicochemical properties, functional properties and sensory evaluation of crackers.
- 2.3 To investigate the physicochemical properties of unripe papaya flour
- 2.4 To investigate the effect of riceberry cracker incorporation with unripe papaya flour on physicochemical properties, functional properties and sensory evaluation.

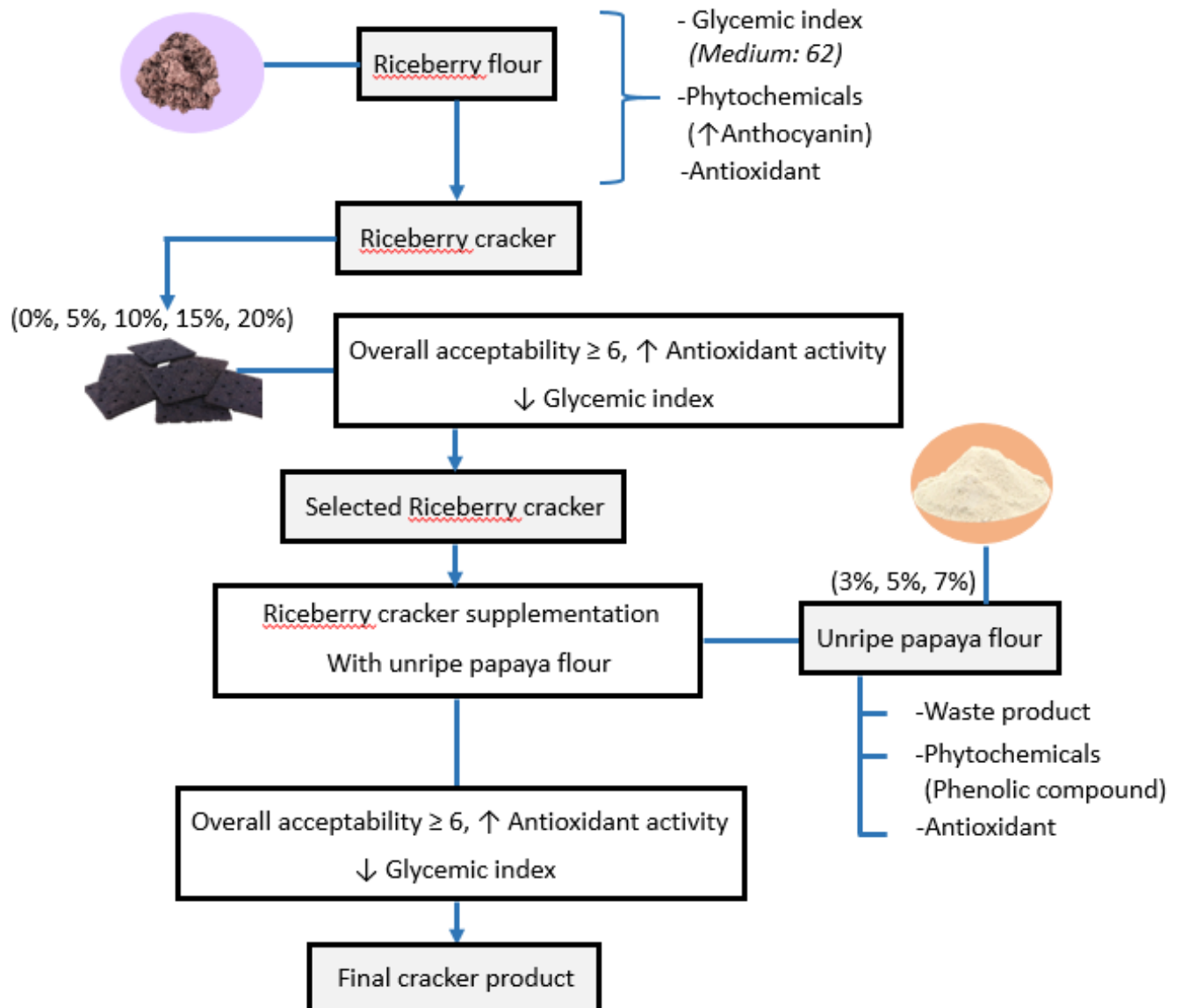
3. Hypotheses of the study

- 3.1 The partial substitution of wheat flour with riceberry flour contains a high amount of bioactive compounds, antioxidant properties and improves the physical properties of crackers.
- 3.2 The development of riceberry cracker supplementation with unripe papaya flour contains a high antioxidant activity, medium glycemic index and accepted by consumers.

4. Benefits of the study

This research provides the scientific information of riceberry flour and unripe papaya flour in terms of the active ingredients for functional food development or dietary supplement. In order to develop foods with high nutritive value as the choice for consumers. In addition, this research may help to promote the consumption of Thai agriculture materials both functional products and fresh foods and raise the value of Thai agriculture.

5. Conceptual framework



CHAPTER II

LITERATURE REVIEW

1. Non-communicable diseases (NCDs)

Non-communicable diseases (NCDs) started causing a major priority health globally (Schmidt, Duncan et al. 2011). NCDs including heart disease, stroke, cancer, diabetes and chronic lung disease. According to the World Health Organization (WHO), an estimated 40 million people died of NCDs each year, equivalent to 70% of all global deaths. It is predicted that NCDs might be causing seven out of every 10 deaths in developing countries in 2020 (Boutayeb and Boutayeb 2005). There are four major modifiable risk factors that driven rise of NCDs: physical inactivity, tobacco use, the harmful use of alcohol and unhealthy diets (increased fat and sodium, with low fruit and vegetable intake). Behaviors (modifiable risk factors) can lead to metabolic risk factors, including raised blood pressure, total cholesterol, elevated glucose and overweight, together with the presence of non-modifiable risk factors such as age, gender, race and family history (genetics), result to develop risk of NCDs.

2. Reactive oxygen species (ROS)

Reactive oxygen species (ROS, also called oxygen free radical) is a phrase of reactive molecules and free radicals derived from molecular oxygen (Held 2012) (superoxide, hydroxyl radical, nitric oxide) and non-radical oxygen derivatives of high reactivity (singlet oxygen, hydrogen peroxide, peroxyxynitrite, hypochlorite). ROS has one or more unpaired electron in an outer orbit which become an unstable molecule. This unstable can create energy that might damage biological system. Most of free radicals are generated both intracellular and external sources (Chance, Sies et al. 1979). The endogenous sources of ROS such as mitochondria (major), cytochrome P450, lipoxygenase, metabolic processes, NADH oxidase and

peroxisomes. Additionally, exogenous sources also can trigger the production of ROS, chemotherapeutics, environmental toxins, ionizing radiation and ultraviolet light (Rahman, Hosen et al. 2012). Accumulation of ROS in the body can lead to cell death or implicate in ageing and age-related diseases such as Alzheimer's disease, cardiovascular disease, cancer and diabetes (Rahman, Hosen et al. 2012). ROS can be eliminated by protective mechanisms and antioxidants.

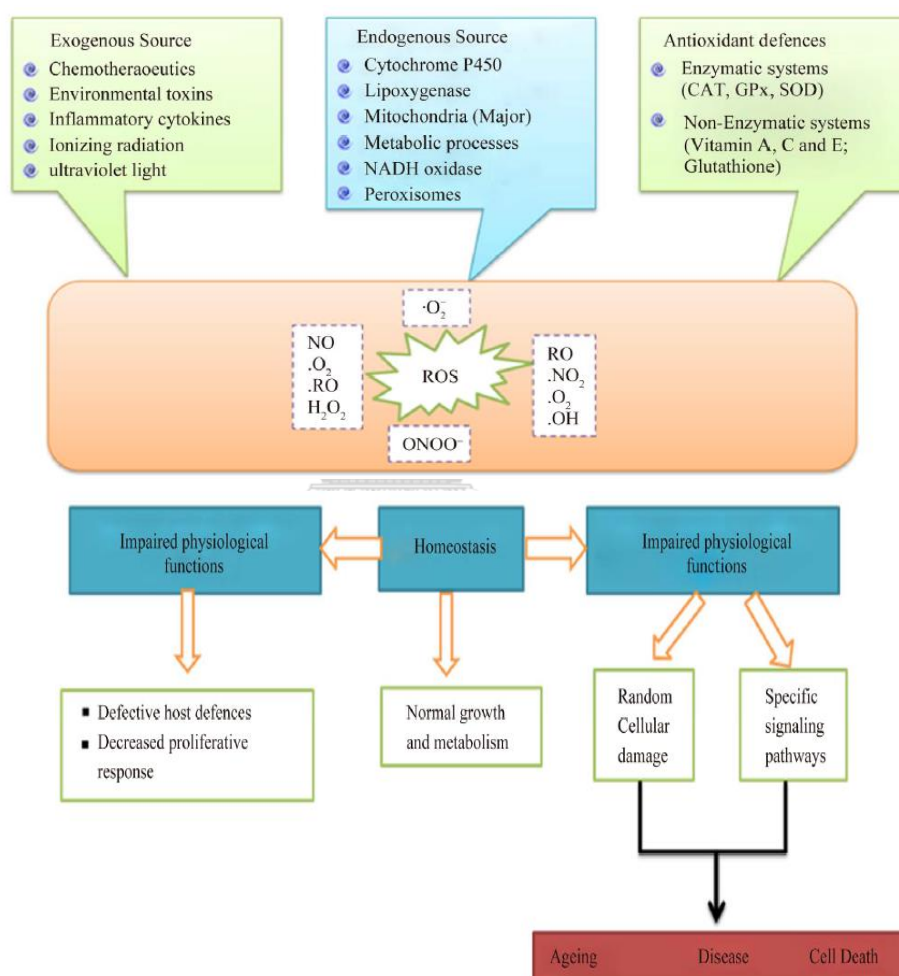


Figure 1 The source and cellular responses to reactive oxygen species

(Rahman, Hosen et al. 2012)

3. Oxidative stress

Normally, our cell has the balance between formation and removal of free radicals. Nevertheless, when the body loses the balance of free radicals or reactive oxygen species and antioxidants, cell will enter a state called “oxidative stress” (Betteridge 2000). Oxidative stress takes place when free radicals generate in the body exceeds the body’s ability to get rid of and neutralize them (Rahman, Hosen et al. 2012). The imbalance of free radicals and antioxidants can lead to damage of crucial cells and biomolecules which impact to the whole organism such as membrane damage, cross linking of proteins or DNA and even lead to cell death. All these consequences might drive to the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases (Pham-Huy, He et al. 2008).

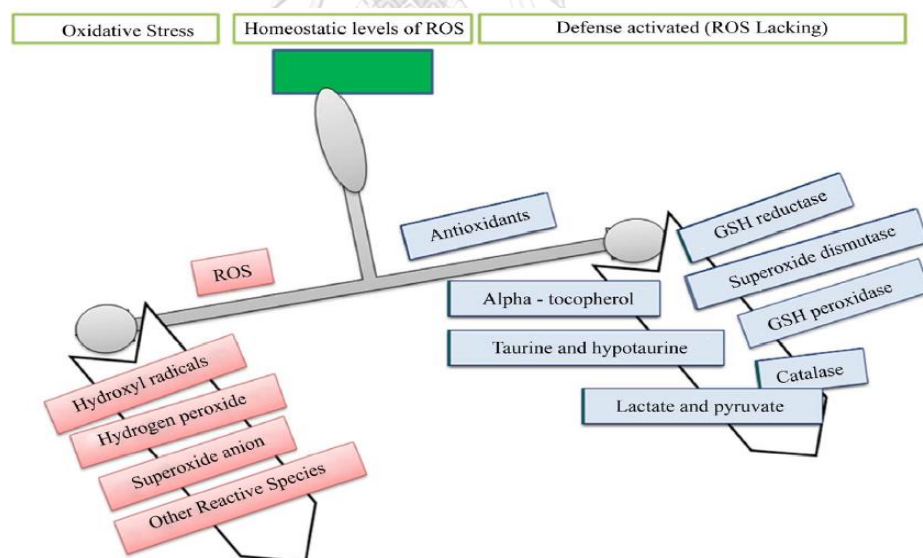


Figure 2 Imbalance between oxidant and antioxidant

(Rahman, Hosen et al. 2012)

4. Antioxidants and prevention of oxidative stress

An uncontrolled overproduction of reactive oxygen species (ROS) or oxygen free radical may result to cause oxidative stress which is implicated in human diseases. When oxidative stress has presence in the cells, the body has several ways or mechanisms to defend against ROS (Pham-Huy, He et al. 2008). The antioxidant protection system which is a system for protecting the cells and organ of the body to against ROS. Antioxidants play an important role in the body oxidative stress defense system (Ou, Huang et al. 2002). Antioxidant is any substance that neutralizes the excess of free radicals and prevent or remove oxidative damages to a target cell. Moreover, antioxidants can be classified as enzymatic antioxidants and non-enzymatic antioxidants (Pham-Huy, He et al. 2008). The antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GRx), catalase (CAT), superoxide dismutase (SOD) which neutralize free radical and require micronutrient cofactors including zinc, iron, selenium, manganese and copper for optimum catalytic activity (Yadav, Kumari et al. 2016). The non-enzymatic antioxidants can separate into metabolic and nutrient antioxidants (Pham-Huy, He et al. 2008). Metabolic antioxidants are a member with endogenous antioxidants including bilirubin, thiols (lipoic acid and N-acetyl cysteine), coenzyme Q10, L-arginine and uric acid which is produced by metabolism in the body. On the contrary, nutrient antioxidants are a member with exogenous antioxidants such as vitamin C, vitamin E, beta carotene, polyphenols (flavonoids, flavones and proanthocyanidins), omega-3 and omega-6 fatty acids which cannot be produced in the body and must be derived from natural food sources or food supplements (Pham-Huy, He et al. 2008). The antioxidant process including the chain-breaking and preventive way. For the chain-breaking, when free radical steals an electron, a free radical is formed. The free radical is stabilized by chain-breaking antioxidants such as vitamin C, E. For the preventive way,

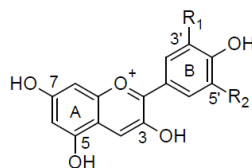
an enzymatic antioxidants can prevent oxidation by reducing the rate of chain initiation by scavenging and stabilizing free radicals (Young and Woodside 2001).

5. Polyphenol

Polyphenols or phenolic compounds are natural substances that widely distributed in plant species. The structure of polyphenols characterized by the presence of phenol units (Lima, Vianello et al. 2014). The polyphenols rich sources, including fruits, vegetables, whole grains and other types of foods such as tea, chocolate and wine. There are many researchers proposed that phenolic substances can act as a strong antioxidants by defending against oxidative stress caused by excess reactive oxygen species (ROS) (Tsao 2010). Moreover, polyphenols have been shown to provide beneficial effects, to protect the development of chronic diseases like cancer, cardiovascular diseases and diabetes (Walle, Ta et al. 2007). Particularly, anthocyanins, which are one group of polyphenols and has many health-beneficial properties (Konczak and Zhang 2004).

5.1 Anthocyanins

Anthocyanins are naturally the most considerably pigments and responsible for red, purple and blue color in many fruits and vegetables (Delgado-Vargas, Jiménez et al. 2000). The predominant source of anthocyanins including cherries, blueberries, raspberries and black currants (Miguel 2011). The common anthocyanins founded in plants such as cyanidin, delphinidin, pelargonidin, peonidin, malvidin, and petunidin (Castaneda-Ovando, de Lourdes et al. 2009). Anthocyanins are water- soluble that belonging to flavonoids of phenolic compounds (Khoo, Azlan et al. 2017). Glycosides are mainly occurring of anthocyanin-chromophores with the sugar molecule normally attached at the 3-position on the C-ring or the 5-position on the A-ring (figure 3) (Miguel 2011).



Anthocyanidin	R ₁	R ₂	Colour
Pelargonidin	H	H	Orange
Cyanidin	OH	H	Orange-red
Delphinidin	OH	OH	Bluish-red
Peonodin	OCH ₃	H	Orange-red
Petunidin	OCH ₃	OH	Bluish-red
Malvidin	OCH ₃	OCH ₃	Bluish-red

Figure 3 Structures of major anthocyanidins

(Miguel 2011)

Anthocyanin stability depends on light, pH (1-3), temperature, enzymes, metal ions, antioxidants and types of anthocyanin (Turturica, Oancea et al. 2015). Moreover, the pH of the solution can affect to color of anthocyanins. In the neutral pH condition anthocyanins will presence in a purple color while when an increasing pH condition the color will change to blue. The previous study finding showed that antioxidant capacity and the amount of anthocyanin are better preserved at low temperature (Bakowska-Barczak 2005). Furthermore, the natural color of anthocyanin pigments can use as food colorants and additives in beverages and processed foods (Khoo, Azlan et al. 2017). Anthocyanins also used to assess the adulteration of some pigmented food products (Delgado-Vargas, Jiménez et al. 2000). Because of its natural pigments, low toxicity and safe to consume when compared to synthetic pigment compounds (Khoo, Azlan et al. 2017).

5.2 Health promoting of anthocyanins

The health-promoting benefits of anthocyanins have been suggested, in part of the antioxidant properties. The antioxidant capacity of anthocyanins presents in a various assay methods such as DPPH free radical scavenging activity, trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant potential (FRAP)

and oxygen radical absorbance capacity (ORAC) (Miguel 2011). The antioxidant action including superoxide scavenging activity (Costantino, Albasini et al. 1992), Lipid peroxidation inhibition capacity (Muselík, García-Alonso et al. 2007), induction of enzymatic antioxidants such as glutathione reductase (GRx), catalase (CAT), superoxide dismutase (SOD) (Fiander and Schneider 2000). The antioxidant activity depend on the chemical structure of anthocyanins, the ring position of the compound which a hydrogen atom from a hydroxyl group can be donated to a free radicals and the ability of anthocyanins to support an unpaired electron.

The anti-inflammatory activity of anthocyanins has been suggested. Inflammation is the regular response of living tissue involved with injury or infection which caused by cyclooxygenases (COXs) (Rankin 2004, Hämäläinen, Nieminen et al. 2007). The research studies in aglycon nucleuses of anthocyanins in reddish fruits and vegetables showed inhibition of cyclooxygenases-2 (COX-2) enzyme and inhibiting inducible nitric oxide (iNOS) protein and mRNA expression (Hou, Yanagita et al. 2005). Moreover, the researcher revealed that the anthocyanidin delphinidin was the most COX-2 expression inhibitor at both protein and mRNA levels (Hou, Yanagita et al. 2005).

In term of diabetes control of anthocyanins, insulin is a cause of type-2 diabetes which secreted from β -cells of pancreas. The abnormal function of several organs can cause by the failure of insulin regulation. Base on experimental evidences, demonstrated that anthocyanins can reduce the blood glucose content, improving insulin resistance and increasing pancreatic insulin production (Guo, Ling et al. 2007, Ataie-Jafari, Hosseini et al. 2008, Takikawa, Inoue et al. 2010). In addition, in an animal study showed that cyanidin-3-glucoside (Cy3-g) which is a refined compound can reduce level of blood glucose and improve insulin sensitivity in types-2 diabetes mice because the effect of Cy3-g to increase glucose transporter 4 (Glu4) expression

and decrease of retinol binding protein 4 in white adipose tissue (Sasaki, Nishimura et al. 2007).

Moreover, anthocyanins consumption was shown to prevent obesity and metabolic syndrome and improve adipocytes function. By the downregulation of fatty acid and triglyceride synthesis enzymes (Tsuda 2008). An additional study to investigate the treatment of anthocyanins in rats. The results showed the reduction of triglyceride accumulation during adipocyte differentiation. The mechanism of anthocyanins might be involved to decrease the gene expression of lipid transcription factors (Lee, Lee et al. 2014).

The daily intake of anthocyanin-rich foods such as red wine , berries, apple, found a decrease of cardiovascular risk and mortality in humans (Mink, Scrafford et al. 2007). Dietary anthocyanins have ability to reduce total cholesterol, low density lipoprotein and triglyceride levels (Alvarez-Suarez, Giampieri et al. 2014). Another examination showed that the consumption of fruits and vegetables containing anthocyanins have ability to inhibit carotid atherosclerosis in elderly men (Ellingsen, Hjerkin et al. 2008).

Furthermore, many previous studies indicated the anticarcinogenic properties of anthocyanins. The fraction of red wine, an anthocyanins-rich foods, can suppress proliferation of HCT-15 human colon cancer cells and gastric adenocarcinoma cells (Kamei, Hashimoto et al. 1998, Shih, Yeh et al. 2005). Fimognari and his college reported that purified cyaniding-3-0-beta-glucopyranoside can induce apoptosis in different human leukemia cell lines (Fimognari C, Berti F et al. 2004).

5.3 Food applications of anthocyanins

Nowadays, consumers increased the expectation in both taste and flavor by the appearance and color of food products. These lead the food industry to use

the colorants ingredient as the raw material for manufacturing of food products (Giusti and Wrolstad 2003).

Song et al. (Song, Zhu et al. 2013) studied the effect of various colors of wheat bran (blue, black and purple red) on the dry noodles qualities. The supplementation of pigmented wheat bran into dry noodles directly affected the firmness, water absorption and dynamic viscoelastic properties of dry noodles. In addition, the color of noodles was changed as the incorporation of pigmented wheat bran. The change of color might be due to the natural color pigment in wheat bran such as polyphenols and anthocyanins (Hu, Cai et al. 2007). Song et al. (Song, Zhu et al. 2013) stated that the pigmented wheat bran could be used as an antioxidant dietary fiber ingredient for food development.

Žilić et al. (Žilić, Kocadağlı et al. 2016) investigated the influence of anthocyanin-rich corn flour on the physicochemical and baking properties of cookies. The content of anthocyanins and total flavonoids in cookies was reduced as the increasing of baking time and temperature. Moreover, the addition of citric acid into cookie dough increased the phenolic compound by the improvement of anthocyanin stability. The findings indicated that Maillard reaction posse the important role on the increment of antioxidant property in corn cookies.

Previous study of Pasqualone et al. (Pasqualone, Bianco et al. 2014) studies the effect of grape marc extract on the physico-chemical qualities of biscuits. Since the grape marc present a good source antioxidant compounds such as anthocyanins, phenolic acids and catechins. The findings showed that the polyphenols content and antioxidant activity significantly increased in the biscuits enriched grape marc extract and also had the highest sensory scores of color, odor and taste. Also, there is the research focus on the functional foods development in

many food systems such as baby foods, beverages and ice cream by using anthocyanins rich foods.

In the study of Steed and Truong (Steed and Truong 2008), the purple-fleshed sweet potato was used for the production of puree and studied on the antioxidant activity and physical properties. The results showed that the purple sweet potato puree product noted as the source of antioxidants as the availability of the high amount of phenolic and anthocyanin components and also the sweet potato puree exhibited a shear thinning property similar to the commercial puree. Hence, the consumption of anthocyanins is stably raising as the anthocyanins extracts and food products from fruits and vegetables with high content of anthocyanins are more available nowadays.

6. Riceberry

Rice is the most important daily meal of people in Asia. Thailand has a various rice types, including riceberry which is an anthocyanin-rich food. Riceberry (*Oryza sativa* L.), deep purple grain, has been recently registered rice variety originated from a cross-breed between Jao Hom Nin (JHN) and Khoa Dawk Mali 105 by Rice Science Center, Kasetsart University (figure 4).

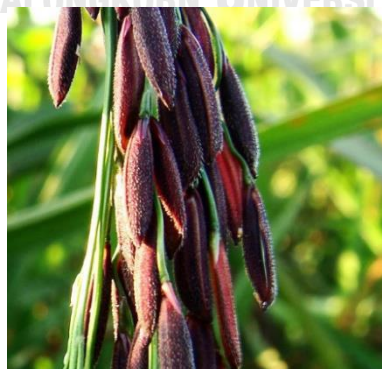


Figure 4 Riceberry rice

(Rice gene discovery & Rice science center)

The areas of riceberry production are in the Northern and North-East of Thailand. The characteristics of riceberry including plant height; 105-110 cm, day of maturity; 130 day, yield; 300-500 kg/rai, amylose content; 15.6% and gel temperature; <70 °C. Riceberry is well known as containing high antioxidant properties. Riceberry is enriched with both water soluble, mainly anthocyanin and lipid soluble antioxidants, such as carotenoid, gamma oryzanol and vitamin E. All nutritional properties of riceberry are contained in rice bran.

6.1 Nutritional value and health benefits of riceberry

Riceberry, a Thai deep purple rice, has been reported to contain a higher phytochemicals compound and dietary fiber than unpigmented rice (Prangthip, Surasiang et al. 2013). The Rice Science Center, Kasetsart University, have been reported nutritive value of riceberry including iron (13-18 mg/kg), zinc (31.9 mg/kg), omega-3 (25.51 mg/100 g), vitamin E (678 µg/100 g), folate (48.1 µg/100 g), β-carotene (63 µg/100 g), polyphenol (113.5 mg/100 g), tannin (89.33 mg/100 g), gamma oryzanol (462 µg/g), water soluble antioxidants (47.5 mg ascorbic acid/ 100 g) and lipid soluble antioxidants (33.4 mg trolox equivalent/ 100 g).

In a study by Sirichokworrakit et al., it was reported that chemical composition of riceberry flour including moisture content: 10.94%, ash: 1.13%, protein: 8.24%, crude fat: 0.91% and crude fiber 2.73% (Sirichokworrakit, Phetkhut et al. 2015). Moreover, the previous study finding showed antioxidants ability and active compound of riceberry, polyphenol content 148.44 mg GAE/100 g flour, flavonoid content 57.35 mg CE/100 g flour, anthocyanin content 33.95 mg C3G/100 g flour and antioxidants ability exhibited DPPH-scavenging activity IC₅₀ = 9.6 g/ml, the FRAP value 0.40 mmol Fe(II)/100 g flour and the TEAC value 0.81 mmol TE/100 g flour (Pramai and Jiamyangyuen 2016).

The development of riceberry can provide nutritional and health benefits to consumers. Leardkamolkarn et al., studied the potential anti-cancer capacity of the bran extracted from riceberry, the results showed that bran extracts from riceberry have cancer chemopreventive ability. Riceberry brans extracted induced apoptosis and inhibited the growth of cancer cells because of the biological modifiers to enhance effectiveness in sensitive cells (Leardkamolkarn, Thongthep et al. 2011).

The riceberry brans extracted are useful as medicinal supplements. Dietary riceberry supplement (up to 41% w/w) has also been examined the effects on hyperglycemia, hyperlipidemia, oxidative stress and inflammation in streptozotocin-induced diabetes rat on a long term (12 weeks). In the treated rats group showed a significant improvement of blood glucose, insulin, HbA_{1c}, IPGTT and GLUT4 levels. Moreover, hyperlipidemia, antioxidant enzyme (SOD, CAT and GPx), antioxidant capacity and pro-inflammation cytokine (TNF- α , IL-6) were improved. The results might be attributed to the bioactive compounds that present in riceberry bran (Prangthip, Surasiang et al. 2013).

Posuwan et al. (Posuwan, Prangthip et al. 2013) studied the effect of long-term supplementation of riceberry bran oil on amelioration of oxidative stress and organ histology changes in streptozotocin-induced diabetics rats fed with high fat diet. Male Sprague-Dawley rats with hyperglycemia were divided into four groups including untreated diabetic group fed a high fat diet alone, untreated diabetic group fed a high fat diet with 5% riceberry bran oil extract, untreated diabetic group fed a high fat diet with 7.5% riceberry bran oil extract and untreated diabetic group fed a high fat diet with 15% riceberry bran oil extract. The results showed that after 12 weeks of riceberry bran oil extract supplementation, the riceberry bran oil significantly restored superoxide dismutase, catalase, glutathione peroxidase,

coenzyme Q10, decreased malondialdehyde and also increased the ORAC scavenging free radical in diabetic rats. Furthermore, riceberry bran oil could improve the regenerative changes of heart, pancreas, liver and kidneys. These illustrated that riceberry bran oil gives the health benefit effect on diabetes by attenuating the oxidative stress and recovering organ systems.

Pannangrong et al. (Pannangrong, Wattanathorn et al. 2011) studied the effect of purple riceberry rice on neuroprotective and enhances cognition in young adult male Wistar rats with alzheimer's disease. Rats were orally supplemented with rice berry once daily at the different dosage including 180, 360, and 720 mg per kg of body weight for 2 weeks before and 1 week after the induction of memory deficit and also cholinergic lesions with AF64A, a specific cholinotoxin, via bilateral intracerebroventricular administration. The findings showed that the riceberry rice had the ability to defend the memory impairment and also improved the hippocampal neurodegeneration in hippocampus. In addition, the riceberry rice could decline lipid peroxidation product formation and hippocampal acetylcholinesterase activity.

Kongkachuichai et al. (Kongkachuichai, Prangthip et al. 2013) studied the effect of deep purple riceberry oil supplementation on the changing in lipid profile, blood glucose, insulin levels and GLUT4 transporter in Streptozotocin-induced diabetic rats fed a high fat diet. Sprague-Dawley rats were divided into 2 groups: the first group of rats were fed with a basal diet and second group of rats were fed with a high fat diet. After 2 weeks, the rats were induced to hyperglycemia condition. The various amounts of riceberry oil (5, 7.5 and 15%) was given to rats. The riceberry oil demonstrated the effectiveness a nutraceutical food as riceberry oil might improve insulin, HbA1C, blood glucose, intraperitoneal glucose tolerance. In terms of lipid

profiles, riceberry oil significantly reduced in LDL-cholesterol in rats fed with riceberry oil.

In the study of Ajinajarn et al. (Ajinajarn, Pongchaidecha et al. 2016) investigated the prevention effects of riceberry bran extract on the renal dysfunction and the expression of renal organic anion transporter 3 in gentamicin-induced nephrotoxicity in rats. Sprague Dawley rats were treated with three dosage including 250, 500 and 1000 mg/kg of riceberry bran extract for fifteen days. All the parameters were evaluated such as the function and expression of organic anion transporter 3, oxidative and antioxidative markers, renal function and histological changes in the kidneys. The decreasing in renal function was observed in treated rats as the reduction of urine creatinine, creatinine clearance and the elevation of BUN and serum creatinine. The decreasing of organic anion transporter 3 expression resulted to the attenuation of organic anion transporter 3 function. In addition, the results found that superoxide dismutase and glutathione activities were reduced in treated rats compared to control. The researcher concluded that the treatment of riceberry bran extract in nephrotoxicity rats could improve the function of renal, oxidative status and organic anion transporter 3 function. Consequently, riceberry bran extract might defend the kidney injury as the antioxidant activity in riceberry bran.

Jittorntrum et al. (Jittorntrum, Chunhabundit et al. 2009) studied the effects of rice bran extracts (brown rice, sinlek rice and riceberry rice) on H₂O₂-induced oxidative stress in Caco-2 cells. Since the oxidative stress plays the important role of the cause of chronic diseases. The human intestinal Caco-2 cells were incubated with all rice bran samples for 3 h. The findings showed that non-toxic concentrations of rice bran extracts could protect the cells against H₂O₂-induced oxidative stress as the antioxidant ability in rice bran samples. Moreover, rice bran

extracts also inhibited cell growth in dose and time-dependent. These indicated that rice bran had the potential power for using as medicine or food supplement.

6.2 Riceberry application in food products

Rice is an important cereal crop which is the staple food of Thailand. Additionally, rice provide a source of nutrients and health benefits. Riceberry which is known to be the most popular rice known for health promoting properties, has been supplement into food products.

Effect of partial substitution of wheat flour with riceberry flour on quality of noodles have been reported by Sirichokworrakit et al. (Sirichokworrakit, Phetkhut et al. 2015), who investigated the effects of riceberry flour addition (10%, 20%, 30% and 40% by flour weight) on cooking quality, texture and sensory attributes of noodles. The authors concluded that increasing the amounts of riceberry flour obstructed the functional dough properties and the cooking quality. In addition, the substitution of wheat flour with riceberry flour up to 30% showed the similar quality and pleasant appearance as normal noodles.

In a study by Chuaykarn et al. (Chuaykarn, Laohakunjit et al. 2013), it was studied the effect of riceberry flour (RF) on physicochemical and sensory properties of low fat ice-cream. Riceberry flour was added into an ice cream with the different levels (3, 5, and 7%). The study finding showed that increasing of RF resulted in increasing viscosity, hardness and decreasing fat destabilization of ice cream. The level of 5% adding of RF had just-about-right score of purple, rice flavor, firmness and highest score of overall acceptability and also help to improve the texture of ice cream. Moreover, ice cream with 5% RF showed total phenolic content (as gallic acid equivalent) was 0.66 mg GAE/g and antioxidant activities as determined by FRAP and ORAC assay were 1.15 and 8.67 $\mu\text{mol TE/g}$, respectively.

According to Tantivirasut et al., it was investigated the effect of pregelatinized riceberry flour (PRF) in reduced fat salad dressing by replacing fat salad dressing with riceberry flour at the different levels including 25%, 30% and 35%. The results demonstrated that the replacement of PRF affect to increase pH, viscosity, total anthocyanin content, total phenolic content and antioxidant properties of salad dressing. Additionally, a salad dressing with 30% PRF replacement had the highest score of overall acceptability. The findings indicated that pregelatinized riceberry flour can be used as a good potential fat replacer in salad dressing.

Researchers have developed and improved the quality of dried rice noodle (Kanom-geen) using riceberry flour and guar gum. Khao Ta Haeng flour was substituted with riceberry flour at the different concentration (10, 15, 20, and 25%). The results presented that high level of riceberry flour increased cooking loss, however decreased hardness of cooked Kanom-geen. Furthermore, Kanom-geen with 25% riceberry flour substitution obtained the highest overall preferences and also had higher crude fiber, total anthocyanin content and antioxidant activities than the commercial Kanom-geen (Bainak, Wongpakdee et al. 2015).

Effect of partial substitution of wheat flour with riceberry flour on quality of fried donut have been studied by Sirichokworrakit et al. (Sirichokworrakit, Phetkhut et al. 2015), riceberry flour used to substitute wheat flour at different proportion of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (wheat flour : riceberry flour). The results of the previous study showed that the crude fat, crude fiber and ash contents increased but protein and moisture contents decreased with high amount of riceberry flour substitution. The texture of fried donut which increased the amount of riceberry flour substitution had increased hardness, but the cohesiveness, springiness, chewiness and specific volume decreased. In addition, the study finding revealed that the substitution of wheat flour with riceberry flour up to 30% could obtain the

similar quality and pleasant appearance as control donut (Supatchalee, Apinya et al. 2016).

According to Sukhonthara and Thipnate, it was studied the replacement of rice flour and sugar in toddy palm cake by using germinated riceberry flour (GRF) and a mixture of maltitol and sucralose (MS). The results showed that the use of GRF increase the level of phenolic, GABA content and antioxidant activity. Moreover, the developed toddy palm cake had the overall liking scores within the range of like highly and 70.8% would buy this product.

In a study by Chamchan et al., it was developed rice papers by using germinated homnin brown rice flour (GHNH) on physicochemical properties and sensory evaluation. The findings indicated that GHNH papers had a quality same as commercial paper product. The antioxidant and phenolic content of GHNH rice papers were higher than control. Furthermore, the developed GHNH rice papers had a lower glycemic index than control and were classified as medium glycemic index food (Chamchan, Chupeerach et al. 2017).

Previous study of Thiranusornkij et al. (Thiranusornkij, Thamnarathip et al. 2019) studied the influence of riceberry rice and hom mali rice flour on physicochemical properties, starch hydrolysis and their application in bread. The results indicated that riceberry rice flour presented a source of phenolic and anthocyanin compounds and had higher the antioxidant activity than hom mali rice flour. Riceberry rice flour also containing lower amylose content, the larger particle size when compared to hom mali rice flour. Furthermore, the slow starch digestion was found in riceberry rice flour. In terms of the rice flour application in bread product, riceberry bread characteristics showed lower gumminess, chewiness, adhesiveness and cohesiveness. Anthocyanin-rich riceberry rice flour exhibited the ability to use as the alternative ingredients for the gluten-free product development.

Thamnarathip et al. (Thamnarathip, Jangchud et al. 2016) studied the protein hydrolysates from riceberry rice bran on the functional properties. The riceberry rice bran protein hydrolysate contained higher amounts of polyphenols than commercial white rice. The functional properties of rice bran protein hydrolysate depend on the main factors including pH and peptide patterns. The results showed that the riceberry bran protein hydrolysate demonstrated the highest stability and displayed more stable at pH 6. These could be concluded that the riceberry bran protein hydrolysate had the desirable emulsification and solubility properties which might be benefit for food and beverage development.

Numfon (Numfon and International 2017) studied the various types of hydrocolloids on the properties of gluten-free bread based on the small broken riceberry flour. Four different types of hydrocolloids including guar gum, xanthan gum and locust bean gum were used for production of bread. The results showed that the using of hydrocolloids showed improved the bread based on small broken riceberry flour properties such as the texture profile, loaf specific volume, microstructure and sensory. Guar gum incorporated in riceberry bread exhibited had the highest score of all sensory attributes.

Riceberry also have been used as the ingredient in terms of the non-dairy synbiotic beverage products to become the functional food beverage for lactose-intolerance consumer (Nakkarach and Withayagiat 2018). Nakkarach and Withayagiat stated that riceberry extract showed the potential as the material for non-dairy symbiotic production for human consumption.

Siripoonpakdee (Siripoonpakdee) studied the effect of using hom mali rice and riceberry rice on the qualities of snack bar. The recommended that riceberry with squid snack bar provided source of fiber, protein and antioxidant activity when compared to the commercial fruit cereal-based snack bars. Researcher stated that

riceberry with squid snack bar could be consumed as an alternative snack any time during the day.

Also Phuwan (Phuwan) developed the instant germinated riceberry beverage as the healthy product for people who health consciously. Ngamdee et al. (Ngamdee, Bunnasart et al. 2019) studied the development of milk chocolate fortified with broken riceberry by-product. The broken riceberry rice is an underutilized by-product which presented the anthocyanins and antioxidant property. The cocoa powder was replaced with riceberry powder for the preparation of milk chocolate. The anthocyanin-rich riceberry was encapsulated in maltodextrin, resulting increased in DPPH scavenging property and also well accepted by consumers. These findings illustrated that the broken riceberry might be used as functional ingredient for healthy diets production.

Previous study of Jiamjariyatam (Jiamjariyatam 2019) studied the effect of tapioca starch and riceberry bran on the oil absorption and physicochemical properties in puffed cracker. The mixture in different ratio between tapioca starch and riceberry rice bran significantly changed the fiber content, expansion ratio, bulk density and sensory parameters of the product. The supplementation of riceberry bran resulted increased the fiber content which affected to decrease the oil absorption in puffed cracker. In terms of texture of cracker samples, the addition of riceberry bran resulted increased hardness value and decreased crispness and brittleness.

7. Functional foods

The demand of foods which improve or give the health benefits have increased in recent years. Because of the consumer desire for healthy life style. Therefore, functional foods play an important role among the foods. Japan was the first country that introduced the positive aspects of food functionality. Functional

foods related to process food containing ingredients that aid specific bodily functions in addition to being nutritious (Arai 1996). Furthermore, the Institute of Medicine's Food and Nutrition Board defined functional foods as, any food or food ingredients that may provide a health benefit beyond the traditional nutrients it contains. The target functions might be sensible to dietary influence including early development and growth, regulation of energy balance and body weight, cardiovascular function, defense against oxidative stress, intestinal function (gut microflora), mental state and/or physical performance and fitness.

Many scientific evidences revealed that a plant-based diet may reduce the risk of chronic disease, especially cancer. People who intake of high amounts of fruits and vegetables presented only one-half of cancer risk when compare to people who consume few of those (Block, Patterson et al. 1992). The researchers mentioned that a various classes of biologically active plant chemicals known as phytochemicals (Steinmetz and Vegetables 1991). The role of phytochemicals in health promoting have been researched in many studies. The cholesterol-lowering properties of β -glucan from oat bran have been investigated in mildly hypercholesterolemic subjects. The results revealed that the consumption of β -glucan which were prepared from oat bran had ability in lowering total and LDL-cholesterol concentrations. The possible mechanism for cholesterol-lowering is the decrease bile acid reabsorption caused by fiber binding or by increased viscosity of intestinal contents (Kerckhoffs, Hornstra et al. 2003). Effect of virgin olive oils (VOO) enriched with phenolic compounds on the protection against oxidative DNA damage and antioxidants enzyme system in hyperlipidemic participants has been studied by Romeu et al., it was found that the consumption of phenol-enriched virgin olive oil had ability to improve DNA protection against oxidation and also improve antioxidant endogeneous enzymatic activity. Virgin olive oil is kind of the Mediterranean diet

which is known as contain approximately 36 phenolic compounds. The health benefits of virgin olive oil phenolics have been reported including improved endothelial dysfunction and reduce oxidative stress, anti-inflammation and antimicrobial activity (Romeu, Rubió et al. 2016).

On the other hand, functional food product development has been done in many researchs. In a study conduct by Ritthiruangdej et al. (Ritthiruangdej, Parnbankled et al. 2011), they investigated the use of unripe banana flour as an ingredient to produce dried noodle products of high nutritional quality with low carbohydrate digestibility and rich in resistant starch. The unripe banana flour was used to substitute wheat flour at five concentration (10, 20, 30, 40 and 50%). The results of developed noodle showed that the appearance of noodle became darker and the stickiness decreased when increased unripe banana flour. The 30% unripe banana flour substitution in the noodle could increase total dietary fiber, resistant starch and be accepted by consumers. Moreover, uncooked noodle composed of 13.7% protein, 0.12% fat and 4.8% dietary fiber (including 2.8% resistant starch). Unripe banana flour has a power source of fiber that beneficial for food product substitution.

Other researchers also substituted wheat flour with black rice powder at various levels (0 to 100%) to make chiffon cakes and determined the physicochemical, antioxidant and sensory quality characteristics of black rice chiffon cakes. The researchers conclude that the substitution of wheat flour with up to 60% black rice powder were moderately accepted by consumers and the total phenols, anthocyanins and scavenging property of black rice chiffon cakes increased with increased black rice powder levels. Black rice might provide a good source of bioactive compounds such as phenolic compounds, γ -oryzanol, tocopherols, anthocyanins and dietary fiber (Mau, Lee et al. 2017). In addition, black rice has been reported as a chemical compound which has antioxidant activity (Ichikawa, Ichiyanagi et al. 2001).

8. Cracker

In the area of bakery products, especially wheat flour processed products, are consumed all over the world. Crackers are the one that popular, convenient and continue growing through the snack products (Sedej, Sakač et al. 2011). Crackers are general word used throughout the world which is described as thin and crisp product with very low sugar and fat content. Moreover, the term of cracker can use when the baked product has a cereal base such as wheat, barley and oat at least 60% and a low moisture content of 1–5% (figure 5). Docking are the holes in crackers which are placed in the dough to stop excessively large air pockets from forming in the cracker while baking (Han, Janz et al. 2010). Normally, the dough of crackers is a hard dough, semisweet and unsweetened. The characteristic of hard dough is hard, but similar consistency to bread dough. The elastic and extensible of cracker dough depend on the gluten network which is well developed during mixing or making dough.

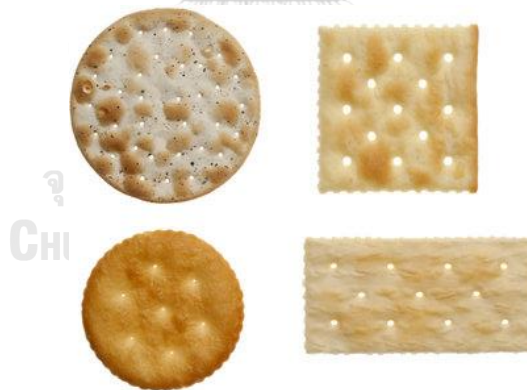


Figure 5 Cracker

Moreover, crackers might be divided into two main categories depended on their differences in ingredients and methods of production (Shukla 1994).

First, fermented (also called soda crackers, saltines and cream crackers). Soda crackers have been consumed and popular in the US. This cracker is normally about

4 mm thick and 50 x 50 mm square shape and contain 8–10% shortening (based on flour). Saltine crackers are a variation of soda crackers, which are smaller and delicious with increased shortening than soda crackers. They are produced using a sponge starter with a long fermentation. Then 1% sodium bicarbonate is added to the cracker dough, which help to increase the alkalinity and gives soda crackers its name. During this time, changes occur in the mixture that contributes to textural and flavor properties. The nine holes docking on each cracker set out in a 3 x 3 grid is the character of soda crackers. The final product texture is crisp. Usually, soda crackers are not eaten alone because of dry and bland, so are eaten with soup. For cream crackers, have been consumed in the UK. Cream crackers have a little higher fat content than soda crackers (12-18%). The shape of cream crackers is about 65 x 75 mm rectangular, which are thicker than soda crackers. They are produced using a long sponge and dough fermentation (4–16 hour) with modern methods include single stage mixing. The moisture content of cream crackers is 3–4%, which is high for crackers and with a higher fat content as well.

Second, chemically leavened (also called snack cracker). These crackers are newer than fermented crackers and have two distinguishing characteristics. Snack crackers are sprayed with hot oil since they leave the oven and a topping is added to the cracker for flavor. The flavor of snack crackers is come from spray the cracker with hot oil and added a topping to the cracker. The crackers normally contain about 4-10% of sugar to help flavor and texture. The proteolytic enzymes or sulphites are added into the dough for relaxing dough because the snack cracker dough does not have a long fermentation process. The shapes and sizes of snack crackers are various but typically they are round and have two docking holes to lift during baking.

8.1 Cracker products development

A good cracker product which contains a high nutritional value depends on the ingredients that use to make a cracker. The development of cracker has been done in many studies. The purpose of a study by Sedej et al., was to develop a new alternative for the use of buckwheat in the formulation of cracker with high nutritional quality. The authors concluded that flour which came from buckwheat showed the ability for using as ingredients to produce crackers. The crackers made from those ingredients presented good nutritional quality with higher total phenolic content, total flavonoids content and tocopherol than wheat crackers. Additionally, showed scavenging activity on DPPH when compare with wheat crackers (Sedej, Sakač et al. 2011).

The objective of a study conducted by Ahmed et al., was to investigate the suitability of incorporating different amount of *Hibiscus sabdariffa calyxes* residue (HSR) (0%, 1.25%, 2.5%, 3.75%, and 5.0%) in the snack cracker formula. The incorporation of HSR in the cracker showed lower protein, fat content and higher content of dietary fiber compared with control crackers. Furthermore, the incorporation of HSR-5% exhibited the highest total phenolic, total flavonoids content which had been shown to possess antioxidant activity. The replacement of wheat flour with HSR was accepted by the panelists (Ahmed and Abozed 2015).

In a study conduct by Mir et al., they studied the influence of apple pomace on the chemical, antioxidant and sensory properties of rice crackers. The addition of apple pomace (0%, 3%, 6% and 9%) in cracker formulation was sensory acceptable. Apple pomace based brown rice flour crackers which prepared from 9% pomace level expressed the highest total phenolic, flavonoid content and had antioxidant properties. Besides, the apple pomace-based cracker contains higher

dietary fiber particularly insoluble fiber than control samples. Crackers also showed rich in minerals especially chlorine, potassium, phosphorus and Sulphur.

According to Slavin et al., it was investigated the effect of soybean seed coat color (yellow and black) and baking time–temperature combinations on the antioxidant properties of a soy cracker food model. The results showed that black soybean crackers had significantly higher total phenolic, total isoflavones and peroxy, hydroxyl, and ABTS⁺ radical scavenging abilities than yellow soybean crackers, at all time–temperature combinations. Further, cyanidin-3-glucoside (C3G) was detected only in black soybean crackers. However, anthocyanin loss is dependent on time and temperature combinations of baking conditions (Slavin, Lu et al. 2013).

Previous study of Oluwamukomi et al. (Oluwamukomi, Oluwalana et al. 2011) investigated the using of composite flour (wheat-cassava flour) on the physicochemical properties of biscuit enriched with soy flour. Wheat flour was replaced with cassava flour at different levels including 0, 10, 20, 30, 40, 50, 60 and 70% in the biscuit. Moreover, the composite flour at levels above 40% were substituted with 10% soy flour in order to increase the protein content of biscuit product. The protein content decreased in the biscuit which increasing the level of cassava flour substitution, whereas the protein content in the biscuit which added with 10% of soy flour increased. The results showed that the addition of soy flour might increase the overall acceptability score and made the nutritious biscuit.

Adeola and Ohizua (Adeola and Ohizua 2018) studied the effect of 14 ratio of flour blends on the qualities of the biscuits. Unripe banana, pigeon pea and sweet potato flour were used as composite flour for the biscuit production. The texture parameters such as hardness and fracturability were affected as the mixture of various ratios of flour. Hardness value decreased when the amount of pigeon pea

flour increased while the fracturability value decreased as unripe banana flour increase. The composition of crude protein, crude fiber, ash and dietary fiber content increased as the pigeon pea flour increased. The biscuits prepared with 21.67% of pigeon pea flour, 21.67% unripe banana flour and 56.67% of sweet potato flour were most preferred in the properties of taste, shape, crunchiness, mouthfeel and overall acceptability. The results indicated that unripe banana, pigeon pea and sweet potato flour could be used as the novel ingredients for biscuit production.

Slavin et al. (Slavin, Lu et al. 2013) studied the effect of black and yellow soybean on the baking qualities including cyanidin-3-glucoside content and antioxidant properties of cracker. Black soybean is known as an ingredient with high content of anthocyanin. The results showed that crackers incorporated with black soybeans had higher total isoflavones, total phenolic content and antioxidant properties than the yellow soybean crackers of all time-temperature conditions. In addition, cyanidin-3-glucoside could only detect in cracker with black soybean and all baking cracker significantly decreased in cyanidin-3-glucoside. The phenolic and isoflavone contents were affected by high temperature condition. This study suggested that the moderate of baking temperature and time might be the best way to preserve antioxidant compounds in crackers.

Olagunju et al. (Olagunju, Omoba et al. 2018) developed the nutritious crackers from value-added blend flour between *acha* (*digitaria exilis*) and pigeon pea (*cajanus cajan*). Four ratios of *acha* and pigeon blend flour were used including 100:0, 80:20, 70:30, respectively. The supplementation of pigeon pea flour into cracker significantly increased the protein content. Also, the predominant amino acids were glutamic and aspartic acids and the lysine and methionine significantly increased as the increasing level of pigeon pea flour formulation. Moreover, the

crackers showed a good antioxidant scavenging properties, low glycemic index and exhibited to inhibit the digestive enzymes.

Sozer et al. (Sozer, Cicerelli et al. 2014) investigated the effect of addition of wheat bran into biscuit on physicochemical characteristics of biscuit. Since wheat bran present a crucial source of dietary fiber and protein. The biscuit was formulated with wheat bran in five concentrations including 0, 5, 10, 15 and 20%, respectively, with two different particle size (450 μm and 68 μm). Hardness of biscuit increased and the compact structure was found as the fine wheat bran (68 μm) incorporation. The coarse wheat bran (450 μm) supplementation up to 20% could improve the nutritional profiles of biscuit. Moreover, the addition of wheat bran up to 5% directly affected the sensory parameters.

Pasqualone et al. (Pasqualone, Bianco et al. 2015) studied the effect of purple wheat on the physicochemical properties of biscuit. Wheat flour was substituted with purple wheat flour for the production of biscuit as the purple wheat flour demonstrated a source of anthocyanins content. The findings illustrated that the biscuit prepared with purple wheat flour exhibited more anthocyanins content and antioxidant activity than the control biscuit (without purple wheat flour substitution). In addition, the presence of volatile compound profile in purple biscuit showed significantly lower in level of lipid-derived carboxylic and also higher in level of alcohols and aldehydes than the control. These results indicated that the low oxidative degradation of lipid in purple biscuit was detected. In the study of

Pasqualone et al. (Pasqualone, Bianco et al. 2014) studied the effect of the grape marc extract on the volatile profiles and physicochemical properties of biscuits. In the wine grape production process generated the huge amount of grape marc byproducts which known as contain a source of bioactive compounds such as catechins, anthocyanins and phenolics. The biscuit enriched with grape marc extract

had higher phenolic content and antioxidant property than the control. In terms of sensory attributes score, the biscuit enriched with grape marc extract had higher color, sour taste and fruity odor score than control biscuit. The using of waste or byproduct from food industry might be a one approach for innovative food development.

Ajila et al. (Ajila, Leelavathi et al. 2008) studied the effect of the incorporation of mango peel powder on the dietary fiber improvement and antioxidant activity in soft dough biscuits. In this study, the researcher used the mango peel which is a by-product from the mango product processing for incorporation into biscuits. Five different concentrations of mango peel powder were used in this study (5.0, 7.5, 10.0, 15.0 and 20.0%). The supplementation of mango peel at 20%, significantly increased the total dietary fiber in biscuit. Furthermore, the phenolic content and carotenoid content increased from 0.54 to 4.50 mg/g and 17 to 247 $\mu\text{g/g}$, respectively of 20% mango peel incorporation. The incorporation of mango peel powder could improve the antioxidant activity. The acceptable biscuit enriched mango peel powder was obtained by the fortification 10%.

Previous study of Tyagi et al. (Tyagi, Manikantan et al. 2007) studied the effect of the fortification of defatted mustard flour on the textural, nutritional and organoleptic characteristics of biscuit. In the biscuit production, wheat flour was substituted with defatted mustard flour in various concentration including 5, 10, 15 and 20%. The addition of defatted mustard flour directly affected to the characteristics of biscuit. The results showed that fat content reduced and protein and fiber content were increased as the level of defatted mustard flour increased. The highest overall acceptability score was found in the biscuit with 15% defatted mustard flour incorporation and also present the most acceptable biscuit.

Onabanjo and Ighere (Onabanjo and Ighere 2014) studied the effect of using the composite of wheat-sweet potato flour on the functional, nutritional and sensory properties of biscuit. The sweet potato flour was used to substitute wheat flour at five levels (50:50, 60:40, 70:30; 90:10 and 100:0) in the preparation of biscuit. Fat and protein content of biscuit were increased as level of sweet potato increased. The highest crude fiber content of biscuit was found in the ratio of 50:50 of wheat-potato flour. Since the sweet potato flour was incorporated into biscuit, the moisture and carbohydrate values were increased. The results revealed that the mineral content including magnesium, calcium, sodium, phosphorous and potassium were higher in biscuits prepared with 50:50, 60:40, 70:30 and 90:10 wheat-sweet potato composite flour than the biscuit prepared with 100:0 wheat-sweet potato composite flour. Moreover, all ratios of wheat-sweet potato composite flour were accepted by the consumer.

9. Papaya fruit description and composition

Papaya (*Carica papaya*) is a fruit that also known to have medicinal properties with high nutritional value throughout the world. It is mainly crop in various parts worldwide, including Hawaii, Sri Lanka, South Africa and tropical regions (figure 6).



Figure 6 ripe and green papaya with embedded seeds

Papaya fruits have been reported to be the source of vitamins (vitamin C, A, folate and riboflavin) and minerals (thiamine, calcium, iron, niacin and potassium)

with low in calories (32 kcal/100g ripe fruit) (Krishna, Paridhavi et al. 2008). Moreover, papaya has been reported various pharmacological actions and medicinal use, including anti-bacterial, anti-inflammatory, anti-hypertensive, free radical scavenging and wound healing. According to Fatema et al., it was studied the glycemic and insulinemic responses to the pumpkin and unripe papaya by estimating the glycemic index (GI) and insulinemic index from Bangladeshi T2DM patients. The results showed that unripe papaya consumption showed significant lower serum glucose response compared to bread and pumpkin. Furthermore, the GI of pumpkin and unripe papaya were 74 and 23, respectively. From this study, unripe papaya is regarded as a low GI vegetable (Fatema, Rahman et al. 2011).

The immature or unripe papaya skin presents green color when fully ripened the skin color will change to reddish-orange (Maisarah, Nurul Amira et al. 2013). Nutritive value of 100g of ripe and green papaya fruit can be seen in (figure 7) (Krishna, Paridhavi et al. 2008)

Constituents	Ripe papaya	Green papaya
Protein	0.6 g	0.7 g
Fat	0.1 g	0.2 g
Minerals	0.5 g	0.5 g
Fibre	0.8 g	0.9 g
Carbohydrates	7.2 g	5.7 g
Energy	32 kcal	27 kcal
Total carotene	2,740 μ m	0
Beta carotene	888 μ m	0

Figure 7 Nutrition content of papaya fruit per 100 g

(Krishna, Paridhavi et al. 2008)

Maisarah et al. (Maisarah, Nurul Amira et al. 2013) studied the antioxidant compounds and antioxidant properties in different parts of *Carica papaya*. Four different parts of papaya including ripe and unripe fruits, seeds and young leaves

were determined total phenolic content, total flavonoid content and also the antioxidant activity. The findings showed that unripe papaya fruits had the highest antioxidant activity through β -carotene bleaching assay followed by young leave, ripe fruit and seed. On the other hand, the highest DPPH scavenging effect was observed in young leaves compared to the others. Moreover, the young leaves also had the highest antioxidant compounds including total phenolic content and total phenolic content. In conclusion, antioxidants in all the samples were noticeable in the sequence of young leaves > unripe fruit > ripe fruit > seed.

Nowadays, fruits byproducts get rid of globally every day by the food industry. Consequently, generating the huge amount of fruit byproducts. Crizel et al. (De Moraes Crizel, Hermes et al. 2016) studied on the fruit byproducts in terms of chemical and bioactive compounds. Papaya, pineapple, olive and blueberry byproducts were evaluated the physicochemical and antioxidant properties. All fruit byproducts presented a good source of total dietary fiber, especially olive byproducts. Papaya powder byproducts exhibited the highest water holding capacity and solubility values among the fruit byproducts. Moreover, the blueberry powder byproducts showed the effectiveness, ability to scavenge the free radical as blueberry powder contained source of anthocyanins. In terms of carotenoid content, papaya powder showed the highest carotenoid content, followed by pineapple and olive powder byproducts. The researcher stated that the fruit byproducts could be used as high potential functional ingredients in food products.

Chukwuka et al. (Chukwuka, Iwuagwu et al. 2013) investigated the nutritional compositions of *Carica papaya* L. in the different stages. Three parts of papaya (pulp, peel and seeds) were determined the mineral and vitamin content and also proximate composition. The results illustrated that unripe papaya fruit presented a desirable source of vitamin, carbohydrate and protein, however, those nutrients

could decrease as the fruits are ripe. The researcher reported that the ripe papaya fruits are not a good source of protein. Hard ripe and very ripe papaya exhibited high content of vitamin C. Every stage of papaya fruit showed the suitable content of vitamin A and minerals including Na, Mg, K and Ca. In addition, the unripe papaya fruit had the highest contents of non-nutritive components such as phenol, tannin, alkaloid, saponin and flavonoid. Nevertheless, in view of consumption the very ripe was recommended.

Previous study of Mahattanatawee et al. (Mahattanatawee, Manthey et al. 2006) studied the florida-grown tropical fruits including ripe and green papaya. The results showed that moisture, phenolic content and ascorbic acid of ripe and green papaya were 89.3%, $442.2 \pm 29.7 \mu\text{g GA/g puree}$, $153.8 \pm 12.1 \text{ mg/100g puree}$ and 92.4%, $311.1 \pm 18.9 \mu\text{g GA/g puree}$, 56.7 ± 3.5 , respectively. Moreover, the ripe and green papaya exhibited the antioxidant properties through the DPPH scavenging and ORAC assays. In terms of total dietary fiber and pectin content of ripe and green papaya were $1.5 \pm 0.1 \text{ g/100g fruit}$, $0.49 \pm 0.01 \text{ g/100g fruit}$ and $2.1 \pm 0.0 \text{ g/100g fruit}$, $0.60 \pm 0.02 \text{ g/100g fruit}$, respectively.

Furthermore, some people in Nigeria used the unripe papaya pulp as a soup ingredient. To know about the nutritional profile of unripe papaya and cooked-unripe papaya, Okon et al. (Okon, Ogrí et al. 2017) aimed to study the nutritional composition of unripe papaya. The findings showed that after cooked the unripe papaya affected to increase the protein, ash, carbohydrate and crude fiber contents, while cooked-unripe papaya resulted to decrease moisture and fat content. In terms of the mineral composition of unripe papaya contained the Ca, P, Na, Mg, K, Fe, Se, Zn and Mn. However, most of the minerals content was decreased after cooked the unripe papaya. The vitamins composition in unripe papaya including vitamin A, E, K, B1, B2, B3, B6, folic acid and vitamin C. The non-nutrient composition of unripe

papaya including saponins, flavonoids, cardenoloids, polyphenols, phenols oxalates, cyanates and phytates. The vitamin and non-nutrient compositions of unripe papaya fruits were affected by the temperature as the decreasing content, both vitamins and non-nutrients content after the cooking process.

The practical food application of papaya has been used as the raw ingredient for cookie production. Varastegani et al. (Varastegani, Zzaman et al. 2015) studied papaya pulp flour on the physicochemical and sensory properties of cookies. In the cookies production process, wheat flour was replaced with papaya pulp flour (15, 30 and 50%). The substitution of papaya pulp flour up to 50%, significantly increased the total dietary content, resistant starch and also the polyphenol contents. The antioxidant activity was improved as the level of papaya pulp flour increased. Furthermore, the cookies incorporation with papaya pulp flour also well accepted by consumers.

Papaya has been used in the food manufacturing industry, especially unripe or green papaya which is used in mixed fruit processing company. However, these processes generate huge amounts of residues (peel, seed, and top/bottom of unripe papaya). The top and bottom residues approximately count for 3.6 – 7.2 tons per year. The several tons of residues have been eliminated in environment system, causing environmental problems. It's beginning to have much interest to use of agroindustry residues to make zero waste production.

CHAPTER III

MATERIALS AND METHODS

1. Materials and equipment

Materials	Company
Unripe papaya (top and bottom)	KCG corporation (Bangkok, Thailand)
Riceberry flour	Sunfood Corp Co., Ltd. (Samutprakan, Thailand)
wheat flour	United Flour Mill Public Co., Ltd. (Samutprakan, Thailand)
Salt	Thai Refined Salt Co., Ltd. (Nakhonratchasima, Thailand)
Sugar	Mitr Phol Sugar Corp Co., Ltd. (Suphanburi, Thailand)
Yeast	Greathill Co., Ltd. (Bangkok, Thailand)
Butter	KCG corporation (Bangkok, Thailand)
Shortening	Thai Vegetable Oil Public Co., Ltd. (Bangkok, Thailand)

Chemicals	Company
Methanol (analytical grade; CH ₃ OH)	JT.Baker (USA)
Hydrochloric acid (HCl)	Merck (Darmstadt, Germany)
Folin-Ciocalteu reagent	Sigma-Aldrich Co. (St. Louis, MO, USA)
Sodium carbonate anhydrous	Ajax Finechem (Taren Point, Australia)
Gallic acid	Fluka™ (Seelze, Germany)
Potassium chloride	Sigma-Aldrich Co. (St. Louis, MO, USA)
L-ascorbic acid	Sigma-Aldrich Co. (St. Louis, MO, USA)
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Sigma-Aldrich Co. (St. Louis, MO, USA)
Trolox (6-hydroxyl-2,5,7,8-tetramethylchromane-2-carboxylic acid)	Sigma-Aldrich Co. (St. Louis, MO, USA)
ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)	Sigma-Aldrich Co. (St. Louis, MO, USA)
α-amylase	Sigma-Aldrich Co. (St. Louis, MO, USA)
Pancreatin	Sigma-Aldrich Co. (St. Louis, MO, USA)
Glacial acetic acid (CH ₃ COOH)	Merck (Darmstadt, Germany)

Chemicals	Company
Pepsin	Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India)
Amyloglucosidase	Megazyme (Illinois, USA)
Glucose liquicolor	HUMAN (GmbH, Germany)
Sodium acetate 3-hydrate	Elago Enterprises Pty. Ltd. (Cherrybrook, Australia)
D-Glucose	Ajax Finechem (Taren Point, Australia)
Sodium chloride (NaCl)	Ajax Finechem (Taren Point, Australia)
Sodium dihydrogen phosphate (NaH_2PO_4)	Ajax Finechem (Taren Point, Australia)
Sodium hydrogen phosphate (Na_2HPO_4)	Ajax Finechem (Taren Point, Australia)
Iron (II) sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	Ajax Finechem (Taren Point, Australia)
Iron (II) chloride anhydrous (FeCl_3)	Ajax Finechem (Taren Point, Australia)
Potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$)	Sigma-Aldrich Co. (St. Louis, MO, USA)
TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine	Sigma-Aldrich Co. (St. Louis, MO, USA)

Equipment	Company
Shaking water bath	Memmert GmbH + Co. KG (Germany)
Rotary evaporator	Buchi (Switzerland)
Spectrophotometer	Biotek (USA)
Vortex mixer	Gemmy industrial (Taiwan)
Sonicator	GT Sonic (China)
Incubator	Wisecube Co., Ltd. (South Korea)
Centrifuge	Hettich zentrifugen (USA)
pH meter	Thermo Fisher Scientific Inc. (USA)
Hot plate	IKA-works (Germany)
Scanning electron microscopy	JEOL (Japan)
Colorimeter	HunterLab (USA)
Infrared Moisture Determination Balance	Kett Electric Laboratory (Japan)
Water activity meter	Decagon Devices, Inc. (USA)
Texture analyzer	Technologies Corp. and Stable Micro Systems Ltd. (USA)
Electronic weighing balance	Sartorius Co. Ltd. (Germany).

2. Methods

2.1 To investigate the effect of partially substituted wheat flour with riceberry flour on physicochemical, functional properties and sensory evaluation of crackers.

2.1.1 Preparation of Riceberry flour

The commercial riceberry flour was purchased from Sunfood corp. (Thailand). Riceberry flour was sieved through a 100-mesh screen, then was kept in sealed container at room temperature until a further analysis.



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Figure 8 Riceberry flour (RBF)
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2.1.2 Preparation of crackers

The formulation for making crackers was presented in Table 1.

Table 1 Formulation of crackers

Ingredients	Amount (g/100 g)
wheat flour	54.4
Salt	0.3
Yeast	0.6
Butter	13.0
Shortening	4.3
Sugar	1.0
Water	26.1

Crackers were prepared in a straight dough process following the procedure of Ahmed and Abozed with slight modification (Ahmed and Abozed 2015). In brief, the ingredients were weighed according to the proportion listed in Table 1. Yeast (0.6 g), sugar (1 g) and salt (0.3 g) were dissolved in water (4.4 g), then added to the wheat flour (54.4 g) and mixed. Butter (13 g), shortening (4.3 g) and the remaining water (22 g) were added and mixed to form cohesive dough. The cracker dough was wrapped with plastic wrap and proofed for 10 min. After that the dough was rolled into 3 mm thickness, cut by pressing a rectangle frame mold and proofed for 40 min. The crackers were baked at 180 °C for 10 min. Baked crackers were cooled at ambient temperature. Finally, the crackers were packed in aluminum bag.

2.1.3 Preparation of crackers substitution with riceberry flour

Control crackers were prepared from 100% wheat flour. The test group crackers were prepared by substituting wheat flour with 5, 10, 15 and 20% of

riceberry flour. Moreover, the amount of all the ingredients that were used in the preparation of both the control and test group are the same except the amount of riceberry flour (Table 2).

Table 2 Formulation of crackers prepared with various levels of riceberry flour replacement in wheat flour

Ingredients (g)	Control	Riceberry flour substitution (%)			
		5	10	15	20
Wheat flour	54.4	51.68	48.96	46.24	43.4
Riceberry flour	-	2.72	5.44	8.16	11.0
Butter	13.0	13.0	13.0	13.0	13.0
Shortening	4.3	4.3	4.3	4.3	4.3
Sugar	1.0	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3	0.3
Yeast	0.6	0.6	0.6	0.6	0.6
Water	26.1	26.1	26.1	26.1	26.1

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Five formulations of cracker used for chemical analysis were ground, sieved through 100-mesh screen, packed in aluminum bag and stored at -20 °C. Crackers were prepared in four batches.

2.1.4 Physicochemical and functional properties of riceberry crackers

2.1.4.1 Weight

Weight of crackers was measured by weighing three individual crackers using an electronic weighing balance. An average of three values was taken for each set of crackers.

2.1.4.2 Height

Height of crackers was determined by measuring three individual crackers with a vernier caliper. Crackers were measured from two sides, measuring once and turning 90° vertically in order to get the average value (Garzóan, Gaines et al. 2003).

2.1.4.3 Diameter

Diameter of crackers was determined by measuring three individual crackers with a vernier caliper, measuring once and turning the crackers 90° horizontally to obtain the average value (Garzóan, Gaines et al. 2003).

2.1.4.4 Moisture

The infrared moisture analyzer was used to determine the moisture content of crackers. The ground cracker (3 g) was weighted on the moisture analyzer and set at 160 °C for 10 min (Pasukamonset, Pumalee et al. 2018).

2.1.4.5 Water activity

The cracker was ground into fine powder and measured the water activity by using a water activity meter.

2.1.4.6 Color analysis

The color values, lightness (L*), redness (a*) and yellowness (b*), of the cracker were carried out using Hunter Lab colorimeter. First, the apparatus was calibrated with black and white standard tiles before color measurement. The lightness (L*), redness (a*) and yellowness (b*) values of the cracker were recorded. Five replicates of crackers were chosen randomly from the four batches. The color measurement of each cracker was calculated to compare with control cracker. Total color difference (ΔE) was estimated by using the formula as shown in the previous study (Ahmed and Abozed 2015).

$$\Delta E = [(L_c^* - L_s^*) + (a_c^* - a_s^*) + (b_c^* - b_s^*)]^2$$

where subscript *c* = control

subscript *s* = cracker containing riceberry flour

2.1.4.7 Texture profile analysis

The texture analysis of the cracker was measured by a texture analyzer TA.XT-Plus. Hardness and fracturability of the cracker were measured using a spherical stainless probe, 5 mm thick. The following settings were used: pre-test speed 5.0 mm s⁻¹, test speed 1.0 mm s⁻¹, post-test speed 5.0 mm s⁻¹, distance 7.0 mm. Fifteen replicates of crackers were chosen randomly from the four batches. (Sattasuwana, Nuengjamnong et al. 2010).

2.1.4.8 Sample preparation for the assays

2.1.4.8.1 Flour extraction

The extraction of riceberry and wheat flour was as described by Shen et al (Shen, Jin et al. 2009). Riceberry and wheat flour (1 g) were extracted with 25 ml of acidified methanol for 24 h at shaking water bath (25 °C). The methanolic extracts were centrifuged at 3000 rpm for 15 min. The supernatant was evaporated by using a rotary evaporator at 50°C. The extracts were stored at -20°C until the phytochemicals and antioxidant capacity analysis.

2.1.4.8.2 Cracker extraction

Crackers were extracted according to the modified procedure reported by Kosutic et al (Košutić, Filipović et al. 2016). Cracker powder (10 g) was extracted with 40 ml of acidified methanol 24 h at shaking water bath (25 °C). It was filtered through No.1 filter paper and then centrifuged at 3000 rpm for 15 min. The supernatant was evaporated by using a rotary evaporator at 50°C. The extracts were stored at -20°C until the phytochemicals and antioxidant capacity analysis.

2.1.4.9 Proximate composition and minerals content determination

The riceberry flour was analyzed for moisture, ash, protein, total fat, carbohydrate, total dietary fiber, iron and zinc according to the official method of AOAC international (2012).

2.1.4.9.1 Determination of moisture content

The moisture content was determined by the official method of 990.19. The moisture of sample was measured by weighing sample, drying sample and weighing dried sample residue. Moisture of sample was determined by using forced air oven. Sample was dried for 3 h at 100 °C and calculated the moisture of sample which is weight of dried sample residue as % of original sample weight.

2.1.4.9.2 Determination of ash content

The ash content was determined by the official method of 945.46. The ash of sample was measured by using gravimetric method. Sample was heated at ≤ 550 °C until ash is C-free, then sample was cooled in the desiccator, weigh and calculated the percentage of ash.

2.1.4.9.3 Determination of protein content

The protein content was determined by the official method of 991.20. The protein of sample was measured by Kjeldahl method. Sample was digested with H_2SO_4 by using $CuSO_4 \cdot 5H_2O$ as the catalyst and K_2SO_4 as the boiling point elevator, in order to release nitrogen from protein and also retain nitrogen as ammonium salt. The concentrated NaOH was added to release NH_3 which was distilled, collected in solution (H_3BO_3) and titrated. The obtain percentage of nitrogen was multiply with factor 6.38, in order to calculate the percentage of protein in sample.

2.1.4.9.4 Determination of total fat content

The total fat content was determined by the official method of 922.06. The total fat of sample was measured by acid hydrolysis method. Sample was hydrolyzed with HCl, then the petroleum ether was added to extract until the upper liquid is clear. Sample was dried in oven at 100 °C to obtain the constant weight and report as the percentage of total fat.

2.1.4.9.5 Determination of carbohydrate content

$$\text{Carbohydrate} = 100 - (\text{Water} + \text{Protein} + \text{Fat} + \text{Ash})$$

2.1.4.9.6 Determination of total dietary fiber content

The total dietary fiber content was determined by the official method of 992.16. The total dietary fiber of sample was measured by enzymatic-gravimetric method. Sample was defatted before the enzymatic digestion and added the α -amylase and phosphate buffer, then sample was incubated at 100 °C for 30 min. Next, the protease enzyme was added in the sample and incubated at 60 °C for 30 min (pH 7.5). The amyloglucosidase was added incubated at 60 °C for 30 min (pH 4.5). The digested sample was washed, filtrated, dried and weighted. The sample was analyzed for the amount of protein and the other sample was determined for the ash content. The total dietary fiber was calculated by weight of residue – weight of protein and ash.

2.1.4.10 Phytochemicals determination

Cracker extracts were weighted and dissolved by methanol before experiments.

2.1.4.10.1 Determination of total phenolic content (TPC)

In this study, to determine the level of polyphenol content in flour and cracker extracts.

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method as described by Shen et al., with a slight modification. Briefly,

the extract (20 μl) was mixed with 150 μl of the freshly diluted 10-fold Folin-Ciocalteu reagent. After incubation at room temperature for 5 min, 150 μl of sodium carbonate (6% w/v) was added to the mixture and incubated at room temperature for 1 h in a dark place. The absorbance at 760 nm was measured using a spectrophotometer). A calibration curve was used standard Gallic acid solution and TPC expressed as mg Gallic acid equivalent (GAE) per 100 g flour. A Gallic standard curve was prepared within the range of 0 – 0.2 mg/ml (Shen, Jin et al. 2009).

2.1.4.10.2 Determination of total anthocyanin content (TAC)

The level of anthocyanin was determined in flour and cracker extracts since it is known as a subgroup of flavonoid and one of the most broadly naturally source of colorants which have antioxidant properties.

The total anthocyanin content (TAC) was determined based on pH colorimetric method of Lee et al (Lee, Durst et al. 2005). The extract was measured at 510 and 700 nm in pH 1.0 of 0.025 M potassium chloride buffer and in pH 4.5 of 0.4 M sodium acetate buffer. Samples (600 μl) were mixed with 600 μl of each buffer and incubated for 20 min at room temperature. The absorbance was measured at 510 nm and 700 nm and then calculated using an equation of $A = (Abs_{510} - Abs_{700})_{\text{pH}1.0} - (Abs_{510} - Abs_{700})_{\text{pH}4.5}$. Anthocyanin pigment was calculated in terms of cyanidin-3-glucoside by the following equation:

$$\text{Anthocyanins pigment} = \frac{A \times MW \times DF}{\epsilon \times l} \times 10^3$$

(Cyanidin-3-glucoside equivalents, mg/L)

$$A = (Abs_{510} - Abs_{700})_{\text{pH}1.0} - (Abs_{510} - Abs_{700})_{\text{pH}4.5}$$

$$MW \text{ (Molecular weight)} = 449.2 \text{ g/mol for cyanidin-3-glucoside (Cy-3-Glu)}$$

$$DF = \text{Dilution Factor}$$

$$l = \text{Path length in cm} = 1 \text{ cm}$$

$$\epsilon = 26,900 \text{ molar extinction coefficient, in L/mol/cm for Cy-3-glu}$$

$$10^3 = \text{Factor for conversion from g to mg}$$

TAC expressed as mg Cyanidin-3-glucoside equivalent (C3G) per 100 g flour

2.1.4.11 Antioxidant capacity determination

2.1.4.11.1 Determination of DPPH radical-scavenging

In this assay, to determine the antioxidant activity in flour and cracker extracts. DPPH, electron transfer based assay, based on the capacity of antioxidant to reduce an oxidant by measuring the decrease of its absorbance (Teixeira, Gaspar et al. 2013).

The effectiveness of the cracker to scavenge DPPH radicals was determined based on the procedure of Jariyapamornkoon et al., with slight modification. Samples (100 μ l) were mixed with 100 μ l of a DPPH solution (0.2 mM in methanol) and then incubated for 30 min in a dark place. The reduction in the absorbance was measured at 517 nm. The extract concentration providing 50% inhibition (IC_{50} value) was calculated from the graph of percentage DPPH scavenge capacity against the concentrations of the extract. Ascorbic acid was used as standard curve which was prepared within the range of 0 – 50 μ g/ml (Alvarez-Suarez, Giampieri et al. 2014).

2.1.4.11.2 Determination of ferric reducing antioxidant power (FRAP)

The antioxidant activity of flour and cracker extracts was determined by using ferric reducing antioxidant power (FRAP). The reducing power of a compound serves as an indicator of its antioxidant activity. Reducing power appears to be related to the degree of hydroxylation and extent of conjugation in polyphenols (Singh, Singh et al. 2003).

The ferric reducing antioxidant power (FRAP) of the cracker was determined according to a modified method described by Jariyapamornkoon et al (Jariyapamornkoon, Yibchok-anun et al. 2013). Concisely, FRAP reagent was mixed

with 10 ml of 0.3 M (pH 3.6) sodium acetate buffer, 1 ml of 10 mM 2,4,6- tripyridyl-S-triazine (TPTZ) in 40 mM HCl and 1 ml of 20 mM FeCl₃. An aliquot (20 µl) of the samples was mixed with 180 µl of FRAP reagent as oxidizing reagent and incubated for 30 min in a dark place. The increasing of solution absorbance was measured at 595 nm. FRAP value was calculated from a standard curve by using FeSO₄. FRAP value expressed as mM of FeSO₄ equivalents per 100 g flour.

2.1.4.11.3 Determination of trolox equivalent antioxidant capacity

This assay was measure of antioxidant activity of mixtures of substances in cracker extracts. The assay based on interaction between antioxidant and ABTS^{•+} which is free radical generator.

Trolox equivalent antioxidant capacity (TEAC) was carry out base on the procedure by Suantawee *et al.*, with a minor modification. The ABTS^{•+} radical solution was originated by mixing 7 mM ABTS in 0.1 M phosphate buffer saline (pH 7.4) with 2.45 mM K₂S₂O₈ in distilled water. The reagent solution was incubated for at least 16 h at room temperature in the darkness to generate the dark green solution and diluted with 0.1 M phosphate buffer saline to an absorbance value of 0.70 ± 0.02 at 734 nm. Antioxidant capacity was measured by an aliquot (10 µl) of the extract was mixed with 90 µl of the ABTS reagent solution. The decrease in the reagent solution absorbance was measured at 734 nm after 6 min. The antioxidant capacity was calculated using the area under the curve (AUC). TEAC value was calculated from AUC of a standard curve of Trolox (Suantawee, Wesarachanon *et al.* 2015).

2.1.4.12 Predicted glycemic index determination

Glycemic index (GI) was determined in the cracker, since it is a property of carbohydrate-containing food that describes the rise of blood glucose occurring after a meal (Ludwig, Majzoub *et al.* 1999). The *in vitro* digestion was

measured according to a modified method of Capriles and Arêas (Capriles and Arêas 2013) and Yousif et al (Yousif, Nhepera et al. 2012).

2.1.4.12.1 *In vitro* gastrointestinal digestion release property

The finely ground samples (500 mg) were mixed 1 ml of α -amylase (250 U per ml of 0.2 M pH 7 carbonate buffer). Then, 5 ml of pepsin solution (40 ml/ml in 0.02 M pH 2 HCl) was added. The mixture was incubated at 37°C in a shaking water bath for 1 h. The gastric digesta was neutralized with 5 ml of 0.02 M NaOH with following by the addition of 25 ml of 0.2 M pH 6 sodium acetate buffer. Then, 5 ml of a mixture of pancreatin (2 mg/ml in 0.2 M pH 6 acetate buffer) and amyloglucosidase (28 U per ml of 0.2 M pH 6 acetate buffer) was added. Incubation at 37°C in a shaking water bath and samples were collected at different times (0, 20, 30, 60, 90, 120 and 180 min). An aliquot was heated at 100°C in heating block for 10 min and centrifuged at 13,000 rpm for 10 min. An aliquot was collected and kept at -20°C until the further study. Glucose content in the aliquots was measured by the glucose Liquicolor test. In brief, working reagent (500 μ l) was mixed with aliquot (5 μ l) of the sample and incubated at room temperature for 10 min. The absorbance was measured at 500 nm. Glucose was used as a standard. The amount of glucose was calculate using the following equation:

$$C = \frac{\Delta Abs \text{ sample}}{\Delta Abs \text{ STD}} \text{ (mg/dl)}$$

(Glucose concentration)

The rate of *in vitro* digestion will express as the glucose concentration at different times (0, 20, 30, 60, 90, 120 and 180 min). The hydrolysis index (HI) will be derived from the ratio between the area under hydrolysis curves of the hydrolyzed sample (cracker) and reference food (glucose) up to 180 min. The areas under hydrolysis curves (AUC, 0 - 180 min) will be calculated, with the equation described below.

$$HI = (AUC_{\text{sample}} / AUC_{\text{glucose}}) \times 100$$

Moreover, The pGI was calculated by using equation below.

$$pGI = 39.71 + 0.549HI$$

For the percentage of rapidly digestible starch (RDS) and slowly digestible starch (SDS) were calculated following the study of Englyst *et al* (Englyst, Kingman et al. 1992) and Zhang and Hamaker (Zhang and Hamaker 2012).

$$\%RDS = (G_{20} - G_0) \times 0.9/TS$$

$$\%SDS = (G_{120} - G_0) \times 0.9/TS$$

$$\%UDS = TS - (RDS+SDS)/TS$$

G_0 : glucose released at 0 min

G_{20} : glucose released at 20 min

G_{120} : glucose released at 120 min

TS : total starch

2.1.4.13 Total starch analysis

Total starch content was determined based on the procedure by Goni et al., with a minor modification (Goñi, Garcia-Alonso et al. 1997). Sample (50 mg) was mixed with 6 ml of 2 M KOH and incubated in a covered shaking water bath for 1 h at room temperature. Then, 3 ml of 0.4 M sodium acetate buffer was added and adjusted pH to 4.75. Amyloglucoidase (3,260 U per ml) was added to the mixture for 60 μ l. After that the mixture was incubated at 60°C in a covered shaking water bath (100 rpm) for 45 min. An aliquot was heated at 100°C in heating block for 10 min and centrifuged at 13,000 rpm for 5 min. Glucose content in the aliquots was measured by the glucose Liquicolor test. The concentration of glucose was converted into amount of starch by multiplying 0.9. Total starch (TS) content was reported in gram per 100 g sample.

2.1.5 Scanning Electron Microscope (SEM) examination

The microstructure of flour and cracker was determined by SEM. Cracker was cut into a cube and adhered on an aluminum stub. Samples were coated with sputter gold and scanned using JSM-IT300 scanning electron microscope. The micrographs were taken at a magnification of 100x for the surface and cross-section parts of the cracker.

2.1.6 Sensory analysis

To determine the acceptability between riceberry cracker and control cracker.

2.1.6.1 Acceptance test

Sensory evaluation was carried out by fifty untrained panelists which were recruited from Chulalongkorn University. The cracker was assessed in a standardized tasting room with individual booths of Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University. The five formulations of crackers were evaluated on various attributes including appearance, color, flavor, taste, hardness, fracturability and overall acceptability using a nine-point hedonic scale. Each panelist was received five formulations of crackers (3 g per piece) in random order which were labeled with three-digit random numbers. All the attributes scored on a scale varying from “9 = like extremely” to “1 = dislike extremely”. Drinking water (330 ml) was provide to the panelists to rinse their mouth between the samples. Crackers were prepared according to good hygiene and manufacturing practices. Panelists were notified about the study and described that their cooperation were totally willing. They can stop the evaluation at any point and the responses were anonymous.

Inclusion and exclusion criteria of panelists involved in the sensory analysis including;

- Inclusion criteria
 - Male/female
 - Age 18-50 years
 - Voluntary participation in research project
- Exclusion criteria
 - color blind
 - Have common cold symptoms including cough, runny nose and sore throat
 - Pregnancy and breastfeeding
 - Gluten allergy
 - Smokers

Hedonic sensory evaluation was used to determine the degree of likeness for the food product. The samples were presented to the panelists on a white plate labeled with three-digit random numbers. Sensory evaluation was carried out around 15 min.

Crackers to be use for sensory evaluation were cooled at room temperature after baking and packed in a plastic box at room temperature until evaluating. The acceptance index (AI) was calculated by the following formula:

$$\text{Acceptance index (AI)} = \left(\frac{\text{Overall acceptability score}}{9} \right) \times 100$$

2.1.7 Statistical analysis

Data was reported as mean of values \pm SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) following by Duncan's multiple range test and using SPSS statistical. The significance different test was used for mean comparison and P-value<0.05 was considered as statistically significant. Calculations were performed with Microsoft Excel 2013. The graphs were generated with Sigma Plot, version 12.0 software.

The test groups of riceberry cracker were considered as acceptable if they have the mean score for overall acceptability above or equal 6.0, the highest antioxidant activity, and the lowest glycemic index value. Therefore, it was selected for further study.



2.2 To investigate the effect of riceberry cracker supplementation with unripe papaya flour on physicochemical, functional properties, and sensory evaluation.

2.2.1 Preparation of unripe papaya flour

Unripe papaya flour was prepared following the procedure of Kumar et al., with slight modification (Kumar, Rajeevkumar et al. 2012). Briefly, the residues (top and bottom) of unripe papaya were supplied by a mixed fruit company in Bangkok province, Thailand. The residues were washed with running water, shredded into a small piece by the machine. The residues were dried until the moisture content reached about 8% in hot air oven at 70 °C for 8 h (Udomkun, Nagle et al. 2015). After that the residues were ground to flour in a grinder for approximately 90 s and passed through a 100-mesh screen. Every batches were collected and mixed into a single one to be used in all experiments. Unripe papaya flour was kept in sealed container at -20 °C until a further analysis.



Figure 9 Unripe papaya flour (UPF)

2.2.2 Preparation of riceberry crackers supplementation with various levels of unripe papaya flour (UPF) replacement in wheat flour

The selected riceberry cracker formulation from part 2.1 was used in further study by substitution of wheat flour with unripe papaya flour in 3 different levels including 3, 5 and 7%, respectively.

Table 3 Formulation of riceberry crackers incorporation with various levels of unripe papaya flour (UPF) replacement in wheat flour

Ingredients (g)	Unripe papaya flour substitution (%)			
	Control	3	5	7
Wheat and riceberry flour	54.4	51.4	49.4	47.4
Unripe papaya flour	-	3.0	5.0	7.0
Butter	13.0	13.0	13.0	13.0
Shortening	4.3	4.3	4.3	4.3
Sugar	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3
Yeast	0.6	0.6	0.6	0.6
Water	26.1	26.1	26.1	26.1

Four formulations of cracker used for chemical analysis were ground, sieved through 100-mesh screen, packed in aluminum bag and stored at -20 °C. Crackers were prepared in four batches.

2.2.3 Physicochemical, functional properties of riceberry crackers incorporation with unripe papaya flour

2.2.3.1 Weight

Weight of crackers was measured by weighing three individual crackers using an electronic weighing balance. An average of three values was taken for each set of crackers.

2.2.3.2 Height

Height of crackers was determined by measuring three individual crackers with a vernier caliper. Crackers were measured from two sides, measuring once and turning 90° vertically in order to get the average value (American Association of Cereal Chemists 2000).

2.2.3.3 Diameter

Diameter of crackers was determined by measuring three individual crackers with a vernier caliper, measuring once and turning the crackers 90° horizontally to obtain the average value (American Association of Cereal Chemists 2000).

2.2.3.4 Moisture

The infrared moisture analyzer was used to determine the moisture content of crackers. The ground cracker (3 g) was weighted on the moisture analyzer and set at 160 °C for 10 min (Pasukamonset, Pumalee et al. 2018).

2.2.3.5 Water activity

The cracker was ground into fine powder and measured the water activity by using a water activity meter.

2.2.3.6 Color

The color values, lightness (L*), redness (a*) and yellowness (b*), values of the crackers were determined by using Hunter Lab colorimeter following

the method in part 2.1.4.6. Total color difference (ΔE) was estimated by using the formula as shown in the previous study (Ahmed and Abozed 2015).

$$\Delta E = [(L_c^* - L_s^*) + (a_c^* - a_s^*) + (b_c^* - b_s^*)]^2$$

where subscript c = riceberry cracker without wheat flour substitution with unripe papaya flour

subscript s = riceberry cracker containing unripe papaya flour

2.2.3.7 Texture profile analysis

Texture analysis of the cracker product was measured by a texture analyzer TA.XT-Plus. The hardness and fracturability of the cracker product were measured following the method in part 2.1.4.7.

2.2.3.8 Sample preparation for the assays

2.2.3.8.1 Unripe papaya flour extraction

Unripe papaya flour was extracted according to the modified procedure reported by Shen, et al (Shen, Jin et al. 2009). Unripe papaya flour (1 g) was extracted with 25 ml of acidified methanol for 24 h at shaking water bath (25°C). The methanolic extracted was centrifuged at 3000 rpm for 15 min. The sample supernatants were removed the solvent using a rotary evaporator at 50°C. The crude extract was kept at -20°C until the phytochemicals and antioxidant capacity analysis.

For the β -carotene content analysis according to the modified procedure described by Ruttarattanamongkol et al (Ruttarattanamongkol, Chittrakorn et al. 2016). Unripe papaya flour (1 g) and ascorbic acid (1 g) were weighed, then extracted with 2 ml of distilled water and 3 ml of 80% potassium hydroxide solution. Moreover, the 3 ml of 0.1% BHT in ethanol was added to the mixture, and heated in the shaking water bath at 50°C for 30 min. The mixture of hexane and ethyl acetate (8:2, 3 ml) were added and left until the separation occurred. The upper clear layer was transferred to another test tube and the residue

was re-extracted until it became colorless. The colorless extracts were blown with nitrogen until becoming dry extracts. After that 0.1 ml of hexane was added and filtered by nylon syringe filter. The filtrate was transferred to the insert vial before injecting to HPLC.

2.2.3.8.2 Crackers extraction

Riceberry crackers incorporation with unripe papaya flour was extracted as described by Kosutic et al., with slight modification (Košutić, Filipović et al. 2016). Riceberry crackers incorporation with unripe papaya flour powder (10 g) was extracted with 40 ml of acidified methanol for 24 h at shaking water bath (25°C). It was filtered through No.1 filter paper and then centrifuged at 3000 rpm for 15 min. The supernatant was evaporated by using a rotary evaporator at 50°C. The crude extract was kept at -20°C until the phytochemicals and antioxidant capacity analysis.

2.2.3.9 Proximate composition determination

The unripe papaya flour was analyzed for moisture, ash, protein, total fat, carbohydrate and total dietary fiber according to the official method of AOAC international (2012).

2.2.3.9.1 Determination of moisture content

The moisture content was determined by the official method of 990.19. The moisture of sample was measured by weighing sample, drying sample and weighing dried sample residue following the method in part 2.1.4.9.1.

2.2.3.9.2 Determination of ash content

The ash content was determined by the official method of 945.46. The ash of sample was measured by using gravimetric method following the method in part 2.1.4.9.2.

2.2.3.9.3 Determination of protein content

The protein content was determined by the official method of 991.20. The protein of sample was measured by Kjeldahl method following the method in part 2.1.4.9.3.

2.2.3.9.4 Determination of total fat content

The total fat content was determined by the official method of 922.06. The total fat of sample was measured by acid hydrolysis method following the method in part 2.1.4.9.4.

2.2.3.9.5 Determination of carbohydrate content

$$\text{Carbohydrate} = 100 - (\text{Water} + \text{Protein} + \text{Fat} + \text{Ash})$$

2.2.3.9.6 Determination of total dietary fiber content

The total dietary fiber content was determined by the official method of 992.16. The total dietary fiber of sample was measured by enzymatic-gravimetric method following the method in part 2.1.4.9.5.

2.2.3.10 Phytochemicals determination

Flour and cracker extracts were weighted and dissolved in methanol before experiments.

2.2.3.10.1 Determination of total phenolic content (TPC)

In this study, to determine the level of polyphenol content in flour and cracker extracts. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method following the method in part 2.1.4.10.1.

2.2.3.10.2 Determination of total anthocyanin content (TAC)

The level of anthocyanin was determined in cracker extracts. The total anthocyanin content (TAC) was determined using pH colorimetric method following the method in part 2.1.4.10.2.

2.2.3.10.3 Determination of β -carotene content

This assay was determine the level of β -carotene in unripe

papaya flour as β -carotene is the major active precursor of vitamin A and described as an antioxidant (Burri B J 1997).

HPLC–DAD–MS will be used to determine the β -carotene content according to a modified method of Zhong et al (Zhong L, Gustavsson K-E et al. 2016). HPLC system equipped with a diode array detector will be used. Sample will be dissolved with 1 ml of hexane. A reverse phase column 5 μ m, 150 mm C₁₈ with gradient elution will be used. The gradient of solvent A [80% acetonitrile, 15% methanol, and 5% dichloromethane (v/v)] and solvent B [30% acetonitrile, 20% methanol, and 50% dichloromethane (v/v)] will be used. The condition as follows: 5–70% B (0–18 min), 70–5% B, (18–20 min), and 5% B, (20–22 min) with 0.8 ml/min flow rate will be performed. The UV–vis detection will be performed at 458 nm wavelength. β -carotene will be detected by comparing between retention time and reference standard. β -carotene content will be calculated from the standard curve of β -carotene.

2.2.3.11 Antioxidant capacity determination

2.2.3.11.1 Determination of DPPH radical-scavenging

In this assay, to determine the antioxidant activity in flour and cracker extracts. DPPH radical-scavenging ability was determined following the method in part 2.1.4.11.1.

2.2.3.11.2 Determination of ferric reducing antioxidant power (FRAP)

The antioxidant activity of flour and cracker extracts was determined by using ferric reducing antioxidant power (FRAP). Ferric reducing antioxidant power (FRAP) was measured following the method in part 2.1.4.11.2.

2.2.3.11.3 Determination of trolox equivalent antioxidant capacity

This assay was measured of antioxidant activity of mixtures of substances in cracker extracts. Trolox equivalent antioxidant capacity (TEAC) was carried out base on following the method in part 2.1.4.11.3.

2.2.3.12 Predicted glycemic index determination

Glycemic index (GI) was determined in the cracker product. The *in vitro* digestion and glucose oxidation were measured following the method in part 2.1.4.12.

2.2.3.13 Total starch analysis

The total starch content was investigated following the method in part 2.1.4.13.

2.2.4 Scanning Electron Microscope (SEM) examination

The microstructure of flour and cracker was determined by SEM. The cracker was cut into a cube and adhered on an aluminum stub. Samples were coated with sputter gold and scanned using JSM-IT300 scanning electron microscope. The micrographs were taken at a magnification of 100x for the surface and cross-section parts of the crackers.

2.2.5 Sensory analysis

To determine the acceptability of four formulations of riceberry crackers incorporation with unripe papaya flour compared with riceberry crackers without unripe papaya flour incorporation.

2.2.5.1 Acceptance test

Sensory evaluation was carried out by fifty untrained panelists which were recruited from Chulalongkorn University. The crackers were assessed in a standardized tasting room with individual booths of Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University. The four formulations of crackers were evaluated on various attributes including appearance, color, flavor, taste, hardness, fracturability and overall acceptability using a nine-point hedonic scale. Each panelist was received four formulations of crackers (3 g per piece) in random order which were labeled with three-digit random numbers. All the attributes were scored on a scale varying from “9 = like extremely” to “1 = dislike

extremely”. Drinking water (330 ml) was provide to the panelists to rinse their mouth between the samples. Crackers were prepared according to good hygiene and manufacturing practices. Panelists were notified about the study and described that their cooperation was totally willing. They can stop the evaluation at any point and the responses was anonymous.

Inclusion and exclusion criteria of panelists involved in the sensory analysis including;

- Inclusion criteria
 - Male/female
 - Age 18-50 years
 - Voluntary participation in research project
- Exclusion criteria
 - color blind
 - Have common cold symptoms including cough, runny nose and sore throat
 - Pregnancy and breastfeeding
 - Gluten allergy
 - Smokers

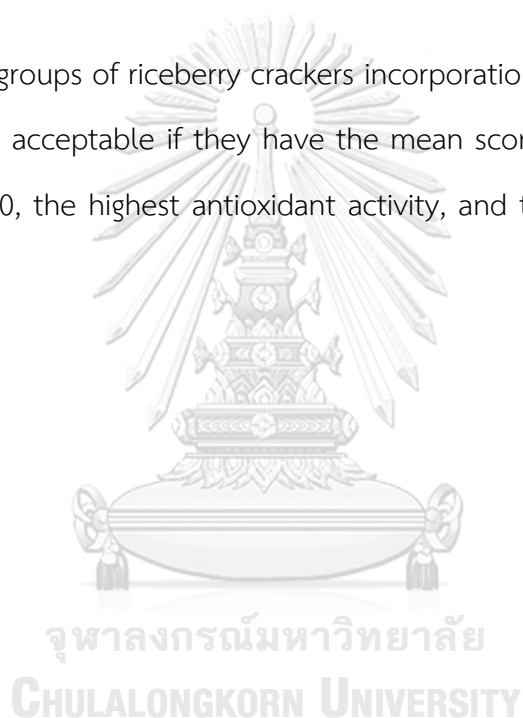
The samples were presented to the panelists on a white plate labeled with three-digit random numbers. Sensory evaluation was carried out around 15 min. Crackers to be use for sensory evaluation was cooled at room temperature after baking and packed in a plastic box at room temperature until evaluating. The acceptance index (AI) was calculated by the following formula:

$$\text{Acceptance index (AI)} = (\text{Overall acceptability score} / 9) \times 100$$

2.2.6 Statistical analysis

Data were reported as mean of values \pm SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test and using SPSS statistical. The significance different test was used for mean comparison and P-value<0.05 was considered as statistically significant. Calculations were performed with Microsoft Excel 2013. The graphs were generated with Sigma Plot, version 12.0 software.

The test groups of riceberry crackers incorporation with unripe papaya flour was considered as acceptable if they have the mean score for overall acceptability above or equal 6.0, the highest antioxidant activity, and the lowest glycemic index value.



CHAPTER IV

RESULTS

1. To investigate the effect of partially substituted wheat flour with riceberry flour on physicochemical, functional properties, and sensory evaluation of crackers

1.1 Proximate composition of riceberry flour

The proximate composition including moisture, ash, protein, total fat, carbohydrate, total dietary fiber, iron and zinc of riceberry flour (RBF) are presented in Table 4. The moisture, ash, protein, total fat, carbohydrate and total dietary fiber content of RBF were 8.87, 4.56, 12.55, 10.63, 63.39 and 13.8 g/100g, respectively.

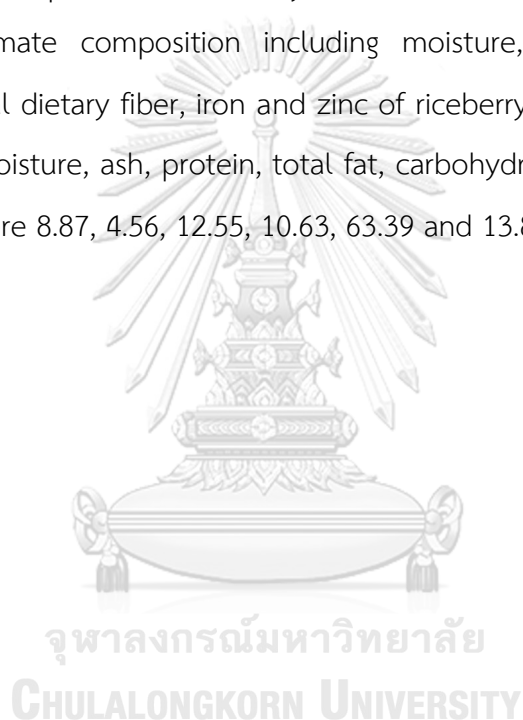


Table 4 Proximate composition of riceberry flour

	Proximate composition (g/100g)
Moisture	8.87
Ash	4.56
Protein	12.55
Total fat	10.63
Carbohydrate	63.39
Total dietary fiber	13.80

1.2 Phytochemical contents and antioxidant properties of flours

All phytochemical compounds, including total phenolic content and total anthocyanins content were quantified by Folin-Ciocalteu and pH colorimetric method. The antioxidant properties were analyzed by Ferric Reducing Antioxidant Power (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC) and DPPH assay. Total phenolic content in wheat flour (WF) and riceberry flour (RBF) were 23.17 ± 1.27 and 505.90 ± 21.46 mg gallic acid equivalent/100g dry weight, respectively (Table 5). The results indicated that total phenolic content in RBF were significantly higher than WF ($P < 0.05$). As shown in Table 5, total anthocyanins content in RBF was 49.95 ± 3.80 mg cyanidins-3-glucosides/100g dry weight but anthocyanins content was not detected in WF. In addition, antioxidant properties of WF and RBF including FRAP, TEAC and DPPH values are shown in Table 5. FRAP and TEAC values of WF were 101.92 ± 0.81 $\mu\text{mol FeSO}_4/100\text{g}$ dry weight and 0.14 ± 0.01 mmol trolox equivalent/100g dry weight, respectively which were significantly lower than RBF ($4,610 \pm 0.18$ $\mu\text{mol FeSO}_4/100\text{g}$ dry weight and 5.19 ± 0.55 mmol trolox equivalent/100g dry weight, respectively). Moreover, the IC_{50} value of DPPH radicals scavenging activity of WF was 0.80 ± 0.02 which was higher than that of RBF (0.23 ± 0.01). As expected, RBF exhibited significantly greater antioxidant activity than WF.

Table 5 Phytochemical contents and antioxidant activities of wheat flour and riceberry flour

Sample	TPC (mg GAE/100g DW)	TAC (mg C3G/100g DW)	FRAP (μ mol FeSO ₄ /100g DW)	TEAC (mmol TE/100g DW)	DPPH IC ₅₀ (mg/ml)
WF	23.17±1.27 ^a	ND	101.92±0.81 ^a	0.14±0.01 ^a	0.80±0.02 ^a
RBF	505.90±21.46 ^b	49.95±3.80	4,610±0.18 ^b	5.19±0.55 ^b	0.23±0.01 ^b

Values are expressed as mean ± S.E.M. (n = 4), ND = data was not detected.

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

TPC = Total phenolic content; TAC = Total anthocyanins content; GAE = Gallic Acid Equivalent; C3G = Cyanidins-3-glucosides;

TE = Trolox Equivalent; DW = dry weight; WF = Wheat flour; RBF = Riceberry flour.

1.3 Determination of starch fraction, hydrolysis index and predicted glycemic index of flours

The determination of starch fraction, hydrolysis index and predicted glycemic index in the wheat flour (WF) and riceberry flour (RBF) by *in vitro* starch digestion were examined by analyzing the glucose released from starch degradation using digestive enzymes within 180 min. The starch digestibility properties are indicated as the rapid digestible starch (RDS), slow digestible starch (SDS), undigestible starch (UDS) and predicted glycemic index (pGI) (Table 6). The total starch and RDS of WF were 71.15 ± 0.32 g/100g sample and $24.60 \pm 0.34\%$, respectively which were more than RBF (67.71 ± 0.41 g/100g sample and $15.21 \pm 0.08\%$, respectively). Moreover, the SDS content of WF ($28.64 \pm 0.41\%$) was significantly lower than RBF ($35.35 \pm 0.14\%$) as shown in Table 6. In addition, the UDS content of RBF (49.44 ± 0.17) was significantly higher than WF (46.77 ± 0.07). As presented in Table 6, the hydrolysis index values of WF (41.50 ± 0.07) was significantly higher than RBF (34.80 ± 0.37). The *in vitro* starch digestograms of WF and RBF are exhibited in Figure 10. At the separate time point on the digestograms, RBF displayed slower amount of glucose release after 20 min and also demonstrated the attenuation of releasing glucose after 60 min ($P < 0.05$). The findings represented the lower amount of hydrolyzed starch and hydrolysis rate of RBF when compared to WF. Consequently, these results indicated the lower starch digestion of RBF than WF. In addition, the pGI between RBF and WF were also affected. As shown in Table 6, the pGI values of WF (62.49 ± 0.04) were significantly higher than RBF (58.82 ± 0.20).

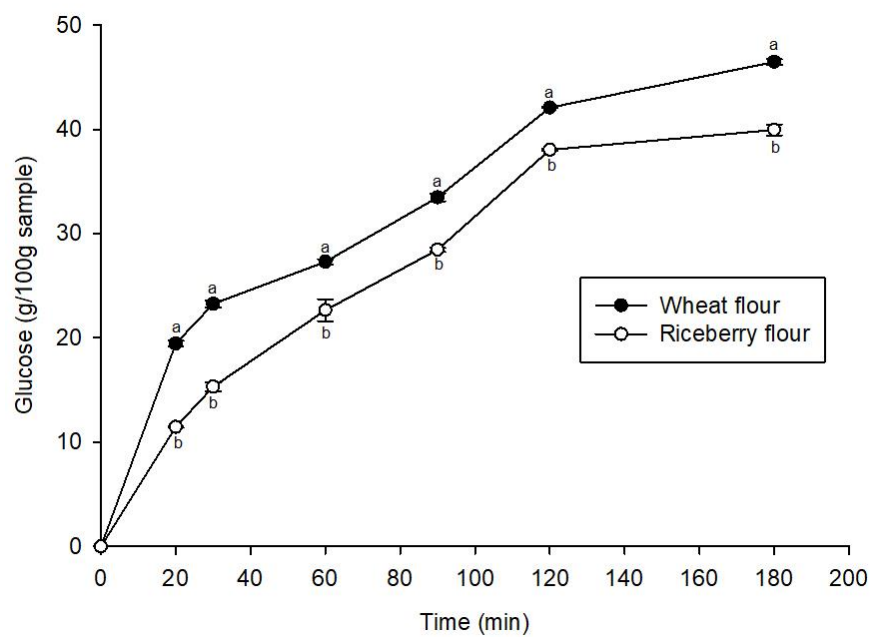


Figure 10 The release of glucose during the hydrolysis of wheat flour and riceberry flour at time intervals of 0, 20, 30, 60, 90, 120 and 180 min. Values are expressed as mean \pm S.E.M. (n = 4).

Table 6 The starch fraction, hydrolysis index and predicted glycemic index of wheat flour and riceberry flour

	WF	RBF
TS (g/100g)	71.15±0.32 ^a	67.71±0.41 ^b
RDS (%)	24.60±0.34 ^a	15.21±0.08 ^b
SDS (%)	28.64±0.41 ^a	35.35±0.14 ^b
UDS (%)	46.77±0.07 ^a	49.44±0.17 ^b
HI	41.50±0.07 ^a	34.80±0.37 ^b
pGI	62.49±0.04 ^a	58.82±0.20 ^b

Values are expressed as mean ± S.E.M. (n = 4).

Mean with different superscript letter within a row are significantly different ($P < 0.05$).

TS = total starch; RDS = rapidly digestible starch; SDS = slowly digestible starch; UDS = undigestible starch; HI = hydrolysis index; pGI = predicted glycemic index; WF = Wheat flour; RBF = Riceberry flour

1.4 Scanning Electron Microscope (SEM) examination of flours

The micrograph of wheat flour (WF) and riceberry flour (RBF) was determined by using SEM. The morphology is shown in Figure 11. The surface of WF starch granules demonstrated a spherical in shape with smooth surface and a loosely packed structure (Figure 11a and b), whereas that of RBF appeared a polygonal in shape with rough surface and a very compact structure (Figure 11 8c and d).



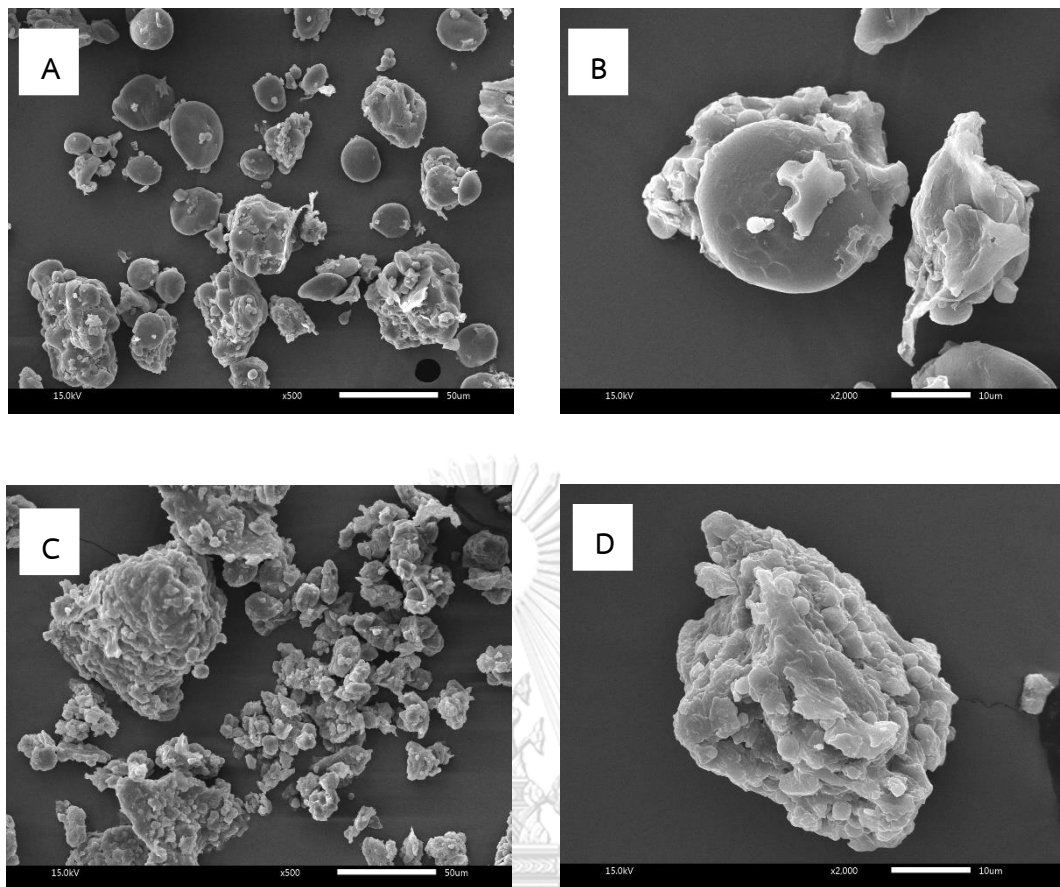


Figure 11 Scanning electron microscope (SEM) micrographs of flours

Wheat flour, (A) magnified 500x and (B) 2000x; Riceberry flour, (C) magnified 500x and (D) 2000x

1.5 Physical characteristics of crackers

The physical characteristics including weight, height, diameter, moisture content, water activity, hardness and fracturability of crackers incorporated with riceberry flour (RBF) are presented in Table 7. The weight of wheat flour cracker (control) was 3.04 ± 0.04 g, whereas that of RBF cracker ranged from 3.12 ± 0.03 to 3.09 ± 0.08 g. The results indicated that the incorporation of RBF into the crackers didn't affect the weight of all samples. As shown in Table 7, the height and diameter of control were 0.34 ± 0.00 mm and 4.26 ± 0.01 cm, respectively, whereas that of RBF cracker ranged from 0.33 ± 0.00 to 0.32 ± 0.00 mm and 4.24 ± 0.01 to 4.21 ± 0.03 cm, respectively. The results illustrated that the replacement of wheat flour (WF) with RBF in the crackers significantly decreased the height and diameter of the crackers ($P < 0.05$). In addition, the moisture content and water activity of crackers significantly decreased from 6.34 ± 0.05 (control) to 5.69 ± 0.04 (20%RBF) and 0.46 ± 0.00 (control) to 0.42 ± 0.01 (20%RBF), respectively when the level of RBF increased (Table 7). In terms of hardness and fracturability of crackers formulated with RBF are presented in Table 7. It was found that the hardness increased from 4088.18 ± 26.74 g (control) to 5208.97 ± 153.11 g (20%RBF) and the fracturability decreased from 1375.36 ± 30.73 g (control) to 1034.07 ± 22.02 g (20%RBF) when the level of RBF increased. The results clearly indicated that texture attributes were affected by the substitution of WF with RBF in the crackers.

Table 7 Physical characteristics of crackers substituted wheat flour with riceberry flour

	Weight (g)	Height (mm)	Diameter (cm)	Moisture (%)	Water activity	Hardness (g)	Fracturability (g)
Control	3.04±0.04 ^a	0.34±0.00 ^a	4.26±0.01 ^a	6.34±0.05 ^a	0.46±0.00 ^a	4088.18±26.74 ^a	1375.36±30.73 ^a
5%RBF	3.12±0.03 ^a	0.33±0.00 ^b	4.24±0.01 ^a	6.15±0.04 ^b	0.47±0.01 ^a	4324.60±99.40 ^{ab}	1245.38±29.13 ^b
10%RBF	3.09±0.04 ^a	0.33±0.00 ^b	4.20±0.01 ^b	6.14±0.05 ^b	0.45±0.01 ^a	4304.89±99.83 ^{ab}	1116.41±9.64 ^c
15%RBF	3.08±0.12 ^a	0.32±0.00 ^c	4.20±0.00 ^b	5.63±0.07 ^c	0.41±0.00 ^b	4461.93±13.35 ^b	1091.23±21.50 ^{cd}
20%RBF	3.09±0.08 ^a	0.32±0.00 ^c	4.21±0.03 ^b	5.69±0.04 ^c	0.42±0.01 ^b	5208.97±153.11 ^c	1034.07±22.02 ^d

Values are expressed as mean ± S.E.M. (n = 4).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

1.6 Color characteristics of crackers

As shown in Figure 12, the addition of riceberry flour (RBF) significantly changed the color parameters including lightness (L^*), redness (a^*) and yellowness (b^*) of the crackers. The incorporation of RBF into crackers directly affected the surface color of crackers which became darker when increasing RBF amounts. The L^* and b^* values of crackers significantly decreased from 39.73 ± 0.50 (control) to 12.49 ± 0.19 (20%RBF) and 10.56 ± 0.66 (control) to 1.36 ± 0.17 (20%RBF), respectively with the level of RBF increased (Table 8). In contrast, the a^* value of crackers significantly increased as the replacement of wheat flour (WF) with RBF level increased. These observations demonstrated that the pigment of RBF had an impact on a reduction of L^* and b^* values and consequence of increasing of a^* value of crackers incorporated with RBF. In addition, the total color difference index (ΔE) of crackers are presented in Table 8. The results showed that ΔE of crackers significantly increased from 19.77 (5%RBF) to 29.07 (20%RBF), respectively when the level of RBF increased.

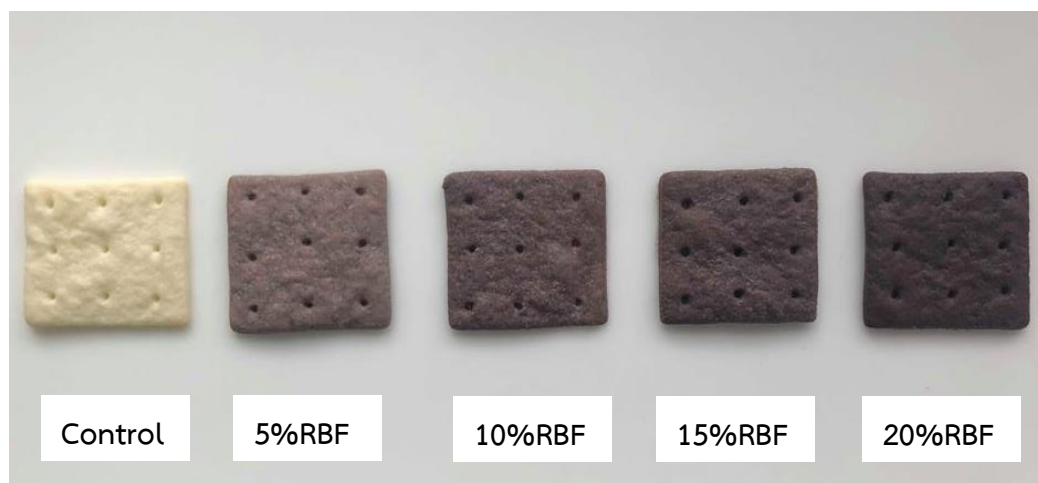


Figure 12 The appearance of control and cracker substituted wheat flour with riceberry flour

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

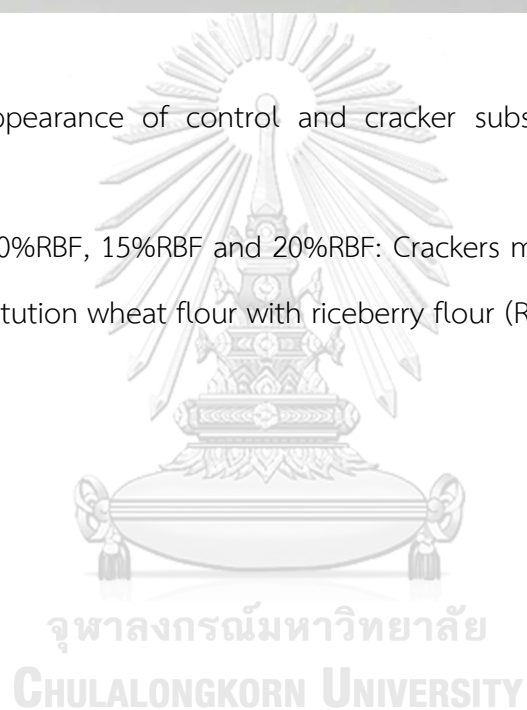


Table 8 Color characteristics of crackers substituted wheat flour with riceberry flour

	Color values			ΔE
	L*	a*	b*	
Control	39.73±0.50 ^a	0.24±0.01 ^a	10.56±0.66 ^a	0.00
5%RBF	21.62±0.87 ^b	3.24±0.09 ^b	3.22±0.18 ^b	19.77
10%RBF	17.26±0.31 ^c	3.65±0.06 ^c	2.49±0.23 ^c	24.12
15%RBF	15.38±0.30 ^d	3.65±0.08 ^c	2.18±0.39 ^c	26.25
20%RBF	12.49±0.19 ^e	3.79±0.19 ^c	1.36±0.17 ^d	29.07

Values are expressed as mean \pm S.E.M. (n = 4).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

ΔE : total color difference between control and treatment.

1.7 Phytochemical contents and antioxidant properties of crackers

The total phenolic content and total anthocyanins content of all cracker formulations demonstrated in Table 9. Total phenolic content of wheat flour (WF) cracker (control) was 2.03 ± 0.36 mg gallic acid equivalent (GAE)/10g dry weight (DW), whereas that of riceberry flour cracker (RBF) ranged from 3.21 ± 0.09 to 8.78 ± 0.19 mg GAE/10g DW, respectively. Total anthocyanins content significantly increased from 153.45 ± 14.77 (5%RBF) to 803.92 ± 5.29 (20%RBF) μg cyanidins-3-glucosides (C3G)/10g DW, respectively when the level of RBF increased. The results indicated that all phytochemical compounds of crackers significantly increased when the level of RBF increased (Table 9). Moreover, antioxidant properties of the crackers are also shown in Table 9. The value of ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) significantly increased from 11.19 ± 0.54 to 79.45 ± 0.83 mM FeSO_4 /10g DW and from 11.95 ± 0.49 to 46.82 ± 1.84 μmol trolox equivalent (TE)/10g DW, respectively (Table 9). In contrast, IC_{50} value of DPPH in the crackers significantly decreased from 0.67 ± 0.02 to 0.39 ± 0.02 mg/ml. The incorporation of RBF (5-20%) into the crackers dramatically increased FRAP and TEAC value about 1 – 7 folds and 1 – 4 folds, respectively. Moreover, IC_{50} value of DPPH markedly decreased about to 1- 0.58 folds as illustrated in Table 9. It was found that the antioxidant ability of crackers was considerably increased when the level of substitution of WF with RBF increased as shown in Table 9.

Table 9 Phytochemical contents and antioxidant activities of crackers substituted wheat flour with riceberry flour

Sample	TPC (mg GAE/10g DW)	TAC (μ g C3G/10g DW)	FRAP (mM FeSO ₄ /10g DW)	TEAC (μ mol TE/10g DW)	DPPH IC ₅₀ (mg/ml)
Control	2.03±0.36 ^a	ND	11.19±0.54 ^a	11.95±0.49 ^a	0.67±0.02 ^a
5%RBF	3.21±0.09 ^b	153.45±14.77 ^a	13.18±0.92 ^b	14.62±0.48 ^b	0.61±0.02 ^b
10%RBF	4.39±0.23 ^c	331.59±44.91 ^b	34.03±1.16 ^c	14.79±1.12 ^b	0.55±0.01 ^c
15%RBF	7.25±0.29 ^d	623.90±31.79 ^c	48.70±1.03 ^d	28.14±1.47 ^c	0.47±0.03 ^d
20%RBF	8.78±0.19 ^e	803.92±5.29 ^d	79.45±0.83 ^e	46.82±1.84 ^d	0.39±0.02 ^e

Values are expressed as mean \pm S.E.M. (n = 4). ND = data was not detected.

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

TPC = Total phenolic content; TAC = Total anthocyanins content; GAE = Gallic Acid Equivalent; C3G = Cyanidins-3- glucosides; TE = Trolox Equivalent; DW = dry weight.

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

1.8 Determination of total starch, hydrolysis index and predicted glycemic index of crackers

The effect of riceberry flour (RBF) on starch digestibility of the crackers was examined by analyzing the glucose released from starch degradation using digestive enzymes within 180 min. The total starch (TS), undigestible starch (UDS), hydrolysis index (HI) and predicted glycemic index (pGI) values are shown in Table 10. TS of control cracker was 44.43 ± 0.25 g/100g sample which were more than RBF cracker ranged from 43.92 ± 0.45 to 41.56 ± 0.37 g/100g sample, respectively. UDS of control cracker was $4.34 \pm 1.40\%$ which were more than RBF cracker ranged from 2.57 ± 1.93 to $16.62 \pm 1.84\%$, respectively. In addition, the HI values of crackers significantly decreased from 46.72 ± 0.25 (control) to 36.99 ± 0.15 (20%RBF), respectively when the level of RBF increased ($P < 0.05$). The *in vitro* starch digestograms of control and RBF crackers are exhibited in Figure 13. At the time intervals of 0, 30, 60, 90, 120 and 180 min on the digestograms, RBF crackers showed slower content of glucose release after 30 min and also displayed the attenuation of releasing glucose after 60 min ($P < 0.05$). The findings indicated that the incorporation of RBF into the crackers illustrated the lower amount of hydrolyzed starch and hydrolysis rate when compared to control. As presented in Table 9, the pGI values among cracker samples were also affected. The pGI values of control cracker (65.36 ± 0.13) were significantly higher than RBF crackers ranged from 65.01 ± 0.12 to 60.02 ± 0.08 .

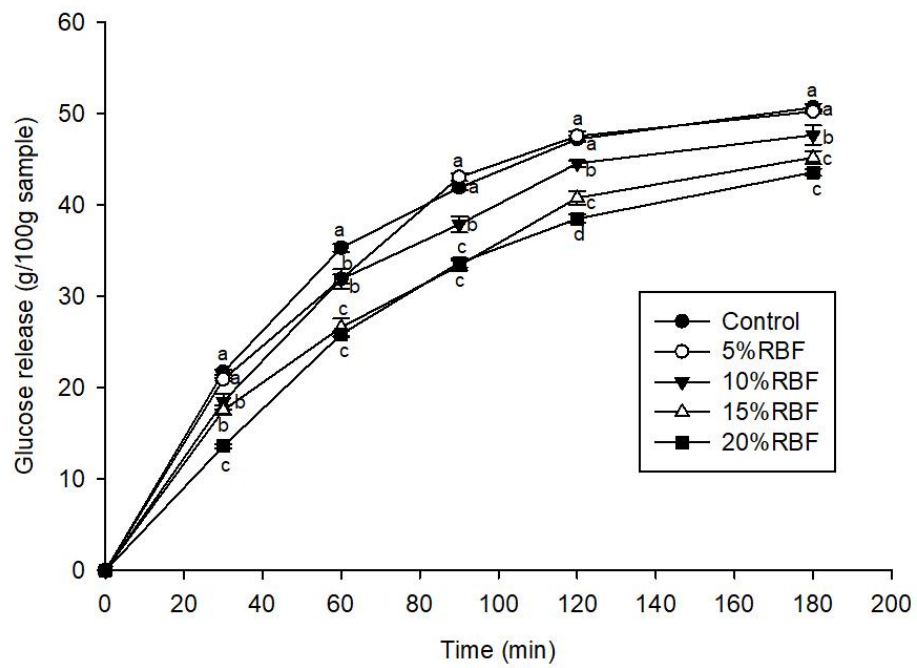


Figure 13 The release of glucose during the hydrolysis of crackers substituted wheat flour with riceberry flour at time intervals of 0, 30, 60, 90, 120 and 180 min

Values are expressed as mean \pm S.E.M. (n = 4).

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

Table 10 The total starch, hydrolysis index and predicted glycemic index of crackers substituted wheat flour with riceberry flour

	TS (g/100g)	UDS (%)	HI	pGI
Control	44.43±0.25 ^a	4.34±1.40 ^a	46.72±0.25 ^a	65.36±0.13 ^a
5%RBF	43.92±0.45 ^a	2.57±1.93 ^a	46.08±0.23 ^a	65.01±0.12 ^a
10%RBF	42.47±0.49 ^a	5.57±1.42 ^a	42.98±0.30 ^b	63.30±0.17 ^b
15%RBF	41.95±0.42 ^b	12.48±1.09 ^b	38.98±0.37 ^c	61.11±0.20 ^c
20%RBF	41.56±0.37 ^b	16.62±1.84 ^c	36.99±0.15 ^d	60.02±0.08 ^d

Values are expressed as mean ± S.E.M. (n = 4).

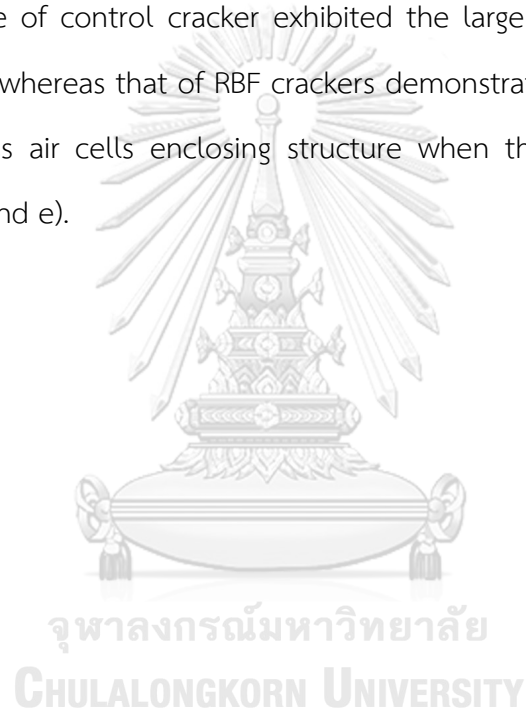
Mean with different superscript letter within a column are significantly different ($P < 0.05$).

TS = total starch; HI = hydrolysis index; pGI = predicted glycemic index.

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

1.9 Scanning Electron Microscope (SEM) examination of crackers

The microstructure characteristics of the surface along with the cross-sectional structure of crackers were determined by using SEM. As shown in Figure 14a, the surface of control cracker demonstrated an uninterrupted surface without the crevice. On the other hand, riceberry flour cracker (RBF) appeared an irregular and scraggly surface (Figure 14b, c, d and e). The results illustrated that the incorporation of RBF into the crackers affected to the surface of crackers. In addition, the cross-sectional structure of control cracker exhibited the large porous structure with air cells (Figure 15a), whereas that of RBF crackers demonstrated the tight and compact structure with less air cells enclosing structure when the level of RBF increased (Figure 15b, c, d and e).



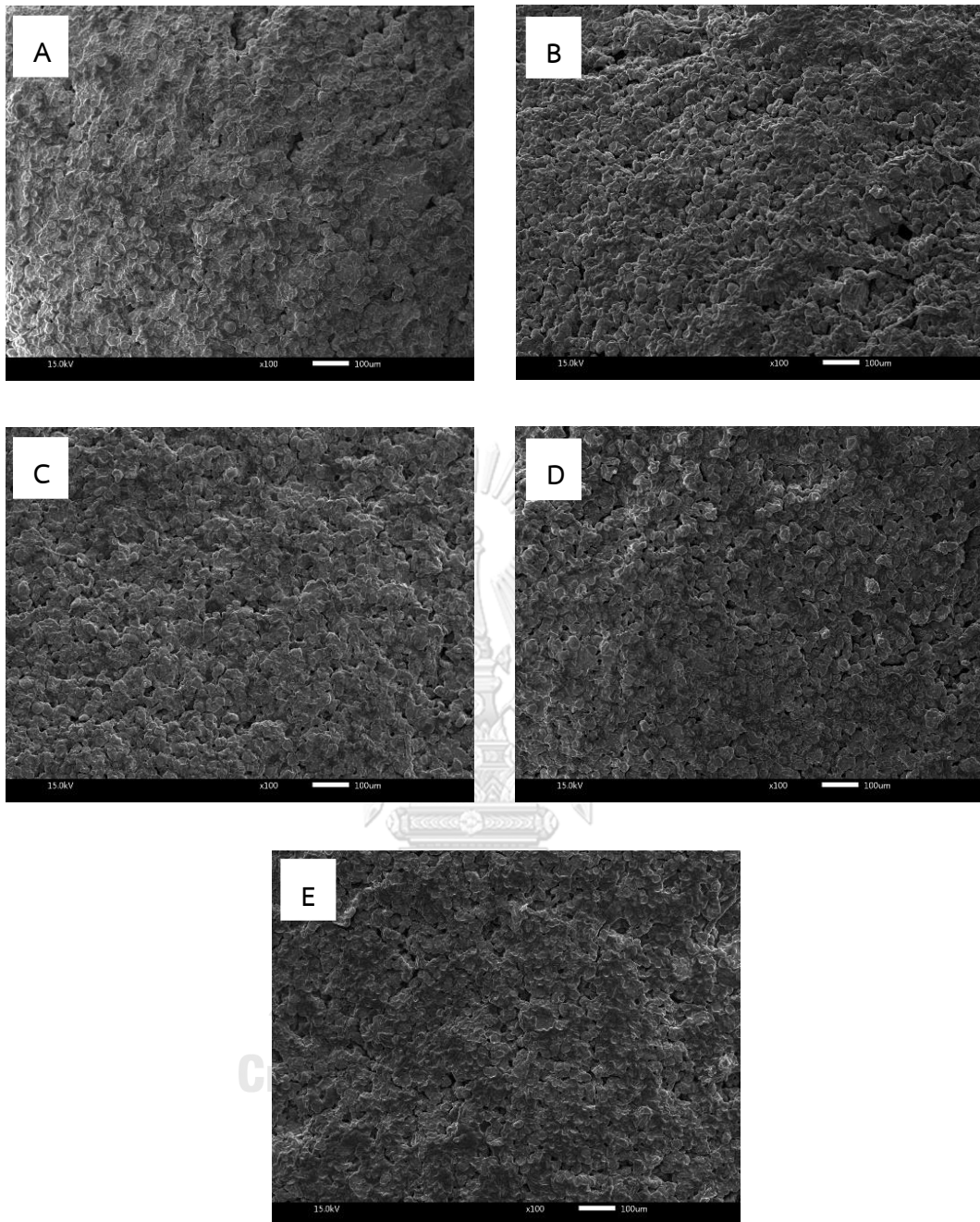


Figure 14 Scanning electron microscope (SEM) micrographs of crackers (100x) for surface structure of crackers; (A), (B), (C), (D) and (E): Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

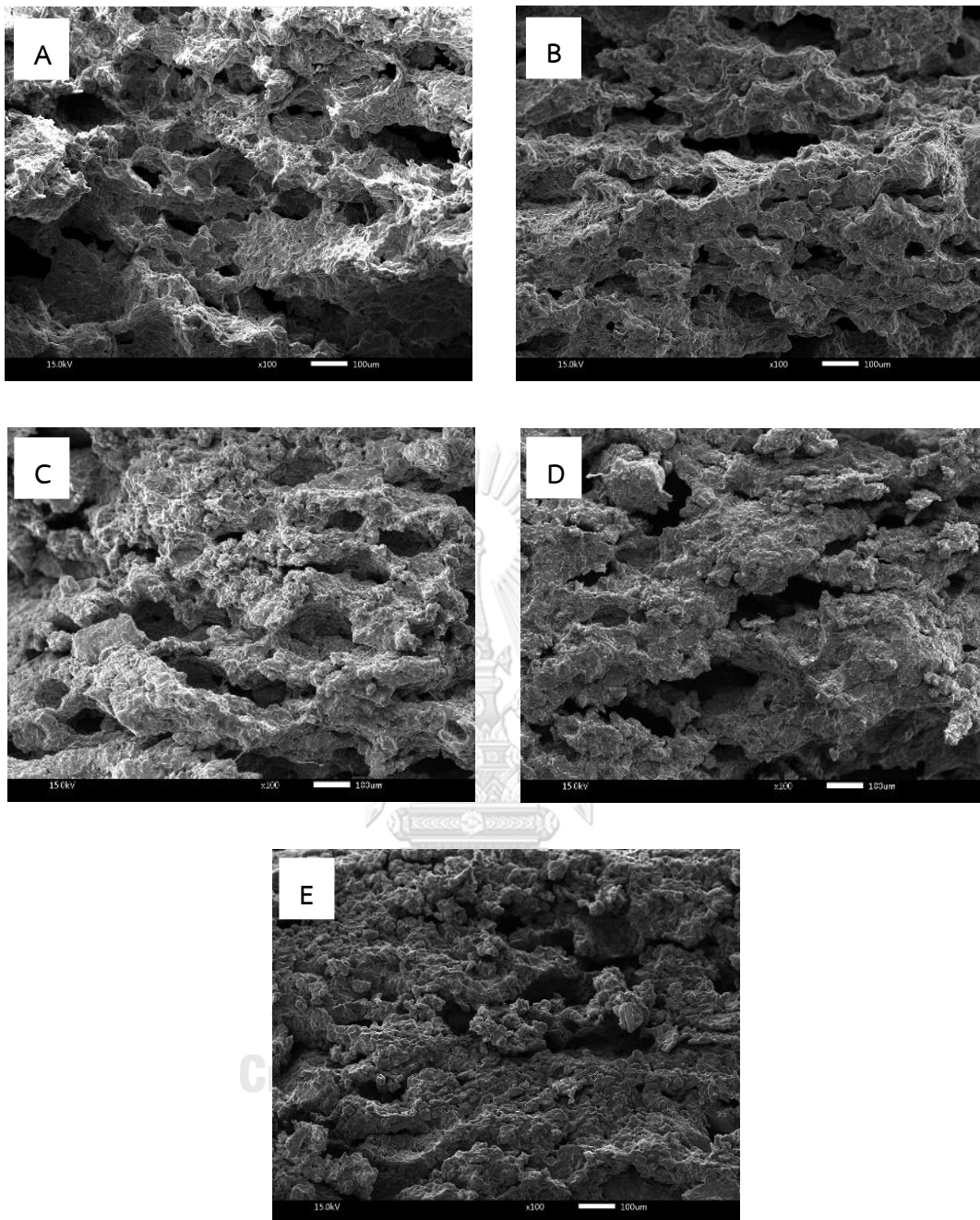


Figure 15 Scanning electron microscope (SEM) micrographs of crackers

(100x) for inner structure of crackers; (A), (B), (C), (D) and (E): Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

1.10 Sensory evaluation

The sensory assessment of the crackers was evaluated by using a nine-point hedonic scale on the sensory parameters including appearance, color, flavor, taste, fracturability, hardness and overall acceptability, as shown in Table 11. The appearance and color scores of control cracker were 7.26 ± 0.15 and 7.12 ± 0.19 , respectively, whereas that of riceberry flour cracker (RBF) ranged from 7.08 ± 0.10 to 7.34 ± 0.14 and 7.04 ± 0.08 to 7.30 ± 0.14 , respectively. The findings indicated that the replacement of what flour (WF) with RBF in the crackers didn't affect the appearance and color scores of all samples. Surprisingly, the sensory scores of flavor and taste of crackers significantly increased from 6.26 ± 0.19 (control) to 7.50 ± 0.09 (20%RBF) and 6.82 ± 0.11 (control) to 7.54 ± 0.10 (20%RBF), respectively when the level of RBF increased (Table 11). The incorporation of RBF into the crackers significantly decreased the fracturability and hardness scores from 7.52 ± 0.09 (control) to 7.02 ± 0.07 (20%RBF) and 7.32 ± 0.11 (control) to 6.90 ± 0.20 (20%RBF), respectively. Nevertheless, the sensory scores of fracturability and hardness were in slightly to moderately preference (score 6-7). Moreover, in terms of overall acceptability scores of crackers significantly increased from 7.12 ± 0.13 (control) to 7.70 ± 0.09 (20%RBF), respectively when the level of RBF increased. The acceptance index (AI) of crackers were ranged from 79.11 to 85.55%.

Table 11 Sensory evaluation scores of crackers substituted wheat flour with riceberry flour

	Appearance	Color	Flavor	Taste	Fracturability	Hardness	Overall acceptability	Acceptance index (%)
Control	7.26±0.15 ^a	7.12±0.19 ^a	6.26±0.19 ^a	6.82±0.11 ^a	7.52±0.09 ^a	7.32±0.11 ^a	7.12±0.13 ^a	79.11
5%RBF	7.08±0.10 ^a	7.04±0.08 ^a	7.02±0.10 ^b	6.94±0.09 ^a	7.44±0.09 ^{ab}	7.38±0.11 ^a	7.08±0.11 ^a	78.66
10%RBF	7.12±0.12 ^a	7.10±0.08 ^a	7.12±0.11 ^{bc}	7.06±0.14 ^{ab}	7.40±0.09 ^{ab}	7.16±0.13 ^{ac}	7.24±0.11 ^a	80.44
15%RBF	7.18±0.14 ^a	7.26±0.12 ^a	7.40±0.11 ^{cd}	7.26±0.14 ^b	7.20±0.10 ^{bc}	7.04±0.19 ^b	7.50±0.13 ^b	83.33
20%RBF	7.34±0.14 ^a	7.30±0.14 ^a	7.50±0.09 ^d	7.54±0.10 ^c	7.02±0.07 ^c	6.90±0.20 ^{bc}	7.70±0.09 ^b	85.55

Values are expressed as mean ± S.E.M. (n = 50).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

Control, 5%RBF, 10%RBF, 15%RBF and 20%RBF: Crackers manufactured with 0, 5, 10, 15 and 20% substitution wheat flour with riceberry flour (RBF), respectively.

2. To investigate the effect of riceberry cracker supplementation with unripe papaya flour on physicochemical, functional properties, and sensory evaluation

The criteria for selection of crackers among five formulations in the first part including the overall acceptability score, antioxidant properties and glycemic index. In the current study showed that the cracker with 20%RBF substitution had the highest score of overall acceptability, antioxidant activities and the lowest glycemic index value. Consequently, the cracker substituted of wheat flour with 20% of riceberry flour was selected for the further study. In the further study, the four ratios were employed to replace wheat flour with unripe papaya flour including 0% (without substitution wheat flour with unripe papaya flour), 3%, 5% and 7%.



2.1 Proximate composition of unripe papaya flour

The proximate composition was obtained from the previous study research in the title of Development of value-added unripe papaya flour from waste product of fruit industry. Proximate composition including moisture, ash, protein, total fat and carbohydrate (available carbohydrate and total dietary fiber) of unripe papaya flour (UPF) are presented in Table 12. The moisture, ash, protein and total fat content of UPF were 11.61, 5.22, 4.65 and 1.36 g/100g, respectively. In addition, the carbohydrate which including available carbohydrate and total dietary fiber content were 21.02 and 56.14 g/100g, respectively. These indicated that the UPF had a plentiful content of total dietary fiber.

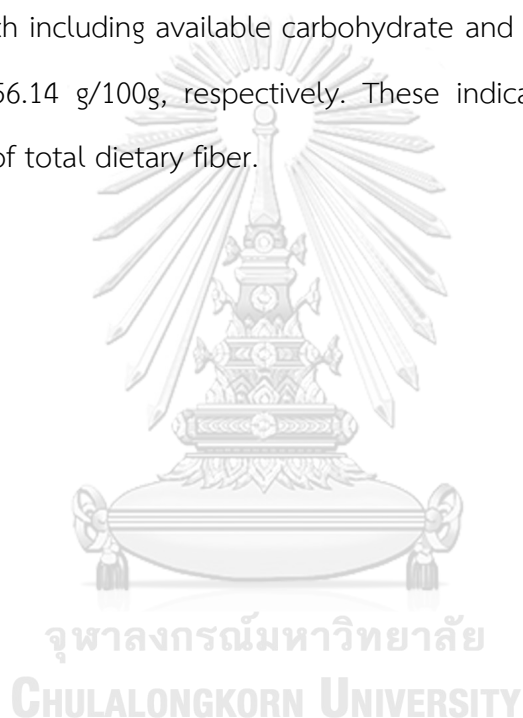


Table 12 Proximate composition of unripe papaya flour

	Proximate composition (g/100g)
Moisture	11.61
Ash	5.22
Protein	4.65
Total fat	1.36
Carbohydrate	
— Available carbohydrate	21.02
— Total dietary fiber	56.14

2.2 Phytochemical contents and antioxidant properties of unripe papaya flour

The phytochemical contents including total phenolic content and β -carotene content were quantified by Folin-Ciocalteu and high-performance liquid chromatography (HPLC) method. The β -carotene content was obtained from the previous study research in the title of Development of value-added unripe papaya flour from waste product of fruit industry. In addition, the antioxidant properties were analyzed by ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) and DPPH assay. As shown in Table 13, total phenolic content in unripe papaya flour (UPF) was 106.87 ± 2.33 mg gallic acid equivalent/100g dry weight (DW). Moreover, the raising of peak area in the similar peak could be assured as β -carotene peak, as shown in Figure 17. The determination of β -carotene was identified at peak 1 with the retention time of 19.833 min. The β -carotene content in UPF was 39 ± 3.3 μ g/100g DW. The standard chromatogram of β -carotene is demonstrated in Figure 16. In terms of antioxidant properties of UPF including FRAP, TEAC and DPPH values are shown in Table 13. FRAP and TEAC values of UPF was 579.90 ± 4.30 μ mol FeSO_4 /100g DW and 0.74 ± 0.01 mmol trolox equivalent/100g DW, respectively. Furthermore, the IC_{50} value of DPPH radicals scavenging activity of UPF was 0.52 ± 0.01 mg/ml.

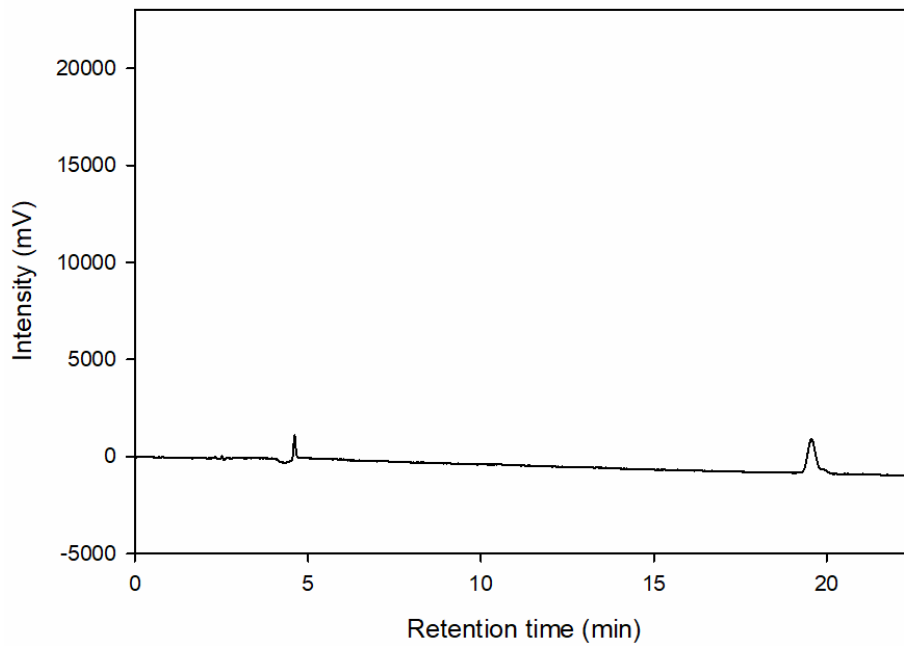


Figure 16 Chromatogram of 0.25 µg/ml β -carotene standard.

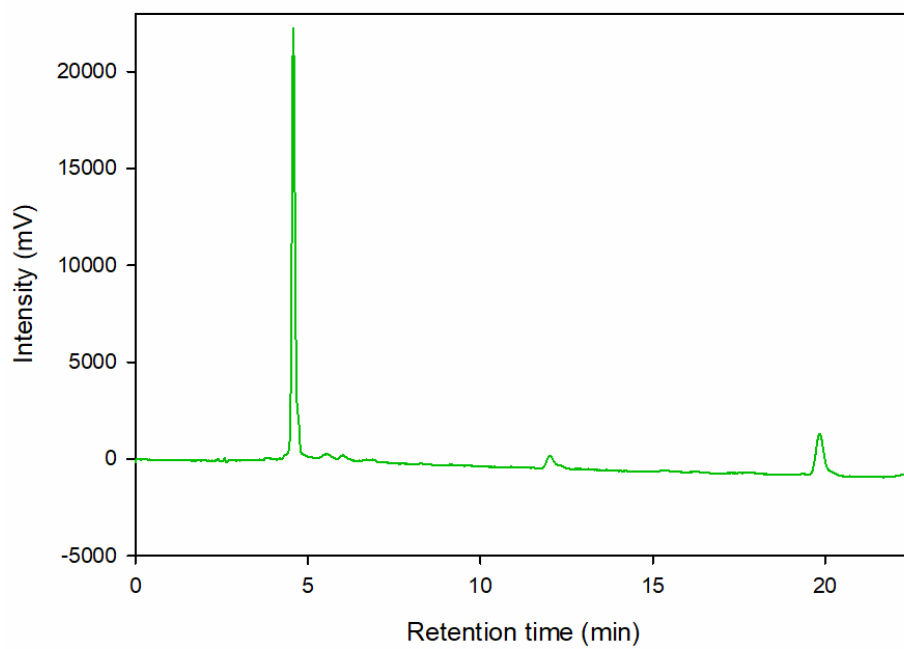


Figure 17 Chromatogram of unripe papaya flour.

Table 13 Phytochemical contents and antioxidant activities of unripe papaya flour

Sample	TPC (mg GAE/100g DW)	FRAP (μ mol FeSO ₄ /100g DW)	TEAC (mmol TE/100g DW)	DPPH IC ₅₀ (mg/ml)
UPF	106.87 \pm 2.33	579.90 \pm 4.30	0.74 \pm 0.01	0.52 \pm 0.01

Values are expressed as mean \pm S.E.M. (n = 4).

TPC = Total phenolic content; GAE = Gallic Acid Equivalent; TE = Trolox Equivalent; DW = dry weight; UPF = Unripe papaya flour.

2.3 Scanning Electron Microscope (SEM) examination of unripe papaya flour

The microstructure of unripe papaya flour (UPF) was determined by using SEM. The morphology is shown in Figure 18. The UPF granules displayed a polyhedral shape with irregular surface (Figure 18a and b).



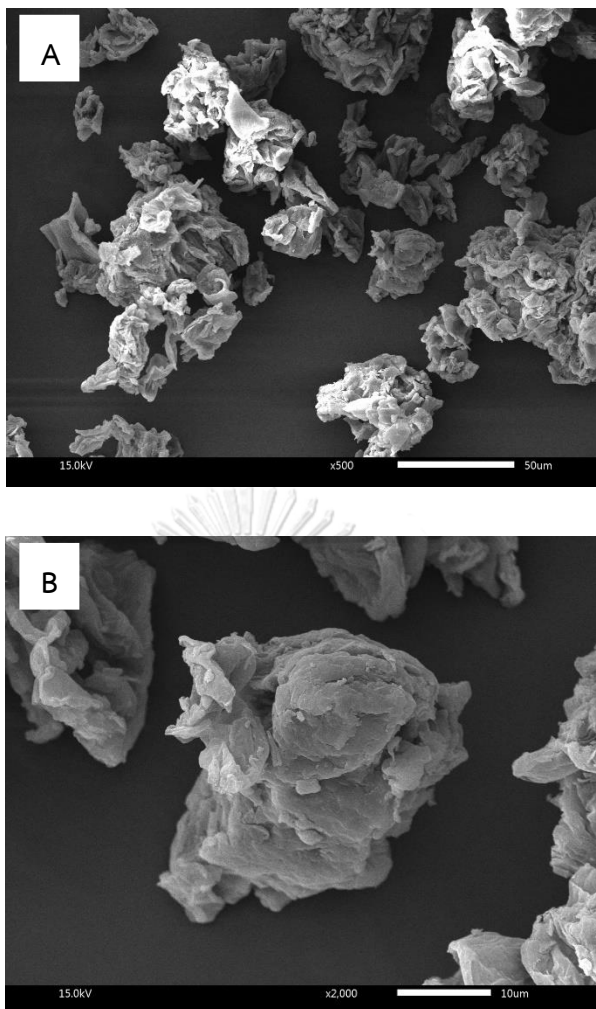


Figure 18 Scanning electron microscope (SEM) micrographs of unripe papaya flour
Unripe papaya flour, (A) magnified 500x and (B) 2000x

2.4 Physical characteristics of riceberry crackers incorporated with unripe papaya flour

The physical characteristics including weight, height, diameter, moisture content, water activity, hardness and fracturability of riceberry flour crackers (RBF) incorporated with unripe papaya flour (UPF) are presented in Table 14. The weight, height and diameter of 20%RBF cracker were 3.08 ± 0.05 g, 0.32 ± 0.00 mm and 4.23 ± 0.01 cm, respectively, whereas that of 20%RBF cracker incorporated with UPF ranged from 3.06 ± 0.07 to 3.15 ± 0.07 g, 0.32 ± 0.00 to 0.31 ± 0.00 mm and 4.24 ± 0.00 to 4.24 ± 0.00 cm, respectively. The results illustrated that the replacement of wheat flour (WF) with UPF in the 20%RBF crackers didn't affect the weight, height and diameter of all samples. As shown in Table 14, the moisture content and water activity of 20%RBF crackers incorporated with UPF significantly decreased from 5.69 ± 0.02 (20%RBF) to 5.35 ± 0.02 (20%RBF+7%UPF) and 0.41 ± 0.00 (20%RBF) to 0.32 ± 0.00 (20%RBF+7%UPF), respectively when the level of UPF increased. Moreover, the hardness and fracturability of 20%RBF cracker formulated with UPF are presented in Table 14. Hardness value of 20%RBF crackers incorporated with UPF ranged from 5385.39 ± 55.53 (20%RBF) to 3676.90 ± 63.87 g (20%RBF+7%UPF), respectively, whereas fracturability value of 20%RBF crackers incorporated with UPF ranged from 1069.83 ± 9.85 (20%RBF) to 1362.13 ± 56.75 g (20%RBF+7%UPF), respectively. The findings demonstrated that the incorporation of UPF into the 20%RBF crackers significantly decreased the hardness and increased the fracturability values of the 20%RBF crackers ($P < 0.05$). The results showed that no statistical difference in weight and diameter between control cracker and 20%RBF cracker formulated with UPF. The incorporation with UPF in RBF cracker significantly decreased the height, moisture content and water activity compared to control cracker. Hardness of RBF crackers with UPF incorporation significantly decreased compared to control.

In terms of physical characteristics (Table 15) among the control cracker, 20%RBF cracker and 20%RBF cracker incorporated with 5 and 7%UPF, the results showed that no statistical difference between weight and diameter of all samples. The height of 20%RBF crackers incorporated with UPF significantly decreased from 0.34 ± 0.00 (control) to 0.31 ± 0.00 (20%RBF+7%UPF), respectively when the level of UPF increased. The moisture content and water activity of 20%RBF crackers incorporated with UPF significantly decreased from 6.34 ± 0.05 (control) to 5.35 ± 0.02 (20%RBF+7%UPF) and 0.46 ± 0.00 (control) to 0.32 ± 0.00 (20%RBF+7%UPF), respectively with the increasing of UPF levels. In addition, the hardness and fracturability of control cracker, 20%RBF cracker and 20%RBF cracker formulated with 5 and 7%UPF are presented in Table 15. Hardness value of crackers ranged from 4088.18 ± 26.74 (control) to 3676.90 ± 63.87 g (20%RBF+7%UPF), respectively, whereas fracturability value of crackers ranged from 1375.36 ± 30.73 (control) to 1362.13 ± 56.75 g (20%RBF+7%UPF), respectively. The findings demonstrated that the incorporation of UPF into crackers significantly decreased the hardness of crackers but no statistical difference in fracturability between control and 20%RBF cracker incorporated with 5 and 7%UPF.

Table 14 Physical characteristics of riceberry crackers substituted wheat flour with unripe papaya flour

	Weight (g)	Height (mm)	Diameter (cm)	Moisture (%)	Water activity	Hardness (g)	Fracturability (g)
20%RBF	3.08±0.05 ^a	0.32±0.00 ^a	4.23±0.01 ^a	5.69±0.02 ^a	0.41±0.00 ^a	5385.39±55.53 ^a	1069.83±9.85 ^a
20%RBF+3%UPF	3.06±0.07 ^a	0.32±0.00 ^a	4.24±0.00 ^a	5.31±0.03 ^b	0.32±0.01 ^b	4500.27±59.52 ^b	1155.06±19.26 ^a
20%RBF+5%UPF	3.11±0.02 ^a	0.31±0.00 ^a	4.24±0.01 ^a	5.37±0.06 ^b	0.33±0.01 ^b	4373.47±71.44 ^b	1332.98±27.94 ^b
20%RBF+7%UPF	3.15±0.07 ^a	0.31±0.00 ^a	4.24±0.00 ^a	5.35±0.02 ^b	0.32±0.00 ^b	3676.90±63.87 ^c	1362.13±56.75 ^b

Values are expressed as mean ± S.E.M. (n = 4).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

20%RBF, 20%RBF+3%UPF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

Table 15 Physical characteristics of riceberry crackers substituted wheat flour with unripe papaya flour

	Weight (g)	Height (mm)	Diameter (cm)	Moisture (%)	Water activity	Hardness (g)	Fracturability (g)
Control	3.04±0.04 ^a	0.34±0.00 ^a	4.26±0.01 ^a	6.34±0.05 ^a	0.46±0.00 ^a	4088.18±26.74 ^a	1375.36±30.73 ^a
20%RBF	3.08±0.05 ^a	0.32±0.00 ^b	4.23±0.01 ^a	5.69±0.02 ^b	0.41±0.00 ^b	5385.39±55.53 ^b	1069.83±9.85 ^b
20%RBF+5%UPF	3.11±0.02 ^a	0.31±0.00 ^{bc}	4.24±0.01 ^a	5.37±0.06 ^c	0.33±0.01 ^c	4373.47±71.44 ^c	1332.98±27.94 ^a
20%RBF+7%UPF	3.15±0.07 ^a	0.31±0.00 ^c	4.24±0.00 ^a	5.35±0.02 ^c	0.32±0.00 ^c	3676.90±63.87 ^d	1362.13±56.75 ^a

Values are expressed as mean ± S.E.M. (n = 4).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

20%RBF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

2.5 Color characteristics of riceberry crackers incorporated with unripe papaya flour

As shown in Figure 19, the addition of unripe papaya flour (UPF) significantly changed the color parameters including lightness (L^*), redness (a^*) and yellowness (b^*) of the 20% of riceberry flour crackers (RBF) incorporated with UPF. The fortification of UPF into 20%RBF crackers affected the surface color which became slightly lighter when increasing UPF amounts. The L^* , a^* and b^* values of 20%RBF crackers incorporated with UPF significantly increased from 12.51 ± 0.04 (20%RBF) to 16.53 ± 0.28 (20%RBF+7%UPF), 3.68 ± 0.07 (20%RBF) to 4.08 ± 0.13 (20%RBF+7%UPF) and 1.27 ± 0.04 (20%RBF) to 1.89 ± 0.07 (20%RBF+7%UPF), respectively (Table 16). The findings indicated that the substitution of wheat flour (WF) with UPF in the 20%RBF cracker significantly increased the L^* , a^* and b^* values. Moreover, the total color difference index (ΔE) of 20%RBF crackers incorporated with UPF are presented in Table 16. The total color difference index (ΔE) of 20%RBF crackers incorporated with UPF significantly increased from 3.77 (20%RBF+3%UPF) to 4.09 (20%RBF+7%UPF), respectively when the level of UPF increased.

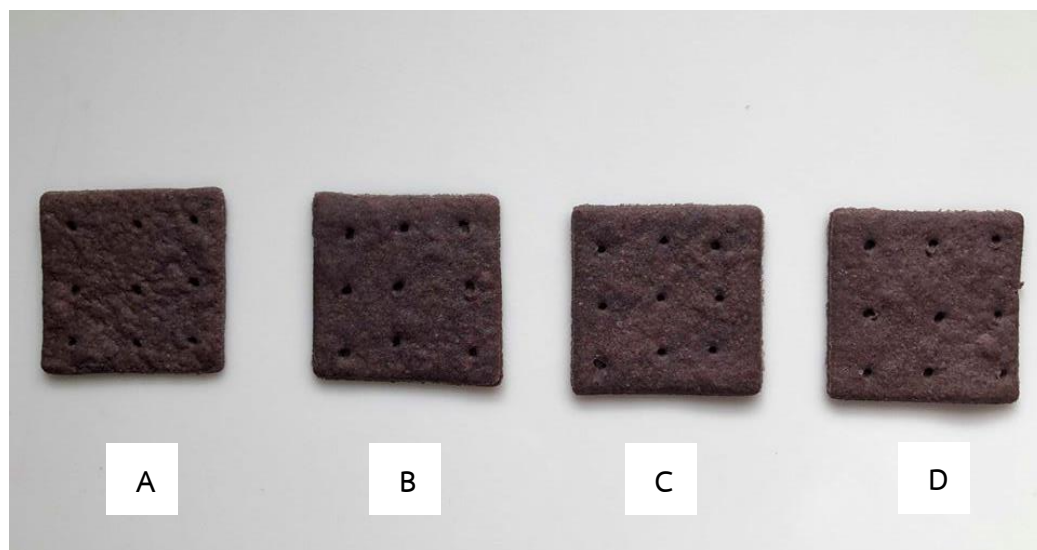


Figure 19 The appearance of riceberry crackers substituted wheat flour with unripe papaya flour

(A), (B), (C) and (D): Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

Table 16 Color characteristics of riceberry crackers substituted wheat flour with unripe papaya flour

	Color values			ΔE
	L*	a*	b*	
20%RBF	12.51±0.04 ^a	3.68±0.07 ^a	1.27±0.04 ^a	0.00
20%RBF+3%UPF	16.25±0.42 ^b	4.03±0.09 ^b	1.61±0.06 ^a	19.77
20%RBF+5%UPF	16.99±0.36 ^b	4.06±0.14 ^b	1.77±0.02 ^b	24.12
20%RBF+7%UPF	16.53±0.28 ^b	4.08±0.13 ^b	1.89±0.07 ^b	26.25

Values are expressed as mean ± S.E.M. (n = 4).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

20%RBF, 20%RBF+3%UPF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

ΔE : total color difference between riceberry cracker and treatment.

2.6 Phytochemical contents and antioxidant properties of riceberry crackers incorporated with unripe papaya flour

The phytochemical contents which are total phenolic content and total anthocyanins content of 20% of riceberry flour cracker (RBF) incorporated with unripe papaya flour (UPF) exhibited in Table 17. Total phenolic content and total anthocyanins content of 20%RBF cracker were 8.61 ± 0.20 mg gallic acid equivalent (GAE)/10g dry weight (DW) and 802.19 ± 1.53 μ g cyanidin-3-glucoside (C3G)/10g DW, respectively, while that of 20%RBF crackers incorporated with UPF ranged from 8.72 ± 0.11 (20%RBF+3%UPF) to 11.59 ± 0.29 (20%RBF+7%UPF) mg GAE/10g DW and from 801.71 ± 1.48 (20%RBF+3%UPF) to 802.48 ± 3.91 (20%RBF+7%UPF) μ g C3G/10g DW, respectively. The findings indicated that total phenolic content significantly increased as the level of UPF increased (Table 17). Furthermore, antioxidant properties of the 20%RBF crackers are also shown in Table 16. The values of ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) significantly increased from 79.99 ± 0.66 to 81.92 ± 0.41 mM FeSO_4 /10g DW and from 45.93 ± 2.80 to 63.97 ± 1.23 μ mol trolox equivalent (TE)/10g DW, respectively. In terms of the IC_{50} value of DPPH radicals scavenging activity of 20%RBF crackers incorporated with UPF ranged from 0.41 ± 0.01 (20%RBF) to 0.42 ± 0.01 (20%RBF+7%UPF) mg/ml, respectively. The incorporation of UPF into the 20%RBF crackers increased FRAP and TEAC values about one fold. As expected, the incorporation of UPF in the 20%RBF crackers showed significantly increased antioxidant properties.

Table 17 Phytochemical contents and antioxidant activities of riceberry crackers substituted wheat flour with unripe papaya flour

Sample	TPC (mg GAE/10g DW)	TAC (μ g C3G/10g DW)	FRAP (mM FeSO ₄ /10g DW)	TEAC (μ mol TE/10g DW)	DPPH IC ₅₀ (mg/ml)
20%RBF	8.61±0.20 ^a	802.19±1.53 ^a	79.99±0.66 ^{ab}	45.93±2.80 ^a	0.41±0.01 ^a
20%RBF+3%UPF	8.72±0.11 ^a	801.71±1.48 ^a	79.15±1.09 ^a	49.02±1.38 ^b	0.43±0.01 ^a
20%RBF+5%UPF	10.62±0.36 ^b	803.81±2.67 ^a	81.59±0.58 ^b	54.09±0.98 ^c	0.44±0.02 ^a
20%RBF+7%UPF	11.59±0.29 ^c	802.48±3.91 ^a	81.92±0.41 ^b	63.97±1.23 ^d	0.42±0.01 ^a

Values are expressed as mean \pm S.E.M. (n = 4).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

TPC = Total phenolic content; TAC = Total anthocyanins content; GAE = Gallic Acid Equivalent; C3G = Cyanidins-3-glucosides; TE = Trolox Equivalent; DW = dry weight.

20%RBF, 20%RBF+3%UPF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

2.7 Determination of total starch, hydrolysis index and predicted glycemic index of riceberry crackers incorporated with unripe papaya flour

The effect of unripe papaya flour (UPF) incorporated into 20% of riceberry flour crackers (RBF) on starch degradation using digestive enzymes was examined to analyze the releasing of glucose. The total starch (TS), undigestible starch (UDS), hydrolysis index (HI) and predicted glycemic index (pGI) values are shown in Table 18. TS of 20%RBF cracker was 41.54 ± 0.17 g/100g sample which were more than 20%RBF crackers incorporated with UPF ranged from 41.41 ± 0.22 to 38.14 ± 0.08 g/100g sample, respectively. UDS of 20%RBF cracker incorporated with UPF were in the range of 19.08 ± 1.08 to $28.54 \pm 1.40\%$, respectively. Moreover, the HI values of 20%RBF crackers incorporated with UPF significantly decreased from 37.14 ± 0.62 (20%RBF) to 30.14 ± 0.38 (20%RBF+7%UPF), respectively when the level of UPF increased ($P < 0.05$). The *in vitro* starch digestograms of 20%RBF cracker and 20%RBF crackers incorporated with UPF are exhibited in Figure 20. At the time intervals of 0, 30, 60, 90, 120 and 180 min on the digestograms, 20%RBF crackers incorporated with UPF showed the attenuation of releasing glucose after 60 min ($P < 0.05$). The results illustrated that the incorporation of UPF into the 20%RBF crackers demonstrated the lower amount of hydrolysis rate when compared to 20%RBF cracker. As shown in Table 18, the pGI values among cracker samples were also affected. The pGI values of 20%RBF cracker (60.10 ± 0.34) were significantly higher than that of 20%RBF crackers incorporated with UPF ranged from 59.22 ± 0.06 to 56.26 ± 0.21 .

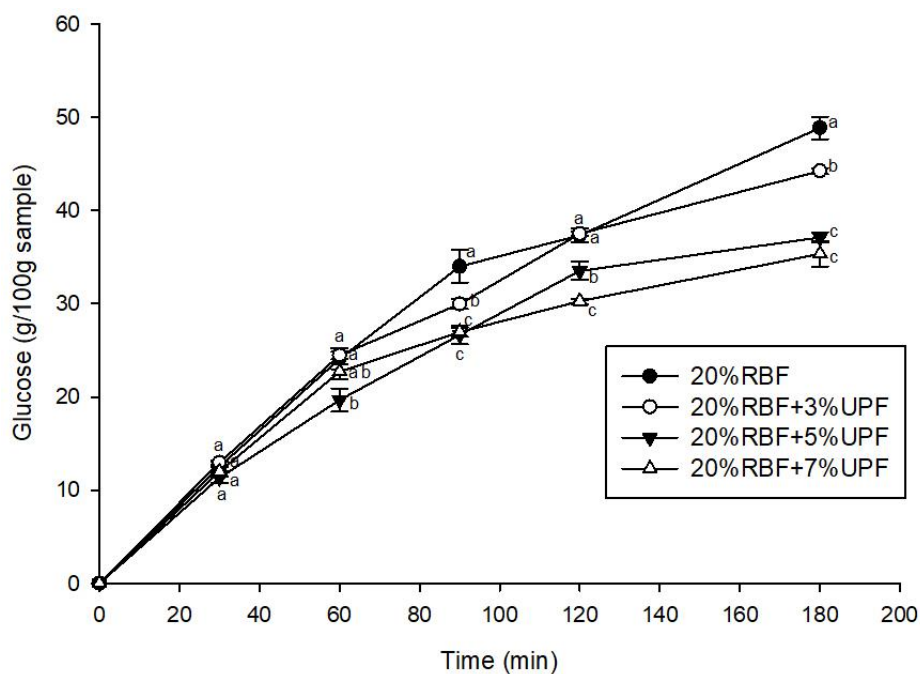


Figure 20 The release of glucose during the hydrolysis of riceberry crackers substituted wheat flour with unripe papaya flour at time intervals of 0, 30, 60, 90, 120 and 180 min

Values are expressed as mean \pm S.E.M. (n = 4).

20%RBF, 20%RBF+3%UPF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

Table 18 The total starch, hydrolysis index and predicted glycemic index of riceberry crackers substituted wheat flour with unripe papaya flour

	TS (g/100g)	UDS (%)	HI	pGI
20%RBF	41.54±0.17 ^a	19.08±1.08 ^a	37.14±0.62 ^a	60.10±0.34 ^a
20%RBF+3%UPF	41.41±0.22 ^a	18.64±1.11 ^a	35.53±0.10 ^b	59.22±0.06 ^b
20%RBF+5%UPF	39.28±0.31 ^b	23.17±1.54 ^a	30.70±0.32 ^c	56.56±0.18 ^c
20%RBF+7%UPF	38.14±0.08 ^c	28.54±1.40 ^b	30.14±0.38 ^c	56.26±0.21 ^c

Values are expressed as mean ± S.E.M. (n = 4).

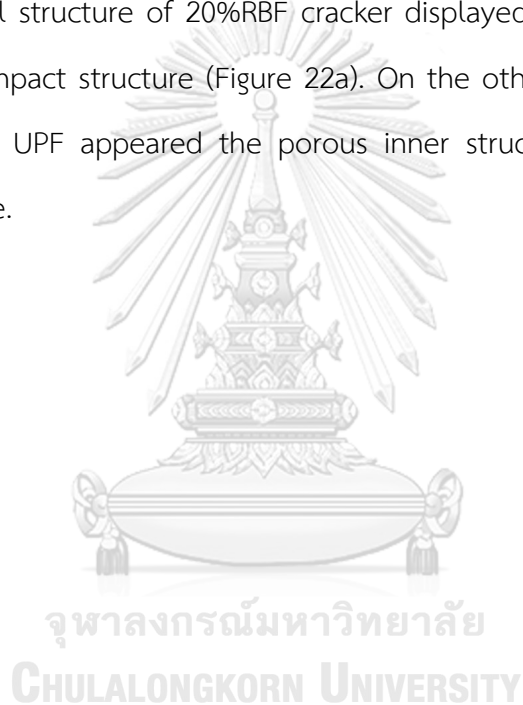
Mean with different superscript letter within a column are significantly different ($P < 0.05$).

TS = total starch; UDS = undigestible starch; HI = hydrolysis index; pGI = predicted glycemic index.

20%RBF, 20%RBF+3%UPF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

2.8 Scanning Electron Microscope (SEM) examination of riceberry crackers incorporated with unripe papaya flour

The microstructure characteristics of the surface along with cross-sectional structure of cracker samples were determined by using SEM. As shown in Figure 21a, the surface of 20% of riceberry cracker (RBF) exhibited rough surface, whereas 20%RBF cracker incorporated with unripe papaya flour (UPF) demonstrated more irregular surface when the level of UPF increased (Figure 21b, c and d). Furthermore, the cross-sectional structure of 20%RBF cracker displayed the lower number of air cells with the compact structure (Figure 22a). On the other hand, 20%RBF crackers incorporated with UPF appeared the porous inner structure with large air cavity enclosing structure.



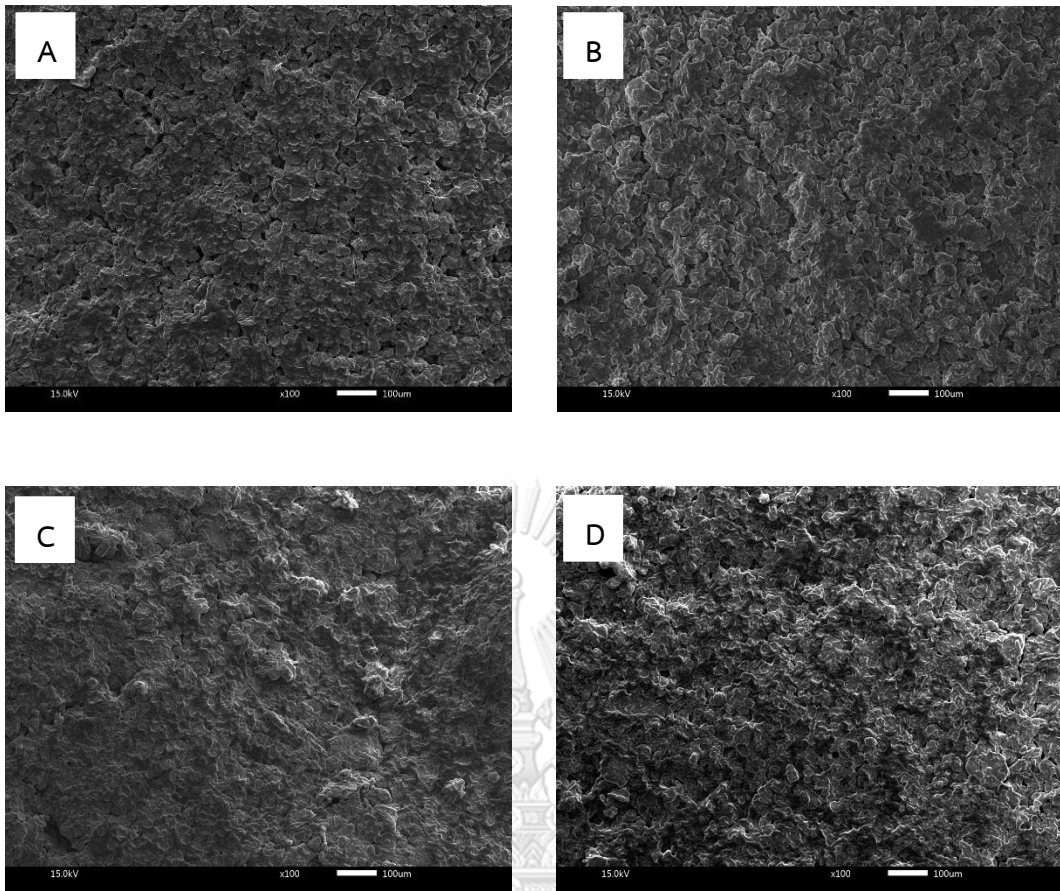


Figure 21 Scanning electron microscope (SEM) micrographs of riceberry crackers (100x) for surface structure of crackers; (A), B), (C) and (D): Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

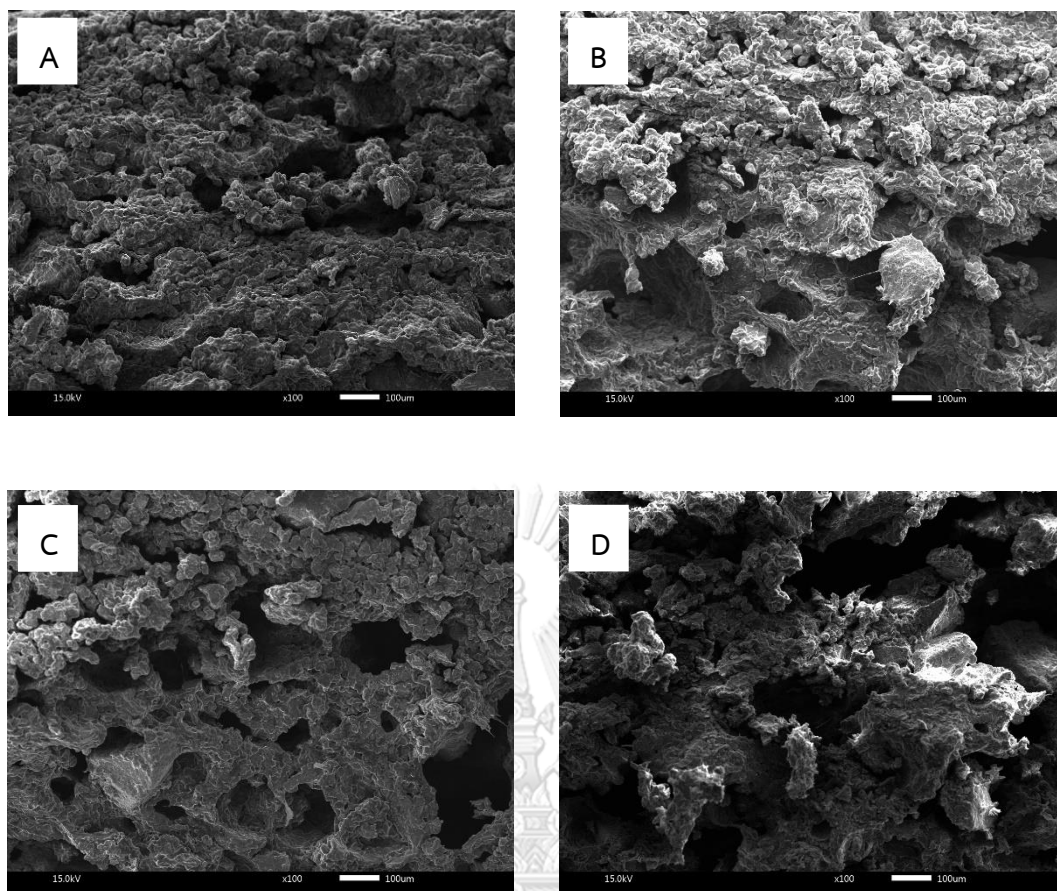


Figure 22 Scanning electron microscope (SEM) micrographs of crackers

(100x) for inner structure of crackers; (A), (B), (C) and (D): Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

2.9 Sensory evaluation

The sensory evaluation of 20% of riceberry flour crackers (RBF) incorporated with unripe papaya flour (UPF) was evaluated by using a hedonic scale (nine-point). The assessment parameters of consumer acceptability including appearance, color, flavor, taste, fracturability, hardness and overall acceptability were shown in Table 19. The appearance, color and flavor scores of 20%RBF crackers were 7.26 ± 0.15 , 7.42 ± 0.12 and 7.38 ± 0.13 respectively, whereas that of 20%RBF crackers incorporated with UPF ranged from 7.28 ± 0.15 to 7.24 ± 0.14 , 7.50 ± 0.14 to 7.42 ± 0.15 and 7.24 ± 0.19 to 6.90 ± 0.19 , respectively. Moreover, the fracturability and hardness scores of 20%RBF crackers were 7.04 ± 0.11 and 6.80 ± 0.11 , respectively, whereas the 20%RBF crackers incorporated with UPF ranged from 7.02 ± 0.17 to 7.16 ± 0.11 and 6.80 ± 0.11 to 7.28 ± 0.17 , respectively. The results indicated that the replacement of what flour (WF) with UPF in the 20%RBF crackers didn't affect the appearance, color, flavor, fracturability and hardness scores of all cracker samples. In addition, the sensory scores of taste and overall acceptability of crackers significantly decreased from 7.60 ± 0.15 (20%RBF) to 7.02 ± 0.17 (20%RBF+7%UPF) and 7.50 ± 0.13 (20%RBF) to 7.02 ± 0.16 (20%RBF+7%UPF), respectively when the level of UPF increased (Table 19). The acceptance index (AI) of 20%RBF crackers incorporated with UPF were ranged from 83.33 to 78.00%.

Table 19 Sensory evaluation scores and acceptance index of riceberry crackers substituted wheat flour with unripe papaya flour

	Appearance	Color	Flavor	Taste	Fracturability	Hardness	Overall acceptability	Acceptance index (%)
20%RBF	7.26±0.15 ^a	7.42±0.12 ^a	7.38±0.13 ^a	7.60±0.15 ^a	7.04±0.11 ^a	6.80±0.11 ^a	7.50±0.13 ^a	83.33
20%RBF+3%UPF	7.28±0.15 ^a	7.50±0.14 ^a	7.24±0.19 ^a	7.14±0.19 ^{ab}	7.02±0.17 ^a	6.06±0.19 ^a	7.42±0.13 ^{ab}	82.44
20%RBF+5%UPF	7.26±0.15 ^a	7.44±0.15 ^a	7.20±0.19 ^a	7.24±0.22 ^{ab}	7.12±0.17 ^a	7.18±0.20 ^a	7.46±0.17 ^{ab}	82.88
20%RBF+7%UPF	7.24±0.14 ^a	7.42±0.15 ^a	6.90±0.19 ^a	7.02±0.17 ^b	7.16±0.11 ^a	7.28±0.17 ^a	7.02±0.16 ^b	78.00

Values are expressed as mean ± S.E.M. (n = 50).

Mean with different superscript letter within a column are significantly different ($P < 0.05$).

20%RBF, 20%RBF+3%UPF, 20%RBF+5%UPF and 20%RBF+7%UPF: Riceberry crackers manufactured with 0, 3, 5, and 7% substitution wheat flour with unripe papaya flour (UPF), respectively.

CHAPTER V

DISCUSSIONS

1. To investigate the effect of partially substituted wheat flour with riceberry flour on physicochemical, functional properties, and sensory evaluation of crackers

1.1 Proximate composition of riceberry flour

The proximate composition is an information about the composition of foods which based on the reliable method. The nutrient composition plays an important information in terms of food product development (Varastegani, Zzaman et al. 2015). Proximate composition of riceberry flour was analyzed in this current study including protein, total fat, carbohydrate, total dietary fiber, moisture and ash. The protein (12.55 g/100g) and total fat content (10.63 g/100g) of riceberry flour in the current study were higher than that of previous studies which stay in the range of 7.54 to 8.65 g/100g and 1.40 to 4.35 g/100g, respectively (Chuaykarn, Laohakunjit et al. 2013, Supatchalee, Apinya et al. 2016, Kraithong, Lee et al. 2018, Tangsrianugul, Wongsagonsup et al. 2019). The amount of carbohydrate and moisture content of riceberry flour were observed which agreed with previous studies (Chuaykarn, Laohakunjit et al. 2013, Kraithong, Lee et al. 2018, Tangsrianugul, Wongsagonsup et al. 2019). In addition, riceberry flour also had higher content of total dietary fiber (13.80 g/100g) than the previous study (Tangsrianugul, Wongsagonsup et al. 2019). Moreover, the total dietary fiber content of riceberry flour exhibited higher than the wheat flour which is in the range of 2.31 to 3.70 g/100g dry weight (Sowbhagya, Soumya et al. 2015, Klunklin and Savage 2018). The compositions of rice flour normally rely on rice variety and may affect by the milling conditions such as dry or

wet milling (Wang, Guo et al. 2016, Kraithong, Lee et al. 2018). During the wet milling process, the soluble protein or non-starch components may bind with lipids and then remove, resulting tend to have higher carbohydrate content with lower the content of other components (Chiang and Yeh 2002). The amount of ash of riceberry flour in the present study was higher than previous studies (Chuaykarn, Laohakunjit et al. 2013, Supatchalee, Apinya et al. 2016, Kraithong, Lee et al. 2018, Tangsrianugul, Wongsagonsup et al. 2019). The percentage of ash is related to the mineral content of food sample (Oloyede 2005).

1.2 Phytochemical contents and antioxidant properties of flours

The riceberry flour obviously had higher phenolic compound than wheat flour and also contained a high content of anthocyanin. In terms of antioxidant capacity, riceberry flour had a higher ferric reducing antioxidant power (FRAP), trolox equivalent antioxidant capacity (TEAC) and DPPH values than wheat flour. Our findings indicated that riceberry flour exhibited the effectiveness to scavenge free radicals than wheat flour. These might be due to the availability of the high amounts of polyphenols in riceberry flour. Chaiyasut et al. (Chaiyasut, Sivamaruthi et al. 2016) examined the anthocyanins content and antioxidant activity in flowers, fruits and vegetables in Thailand, the findings reported that ma-kiang extract presented a good source of anthocyanins especially cyanidin-3-glucoside and had the highest antioxidant capacity when compared to the other plant extracts. The previous studies have been done the antioxidant capacity in plant-based extract which the results obtained from the different assays. Mostly, the results of antioxidant capacity done through the single electron transfer (ET) reaction based assays which determine an antioxidant's reducing capacity including TEAC, FRAP and DPPH (Huang, Ou et al. 2005). Since free radicals are molecules with unpaired electrons that can cause the chain reaction to seek out the electrons from other substances for neutralization themselves to

become stable (Percival 1996). Polyphenols are a large group of phytochemicals which normally present in plant-based foods (Tsaio 2010). Phenolic compounds can be categorized as the dietary antioxidants which are one of the antioxidant protection systems. The mechanisms of phenolics in order to act as antioxidants are exhibited in a number of ways (Percival 1996). Hydroxyl groups of phenolics present as the hydrogen-donating antioxidants that able to react with reactive oxygen species, result to break the cycle of new free radical generation (Valentão, Fernandes et al. 2003). Phenolic compounds also exhibit the capacity to chelate metal ions such as Fe^{2+} which related to the production of radicals (Yang, Landau et al. 2001). Hence, the chelation able to reduce the rate of Fenton reaction and preclude the oxidation (Pietta 2000). Consequently, the suppression of radical production may reduce the oxidation by deactivating the precursors of free radicals (Tsaio 2010). Furthermore, in terms of antioxidant ability in pigmented rice also have been done in many previous studies. The purple rice known as to be the source of phenolic compounds especially anthocyanins (Shao, Hu et al. 2018). The major anthocyanin compounds that found in riceberry bran were cyanidin-3-glucoside and peonidin-3-glucoside (Leardkamolkarn, Thongthep et al. 2011). Shao et al. (Shao, Hu et al. 2018) evaluated the bioactive compounds including phenolics and anthocyanins of black rice, the results reported that the phenolic compounds significantly conducted to antioxidant activity. In the studies of Ichikawa et al. (Ichikawa, Ichiyanagi et al. 2001) reported that the anthocyanin extract contributed to the antioxidant ability of purple black rice. Klinklin and Savage (Klunklin and Savage 2018) also stated that the antioxidant compounds (phenolic and anthocyanin contents) exhibited the antioxidants scavenging capacity in purple rice variety named Sanpatong. Those findings in agreement with our results. In addition, Ratseewo et al. (Ratseewo, Warren et al. 2019) stated that the phenolic acids which were identified in riceberry rice including ferulic acid, protocatechuic, *p*-coumaric, vanillic, gallic, sinapic, chlorogenic

and syringic. Consequently, the antioxidant capacity of riceberry flour in the current study might come from a group of anthocyanins coordinate with phenolic compounds.

1.3 Determination of starch fraction, hydrolysis index and predicted glycemic index of flours

In the current study showed that the amount of total starch of riceberry flour was less than wheat flour. Moreover, riceberry flour had a low content of rapidly digestible starch (RDS: amount of glucose released after 20 min) and high content of slowly digestible starch (SDS: amount of glucose released between 20 to 120 min of digestion) compared to wheat flour (Gourineni, Stewart et al. 2017). These might affect to the hydrolysis index of riceberry flour. The results indicated that hydrolysis index of riceberry flour was lower than wheat flour. Riceberry flour showed a strong resistance to starch digestion which resulted in delay and lower of released glucose. Consequently, the riceberry flour had the lowest values of the predicted glycemic index when compared to wheat flour. Our finding was accordance with the previous study of Klinklin and Savage (Klunklin and Savage 2018), reported that the presence of a low proportion of RDS and high proportion of SDS in purple rice flour (Sanpatong) also had the lowest amount of the predicted glycemic index. In addition, a similar result was found in the study of An et al. (An, Bae et al. 2016), who reported that black rice flour had the lower predicted glycemic index value than wheat flour during starch digestibility. Therefore, the use of rice with low glycemic index as an ingredient for the production of foods might be good for diabetic patients by attenuating the raising of blood glucose (Ludwig 2002). Also, Guo et al. (Guo, Ling et al. 2007) studied the effect of anthocyanin-rich extract (AREBR) from black pigmented rice on insulin resistance in fructose-fed Sprague-Dawley rats. AREBR (5 g/kg of high-fructose diet) was fed for 8 weeks and results showed that AREBR exhibited the

increasing of insulin sensitivity, thus AREBR may prevent the progress of the fructose-induced insulin resistance in animal models. The previous study of Chaiyasut et al. (Chaiyasut, Sivamaruthi et al. 2017) studied the phytochemicals content in germinated black rice extract (GBRE) and the effect of GBRE on anti-diabetic properties in streptozotocin-induced diabetic Wistar rats. GBRE (500 mg and 1000 mg/kg body weight) was fed for 12 weeks and results exhibited that the GBRE could improve the antioxidant ability and antioxidant enzymes. Moreover, the reducing in plasma glucose, insulin resistance and glucose tolerance were found in diabetic rat. The plasma insulin secretion also was significantly increased upon GBRE treatment in diabetic rat. Furthermore, Sun et al. (Sun, Spiegelman et al. 2010) stated that the consumption of brown rice might associate with a lower risk of Type 2 diabetes, whereas white rice consumption could increase the risk. However, there are several factors that might affect to starch digestibility. The previous study reported that the different type of cultivars may exist in the different of chemical compositions such as dietary fiber and protein content. Klunklin and Savage (Klunklin and Savage 2018) studied the proximate composition of wheat flour and purple rice flour. The results showed that purple rice flour had two-fold higher total dietary fiber content than wheat flour which may result to restrict the starch hydrolysis. Khatun et al. (Khatun, Waters et al. 2019) stated that starch digestibility might be affected by protein in rice flour. Rice protein could form the protective layer surrounding rice starch, then altering the properties of starch. In addition, starch of purple rice has been reported to have high levels of amylopectin content. The amylopectin chain might be retrograded during the starch hydrolysis, resulted to form the double helix with a compact crystalline structure which make the flour resists the enzyme to access (Gidley and Bulpin 1987, Klunklin and Savage 2018). Moreover, the content of antioxidant compounds, phenolic and anthocyanin content, that presented in black rice flour (Josaengheugchal) also had the impact on the digestibility by inhibiting the

main carbohydrates digestive enzymes, then decreased rate of glucose released and attenuated the starch digestibility (An, Bae et al. 2016). In the recent study of Ratseewo et al. (Ratseewo, Warren et al. 2019) studied the influence of Thai pigmented rice on the starch digestibility. The results showed that Thai pigmented rice flour (red and purple rice) exhibited lower starch digestibility compared to non-pigmented rice (white rice). Phenolic compounds and anthocyanin contents which widely found in pigmented rice have been shown the possible effect on starch digestibility (Ratseewo, Warren et al. 2019). The main anthocyanins in pigmented rice including cyanidin-3-glucoside and peonidin-3-glucoside which was reported to possess inhibition of α -glucosidase activity (Adisakwattana, Charoenlertkul et al. 2009, Chatthongpisut, Schwartz et al. 2015). Furthermore, Ramdath et al. (Ramdath, Padhi et al. 2014) reported that polyphenols and anthocyanins in pigmented potato extract showed the effectiveness to reduce *in vitro* starch digestion. Hence, the results of our present study might agree with those studies that phenolic and anthocyanin contents in riceberry flour could play an important role in enzymes inhibiting which affected to slow the starch digestion. In addition, riceberry flour had a higher content of undigestible starch than wheat flour. The undigestible starch is the component that was not being digested by carbohydrate digestive enzymes and known as the dietary fiber. Dietary fiber may affect the functional properties of flour. Fiber in rice flour exhibited the ability to be a barrier during the starch digestion which can reduce the food to access the enzyme and cause slow starch digestibility (Sozer, Cicerelli et al. 2014). In the study of Sozer et al. (Sozer, Cicerelli et al. 2014) studied the effect of wheat bran addition on *in vitro* starch digestibility and physicochemical properties of biscuits. Since the wheat bran considered as the source of dietary fiber and protein. The results showed that the increasing of dietary fiber content in biscuits formulation, resulted increasing the dietary fiber content in biscuits and reducing the starch digestibility. Furthermore, the particle size of flour is one of the factors that

may affect the starch digestion. Riceberry flour granules of the current study presented bigger and larger than wheat flour granules. The large surface area of flour granules exhibits more possible surface to be attacked by the digestive enzymes. Consequently, the increasing of particle size of flour granule, resulted decreasing the surface space to face the enzymes (De la Hera, Martinez et al. 2013).

1.4 Scanning Electron Microscope (SEM) examination of flours

The morphology of riceberry flour exhibited a rougher and irregular shape. In contrast, wheat flour showed a smooth surface. The difference in flours microstructure might be due to the dissimilarity of the intensity of mechanical work in the flour preparation process (De la Hera, Martinez et al. 2013). Moreover, the starch granules might be affected by the milling method and chemical characteristics of flours (Sakhare, Inamdar et al. 2014). Ahmad et al. (Noorlaila, Asmeda et al. 2016) studied on the effects of different grinding methods including dry, semi-wet and wet on morphology and characteristics of rice flour. The results showed that all rice flours with different grinding methods displayed irregular pentagonal shaped particles adhered with protein matrix. The starch granules from wet grinding method had small particles and free starch granules. On the other side, the damaged granules were found in the dry grinding method. Normally, the starch granules exhibited a unique structure. The difference of botanical species and environmental condition have an impact on the shape and size of granules. Generally, maize and rice starch present polyhedral or angular shape. Wheat and potato starch have flat circular or spherical granules and oval-shaped, respectively. Furthermore, the size of each starch granule also varies depending on the botanical source. In addition, Narvaez-Gonzalez et al. (Narváez-González, de Dios Figueroa-Cárdenas et al. 2006) reported that the morphology of starch granule (polygonal, spherical and round) had an impact on the physical property, degree of compaction

and chemical composition. Kang et al. (Kang, Sohn et al. 2015) studied the effect of rice varieties with different starch shapes on gluten-free bread qualities, the results illustrated that the rice grain with polygonal starch shape had high hardness values of bread. Commonly, rice granules displayed a polygonal with compactly packed structure, resulting in a hard endosperm which require high mechanical force for bakery production. Contreras-Jimenez et al. (Contreras-Jiménez, Torres-Vargas et al. 2019) studied the physicochemical properties and morphology of quinoa starch, reported that the shape, size and conformation of starch might influence to the physical property such as texture of food products. In addition, Lorenz (Lorenz 1990) reported that cakes or bread baked products with quinoa starch which displayed the oblong or spherical structure, resulting poor quality in baked goods (compact and dense in texture).

1.5 Physical characteristics of crackers

In this study, researcher aimed to study the effect of partially substituted wheat flour with riceberry flour in cracker product. The five ratios were employed to replace wheat flour with riceberry flour including 0%, 5%, 10%, 15% and 20% replacement. The incorporation of riceberry flour affected to the height and diameter of crackers. The significantly decreased in height and diameter were observed in the cracker with increasing the riceberry flour fortification. It might be due to the working of gluten which is the protein in wheat flour and normally refers to the glutenin and gliadin (Ahmed and Abozed 2015). Gluten network was generated when wheat flour is blended with water during the mixing process, then resulted in the entrapping of air, CO₂ and moisture. Under the baking condition, the increasing of temperature leads to the expansion of dough, therefore, increasing in height and diameter (Ahmed and Abozed 2015, Mau, Lee et al. 2017). In contrast, the presence of low gluten protein proportion as the replacement of wheat flour with riceberry flour

resulting in declining the CO₂ holding capacity which originated by the yeast. Hence, reduced height and diameter of baked crackers (Wu, Sung et al. 2009, Ahmed and Abozed 2015). The dilution of gluten protein is the limitation of gluten strength as the weakness gluten caused by low in gas retention of wheat dough of baked product (Wu, Sung et al. 2009). The results are in accord with the previous findings of the crackers incorporated with *Hibiscus sabdariffa* by-product (Ahmed and Abozed 2015). The decreasing of thickness was also found in the biscuits with increasing the purple rice flour fortification (Klunklin and Savage 2018). Moreover, the increasing level of the riceberry flour incorporated into the crackers slightly decreased moisture content and water activity. It might because the existing of moisture content of riceberry flour (7.2%) which is ample lower than wheat flour (11.8%). The content of moisture which contain in the crackers might affect to the quality of the product, for instance, the low moisture content of the product is crucial for extending the shelf life of products (Ahmed and Abozed 2015). Our results are in agreement with a study of biscuit replacement wheat flour with potato flour Seevaratnam et al. (Seevaratnam, Banumathi et al. 2012). The results indicated that the texture parameters of crackers were affected by the fortification of riceberry flour. Since the level of riceberry flour increased, the hardness value was increased while the fracturability of crackers was decreased. Hardness is defined as the penetrating force to break the sample while fracturability represents the force getting from the maximum force needed to break the sample (Gaines, Kassuba et al. 1992). Moreover, the term of hardness is a contrary term of fracturability or crispiness as the low hardness value showed to present high fracturability value (Nurul, Boni et al. 2009). The increasing of hardness in composite riceberry crackers (5-20%) might be owed to the lower amount of gluten protein. Normally, the ability of wheat flour which be processed to become the bakery products is mainly determined by the protein. Gluten plays an important role to determine the baking quality of wheat flour

including water absorption ability which result in elasticity, viscosity, and cohesivity properties of dough. During the dough fermentation, the gluten network trap the CO₂ bubbles to make the dough rising (Mau, Lee et al. 2017). Consequently, riceberry which is gluten-free flour cannot entrap the CO₂ and generate a viscoelastic network, then result in the tight structure of crackers. Our findings are in conformity with the previous study of the cookies fortified with chestnut flour (Šoronja-Simović, Pajin et al. 2017). Mau et al. (Mau, Lee et al. 2017) reported that the substitution of wheat flour with black rice flour into chiffon cakes showed the increasing of hardness value with the increased in black rice flour. Since the morphology of flour is considered important as the shape of starch could affect to the texture characteristic of the final product (Kang, Sohn et al. 2015). The polygonal in shape with rough surface and a very compact structure of riceberry flour morphology had the impact of increasing the hardness values of crackers incorporated with riceberry flour.

1.6 Color characteristics of crackers

For the color characteristics of crackers, the cooperation between ingredients and baking process play a crucial role in the final product (Sudha, Vetrmani et al. 2007). Since color is one of the considerable parameters that affect to the consumer in the purchase process (Sozer, Cicerelli et al. 2014). Moreover, the using of natural colorant from plants, fruits and vegetables consolidates into food products is impact to increase the consumer acceptability (Khoo, Azlan et al. 2017). The color parameters showed that the lightness (L^*), redness (a^*) and yellowness (b^*) values gave variation depended on the addition of riceberry flour. The incorporation of riceberry flour into crackers directly affected the color of crackers which became darker when increasing riceberry flour amounts. Moreover, the total color difference index (ΔE) exhibited the relation with the amount of riceberry flour incorporation into the cracker. The results showed that the values of ΔE increased in the cracker with

high level of riceberry flour incorporation. Consequently, cracker with high ΔE values demonstrated the higher difference in total color difference index when compared to control. The formation of color in bakery product might correlate with many factors. However, in terms of the baking process widely known as resulting from browning reaction (Purlis 2010). Since the non-enzymatic browning reaction is related to the degradation of carbohydrate such as Maillard reaction which generated when the reducing sugars and amino acids are heated together (BeMiller and Whistler 1996). Under the baking process, the elevation of temperature and baking time, might result in the development of color in the cracker (Sharma, Saxena et al. 2016). Moreover, it might be assumed that the raw materials had the impact on the color of crackers as the increasing of levels of riceberry flour (Čáslavková, Bednářová et al. 2015). The presence of dark purple color in crackers originated from the available anthocyanins pigments in the riceberry flour (Yodmanee, Karrila et al. 2011). Anthocyanins are water-soluble pigments which are under the phenolic group and responsible for most of the purple, red and blue colors in edible fruits and vegetables (Khoo, Azlan et al. 2017). Furthermore, the change of color has undergone the oxidation reaction of phenolic compounds which showed in riceberry flour during the thermal process (Mau, Lee et al. 2017). The similar findings have been found out in the previous studies of chiffon cake fortified with black rice powder (Mau, Lee et al. 2017), crackers incorporated with purple rice (Klunklin and Savage 2018) and noodles prepared with riceberry flour as replacement of wheat flour (Sirichokworrakit, Phetkhut et al. 2015). All those ingredients that were used in the previous studies including black rice and purple rice are contain the color pigment known as anthocyanins. The addition of the anthocyanins rich ingredient into food product might contribute to the color change which result to increase the redness and decrease lightness and yellow of foods.

1.7 Phytochemical contents and antioxidant properties of crackers

All cracker formulations were determined for the phytochemical compounds and antioxidant activities. The elevation of all phytochemical compounds (total phenolic content and total anthocyanins content) and antioxidant properties (DPPH, FRAP and TEAC) of the crackers acquired from the various amounts of riceberry flour incorporation. It could be stated that after baking, crackers enriched riceberry flour demonstrated the desirable source of bioactive compounds. The reason is the main component of phenolic compounds that presented in color rice as the earlier results of riceberry flour. This could be indicated that polyphenols in riceberry flour might give the potential free radical scavenging activity in crackers. Consequently, these could raise the phytochemical compounds and antioxidant activities in the crackers as well. Our findings were coincident with other studies of brown rice based cracker supplementation with apple pomace flour (Mir, Bosco et al. 2017), crackers made from with *Hibiscus sabdariffa* as replacement of wheat flour (Ahmed and Abozed 2015) and gluten-free crackers fortified with buckwheat flour (Sedej, Sakač et al. 2011). Although, the stability of phenolic and anthocyanins compounds in riceberry flour was affected as the thermal procedure. However, phytochemical antioxidant substances still remain in the crackers. For those reasons, riceberry flour showed abilities that might be used as an optional ingredient for developing the foods containing potential antioxidants.

1.8 Determination of total starch, hydrolysis index and predicted glycemic index of crackers

For the *in vitro* digestion of crackers, the results showed that the high levels of riceberry flour fortification in crackers exhibited the low amount of total starch. A similar trend of total starch was observed in the biscuits supplemented with purple rice flour (Klunklin and Savage 2018). Moreover, the crackers enriched riceberry flour

(20%) demonstrated the lowest hydrolysis index and predicted glycemic index as the low hydrolyzed starch content was determined. The cracker with riceberry flour incorporation could delay the releasing of glucose when compared to control. It might be due to the low rapidly digestible starch and the highest slow digestible starch proportions of riceberry flour as previously showed. In addition, the riceberry cracker presented the high content of undigestible starch when compared to control cracker. Undigestible starch is the component of dietary fiber which restrict the starch digestion process and reduced the releasing of glucose during crackers digestion at time in intervals. Furthermore, the polyphenol compounds which contained in the cracker with riceberry flour could retard the starch digestion. Sun and Miao (Sun and Miao 2019) reported that the dietary polyphenols might modulate the starch digestibility by inhibition of the main digestive enzymes such as α -amylase as well as the interaction between starch and phenolic compounds. Previous study of Bae et al. (Bae, Choi et al. 2016) stated that the noodles from buckwheat enriched with flavonoids extract and rutin-enhanced with flavonoids extract demonstrated the effectiveness of suppressing starch hydrolysis in the noodles. Similar to the observation of Klunklin and Savage (Klunklin and Savage 2018), biscuit enriched purple rice flour significant lower starch digestibility and predicted glycemic index than wheat biscuit. The presence of phenolic and anthocyanin contents in purple rice flour might contribute to the attenuation of starch hydrolysis.

1.9 Scanning Electron Microscope (SEM) examination of crackers

In terms of the microstructure characteristics of crackers, the surface and cross-sectional structure was examined. The fortification of riceberry flour into crackers directly affected to the surface of crackers. The rough surface of crackers was found that might be due to the difference in the morphology of the flour

particles between riceberry flour and wheat flour. Since the morphology of riceberry flour displayed an irregular shape than wheat flour particles. Hence, the elevation of riceberry flour contents incorporated in crackers, the surface of crackers presented the cracked and rough texture. For the internal structure, a large porous texture was found inside the crackers without riceberry flour incorporation. However, wheat flour substituted crackers by riceberry flour presented with the contrast structure. The reducing in the proportion of wheat flour in the cracker formulation obviously affected to change the microstructure of crackers. Hence, the lack of gluten matrix development resulted in less number of large air porous surrounding structures. Eventually, the riceberry enriched crackers became more denser of inner structures than control cracker. In addition, the degree of compaction in baked goods was affected by the shape of starch (Narváez-González, de Dios Figueroa-Cárdenas et al. 2006). Consequently, the fortification of riceberry flour with polygonal and spherical conformation might result of the compact structure of crackers when the level of riceberry flour increased. Previous study of Torbica et al. (Torbica, Hadnađev et al. 2012) studied the rice and buckwheat flour characteristics on cookie quality, the results indicated that the morphology of rice flour displayed the polygonal starch. Moreover, rice flour was used as the ingredient for gluten-free cookie production, the findings showed that the scanning electron micrograph of rice flour cookies exhibited densely packed rice starch. Our results agreed with the research of Rajiv et al. (Rajiv, Lobo et al. 2012) who stated that the addition of green gram flour into cookies appeared to be a compact structure. In addition, Ahmed and Abozed reported that the use of *Hibiscus sabdariffa* powder supplementation in crackers showed more cramped structures and less air cells of crackers (Ahmed and Abozed 2015).

1.10 Sensory evaluation

The sensory analysis was evaluated among the cracker to examine probable effects on the sensory profile of each formulation of crackers. Nine-point hedonic test is one of the most often used to assess food products as its simple and effective method (Tuorila 2015). The appearance and color scores showed no difference between cracker fortification with riceberry flour and control cracker. Khoo et al. (Khoo, Azlan et al. 2017) stated that the utilization of anthocyanins which is a natural color pigment might affect to increase consumer acceptability in food products. The terms of hardness and fracturability were used to evaluate the baked products since their related to freshness which is the perception of human (Kuchtová, Kohajdová et al. 2018). The findings found that sensory profile scores related to the changes in physical characteristics of crackers. The texture scores (hardness and fracturability) were in agreement with the texture profile analysis. Since the addition of riceberry flour increased from 5 to 20%, the scores of hardness and fracturability were significantly decreased. Nevertheless, the texture scores were in slightly to moderately preference (score 6-7). It was found in the same trend of sponge cake fortification with *Clitoria ternatea* extract (Pasukamonset, Pumalee et al. 2018). Moreover, the taste, flavor and overall acceptability scores of crackers significantly increased as the elevation in the riceberry flour incorporation. Similar results were also detected in a study of gluten-free biscuits from various ratios of brown rice flour. The previous study of Mancebo et al. (Mancebo, Rodriguez et al. 2016) reported that the cookies which prepared from the mixing of rice flour were showed desirable overall acceptance. The flavor could be defined as the impression perceived through the chemical senses in the mouth from food product (Caul 1957). Term of flavor is including the aromatic (olfactory perceptions which related to the volatile compounds from the product released into the mouth), taste (salty, bitter, sour and sweet) and chemical feeling factors (umami taste, bite, cooling and heat).

Borriboon et al. (Borriboon, Lontom et al. 2018) stated that when riceberry rice is cooked, it could release a distinctive natural aroma that might affect to the taste and flavor attributes. Normally, the odor of rice is detected by the volatile compounds entering the nasal cavity (Champagne 2008). Yang et al. (Yang, Lee et al. 2007) studied the volatile aroma compounds in black rice, black rice had unique of odor-active compounds. In terms of black rice flavor chemistry, the thirty-five volatile compounds were found in black rice which composed of 10 aldehyde, 10 aromatic, 6 alcohol, 4 nitrogen-containing, 3 ketone and 2 terpenoid compounds. Yang et al. (Yang, Lee et al. 2007) reported that the aldehydes were noticed as the crucial contributors to the unique aroma of black rice. In the previous study of, More et al. (More, An et al. 2013) investigated the effect of white rice and brown rice flour for biscuits production. Three biscuit samples were prepared with 100:50, 100:100 and 50:100 proportions of brown rice and white rice flour and determine the chemical and sensory characteristics. The findings shown that biscuits with 100:50 proportion of brown rice and white rice flour had the highest preference scores of taste, flavor and overall acceptability. Moreover, the overall acceptability score of gluten free biscuits were in the range of 7.3 to 8.0. In term of foods acceptance index (AI), the AI of crackers was increased as the levels of riceberry flour increased. AI is the consideration of foods when adding the new ingredients which can reflect the preference liking or dislike score of the product. These may predict the acceptability in the food product development process. Nevertheless, in food industries, only the acceptance is not enough parameter to analyze for launching the product. The other parameters including flavor, taste, appearance, food stability, nutritional composition and price also considered as important (Granato, Masson et al. 2012). Since the rising score of taste, flavor, overall acceptability and acceptance index in the riceberry enriched crackers, it might be indicated that the use of riceberry flour has enhanced the satisfying of the product. However, it should be stated that the crucial effect on

the quality of the final product might come from the combination between the ingredients and baking process (Sudha, Vetrmani et al. 2007). According to the obtained results that the overall acceptability scores significantly increased along with the higher ratios of riceberry flour consolidated in crackers. Consequently, a partial substitution of wheat flour with 20% riceberry flour in the cracker is well satisfied by the panelist.



2. To investigate the effect of riceberry cracker supplementation with unripe papaya flour on physicochemical, functional properties, and sensory evaluation

Since the 20%RBF cracker was selected among the five formulations of cracker for the further study as the cracker with 20%RBF substitution had the highest score of overall acceptability, antioxidant activities and the lowest glycemic index value. In the further study, researcher, aimed to use unripe papaya which is a waste product from agro-industry as the material for the production of riceberry crackers.

2.1 Proximate composition of unripe papaya flour

The proximate composition also plays a crucial information for the development of new bakery products (Varastegani, Zzaman et al. 2015). Proximate composition of unripe papaya flour was analyzed in this study including protein, total fat, carbohydrate, total dietary fiber, moisture and ash. The low amount of protein was found in unripe papaya flour of the present study. Oloyede (Oloyede 2005) reported that the unripe papaya present not a good source of protein as the low amount of protein was detected. The low fat content of unripe papaya flour was observed which agreed with the other studies (Oloyede 2005, Okon, Ogri et al. 2017). Moreover, unripe papaya flour also had the considerable content of total dietary fiber (56.14g/100g) and showed higher than the previous study (De Moraes Crizel, Hermes et al. 2016). Fruit and vegetable by-products are considered as the noticeable source of dietary fiber (O'Shea, Arendt et al. 2012). The moisture content of UPF was lower than the previous study of Okon et al. (Okon, Ogri et al. 2017). However, our findings are consistent with Oloyede (Oloyede 2005). It might depend on the difference in the initial amount of moisture and the drying temperature and time in the flour production process of each unripe papaya fruit (De Moraes Crizel, Hermes et al. 2016). The percentage of moisture content of unripe papaya flour in this study presented in very low, indicated that unripe papaya flour could be kept

for a period of time which it might not lead to microbial attack and spoilage (Oloyede 2005). The ash content of unripe papaya flour was 5.22 g/100g dry weight which is in good agreement with Okon et al. (Okon, Ogri et al. 2017). The low percentage of ash related to the content of total mineral that present the fruit (Oloyede 2005). However, the important minerals in unripe papaya including potassium, magnesium, calcium and phosphorous (Okon, Ogri et al. 2017). Consequently, the use of unripe papaya flour as the ingredients for food production might show a good nutritional and functional properties.

2.2 Phytochemical contents and antioxidant properties of unripe papaya flour

The phytochemical compounds and antioxidant properties of unripe papaya flour also was evaluated. The total phenolic content of unripe papaya flour was lower than the riceberry flour but showed higher than wheat flour. For the antioxidant capacity, unripe papaya flour also showed had a lower FRAP, TEAC and DPPH values than riceberry flour but showed higher than wheat flour. Phenolic compounds known as the natural antioxidants which widely distributed in plants (Li, Smith et al. 2006). The results indicated that the presence of phenolic compound and β -carotene contents in unripe papaya flour might contribute to an ability to scavenge the free radicals. However, the antioxidant properties that presented in the unripe papaya flour not only due to the phenolic compounds itself, but also with the various other types of active compounds (Khamsah, Akowah et al. 2006). Oboh et al. (Oboh, Ademosun et al. 2015) reported that the polyphenolic phytoconstituents present in the papaya including *p*-Coumaric acid, procyanidin, epicatechin and quercetin. Similar observation is found by Maisarah et al. (Maisarah, Nurul Amira et al. 2013), who reported that the unripe papaya contained the biologically polyphenols and also demonstrated the antioxidant effects. Phenolic antioxidant compounds in unripe papaya flour considered as the competent

hydrogen-donating antioxidants which can interact with free radical and break the radical generation system (Valentão, Fernandes et al. 2003). They also had ability to chelate the metal ions and donate a hydrogen atom to DPPH radical that involve in the production of free radical (Yang, Landau et al. 2001). Since β -carotene known as found in yellow and orange vegetables and fruits and also has high provitamin A activity. Furthermore, β -carotene contents in unripe papaya flour exhibit the property to inactivate the singlet oxygen (1O_2) which is the non-radical molecule that has high sensitivity for the oxidation reaction (Krinsky and Johnson 2005). In the previous study of Liebler et al. stated that β -carotene demonstrated the ability of antioxidant against AAPH-induced lipid peroxidation (Liebler, Stratton et al. 1997). However, the crucial factors that may impact on the levels of β -carotene content in fruits including variety cultivar, ripen stage, weather conditions season of year and geographical area (Setiawan, Sulaeman et al. 2001).

2.3 Scanning Electron Microscope (SEM) examination of unripe papaya flour

The morphology of unripe papaya flour showed a gnarled surface and irregular shape. The starch granules of flour structure might affect from the preparation process of the raw material and milling method which directly depend on the intensity of mechanical work (De la Hera, Martinez et al. 2013, Sakhare, Inamdar et al. 2014). Previous study of Yu et al. (Yu, Chen et al. 2018) the effects of different milling methods on properties of buckwheat flour. Three different milling methods were used to prepare the buckwheat flour including high-speed universal grinder, wet-milling and stone mill. The results showed that the morphology of buckwheat flour with wet-milling method displayed more intact granular and had a low content of damaged starch when compared to high-speed universal grinder and stone mill methods. Moreover, the conditions of milling such as milling intensity and milling force also had the influence to the morphology of flour. The morphology of

starch granules with differences in the chemical compositions and botanical species of flour could display the difference in the starch morphology. Some granule exhibited the mixture of polyhedral, spherical and irregular shapes. On the other hand, some granule appeared more aggregation of the protein matrix than single starch granule (Sakhare, Inamdar et al. 2014).

2.4 Physical characteristics of riceberry crackers

In the current study, researcher, aimed to use the unripe papaya flour as partially substituted wheat flour in 20%RBF cracker. The four ratios were employed to replace wheat flour with unripe papaya flour including 0%, 3%, 5% and 7% replacement. For the physical characteristics of riceberry crackers, the incorporation of unripe papaya flour did not affect to the weight, height and diameter of crackers. However, the increasing content of the unripe papaya flour incorporated into the riceberry cracker slightly decreased moisture content and water activity. Since water considers as the important component in foods that provides the information for predicting the potentiality of microorganisms to the spoilage and infection. Nasir et al. (Nasir, Butt et al. 2003) stated that moisture content of flour is crucial element regard to its shelf life. The lower moisture containing in flour exhibited the better its storage stability which might affect to the lower deterioration of bakery product quality (Nasir, Butt et al. 2003). The texture of food products can be noticed as the essential constituent of customer for consideration of buying the diets. Moreover, the analysis of texture profile is an important way for determining the acceptance of products as this can predict the intense force needed to break through crackers (Klunklin and Savage 2018). In this study, the substitution of wheat flour with unripe papaya flour in 20%RBF crackers obviously changed the texture of crackers. The findings showed that the hardness value significantly decreased since high levels of unripe papaya flour were incorporated into 20%RBF crackers. On the other hand, the

fracturability value significantly increased as increasing of unripe papaya flour. Nguyen et al. (Nguyen, Le et al. 2013) stated that the hardness and fracturability showed the correlate; more crispiness or fracturability resulted in the lower harness of food samples. It might be due to the fact that higher fracturability related with a high air porous conformation inside the crackers. Similar results also found in the previous done by Norhidayah et al. (Norhidayah, Noorlaila et al. 2014), the study findings indicated that unripe banana flour incorporated cookie had decreased hardness with the increasing of the unripe banana flour level. Another previous study conducted by Singh et al. (Singh, Riar et al. 2008) be observed that the hardness of biscuit fortification with sweet potato flour decreased with increasing the content of sweet potato flour. In addition, the similar observation was found in the study of Bhatawale et al. (Bhatawale, Mohammad et al. 2012), the hardness of rice papad decreased as the substitution of banana flour concentration is increased. The reason of explanation might be due to the increasing of unripe papaya proportion in the riceberry cracker formulation. Since the unripe papaya flour contains high content of dietary fiber which contributed to increase water holding property of dietary fiber. The riceberry cracker dough might hold high content of water during dough fermentation. The beginning of the heat transfer start during the baking process, resulted water in cracker dough starts moving and causing pore formation. As the removal of water, resulting the free space within crackers which are affecting to decrease hardness and moisture content of riceberry crackers incorporated with the unripe papaya flour. Porosity is considered as the free space within a material which may define food material as porous or nonporous structure. In addition, Crizel et al. (De Moraes Crizel, Hermes et al. 2016) stated that the papaya powder byproducts also had high values of water holding capacity compared to the others. The water holding capacity might associated with the dietary fiber content in papaya powder. Since dietary fiber clearly conduce to the hydration property. These contributed to

the hydroxyl groups in structure of fiber which allow more water interaction through hydrogen bond (Rosell, Santos et al. 2010). Consequently, unripe papaya flour had ability to absorb the water during the preparation of crackers. The supplementation of ingredient with high water holding capacity could improve characteristics of food such as changing the texture and viscosity of the final product (Grigelmo-Miguel and Martin-Belloso 1999). Since water plays an important function to generate the formation of gluten as the mixing between water and wheat flour. Finally, the hydrated protein begins to interact together to become the stabilizing gluten network which provides ability for retention of gas and give the airy texture of final products (Shewry, Halford et al. 2002). Farkas (Farkas 1994) stated that the formation of air cells inside cracker coordinated with the higher porous conformation, resulting the lower of hardness of cracker.

2.5 Color characteristics of riceberry crackers

Since the food appearance plays an important role for the consumer. Color of food considers as the first parameter which was evaluated by consumers and it is also essential in term of product acceptability (Tanner 2016). The color surface of riceberry cracker was affected by the incorporation of unripe papaya flour. The lightness (L^*), redness (a^*) and yellowness (b^*) values were significantly increased with the increasing of unripe papaya flour levels. These might relate to the available color pigment in unripe papaya flour. Furthermore, the results of total color difference index (ΔE) exhibited that the ΔE values increased in the riceberry cracker with high level of unripe papaya flour incorporation. Hence, riceberry cracker incorporated with unripe papaya flour with high ΔE values showed the higher difference in total color difference index when compared to riceberry cracker without unripe papaya flour incorporation. Carotenes are one of the carotenoid food colorant pigments which give the yellow-orange-red pigments and mostly found in plants. β -carotene which

presented in the unripe papaya flour are predominant of carotene pigments (Solymosi, Latruffe et al. 2015). Moreover, the applications of β -carotene in point of food-coloring agent such as margarine (Del Campo, García-González et al. 2007). In addition, during the unripe papaya preparation, endogenous enzyme might have an effect on the fruit quality. Polyphenol oxidase (PPO) is one of the endogenous enzymes that catalyzes the oxidation reaction of phenolic compounds. PPO enzyme is responsible for the development of brown pigments which could affect to the color and appearance of the fruit (Cano, Lobo et al. 1996). In the study of Falguera et al. (Falguera, Sánchez-Riaño et al. 2012) reported that PPO enzyme in papaya fruit could induce the browning color production during fruit processing. However, the PPO enzyme inhibition might be achieved by thermal treatment at 60 to 115 °C. Consequently, the color of unripe papaya flour might be affected by the PPO enzyme during the flour preparation, then resulting in the change of color parameter of riceberry cracker product. The results from previous study of Varastegani et al. (Varastegani, Zzaman et al. 2015) showed that the using of the high level of papaya flour fortification into cookies affected to increase the yellowness and lightness of cookies.

2.6 Phytochemical contents and antioxidant properties of riceberry crackers

Four formulations of riceberry crackers also determined for the phytochemical compounds and antioxidant activities. Since the increasing of unripe papaya flour levels incorporated into 20%RBF crackers, the results of antioxidant compounds and activities showed significantly increased. These might be demonstrated that after the baking process, riceberry crackers had a desirability of phytochemical compounds which provided the antioxidant properties. The reason of explanation is the cooperation of the active compounds which presented in riceberry flour and unripe papaya flour including phenolic content, anthocyanins content and

β -carotene content. Hence, the effectiveness of antioxidant ability is associated with the presence of antioxidant compounds in riceberry flour and unripe papaya flour which had the capacity to break the free radical chain reaction by devoting hydrogen atom (Sharma and Gujral 2011). Nevertheless, upon the baking process, the active compounds and antioxidant capacity in riceberry crackers decreased in comparison to the riceberry flour and unripe papaya flour. In the study of Varastegani et al. (Varastegani, Zzaman et al. 2015) reported that cookies incorporated with papaya pulp flour showed increased in the content of polyphenols and antioxidant activity. Also, Klunklin and Savage (Klunklin and Savage 2018) indicated that the substitution of wheat flour with purple rice flour into biscuit could increase the antioxidant compounds and antioxidant activity. It could be stated that the riceberry cracker incorporated with the unripe papaya flour possesses the antioxidant activity as the cooperation of the bioactive compounds in the composite flour. These might potential for application in term of the nutritious bakery products.

2.7 Determination of total starch, hydrolysis index and predicted glycemic index of riceberry crackers

For the *in vitro* digestion of riceberry crackers, the incorporation of unripe papaya flour into 20%RBF crackers directly affected to the starch digestion. The riceberry cracker with high amount of unripe papaya flour incorporation showed the lower content of total starch, hydrolysis index and predicted glycemic index comparing to riceberry cracker without unripe papaya flour incorporation. These might be stated that the unripe papaya flour incorporation could delay the glucose release of riceberry crackers. However, the riceberry cracker with unripe papaya incorporation had higher content of undigestible starch when compared to the riceberry cracker without unripe papaya incorporation. The presence of undigestible carbohydrates such as dietary fiber might responsible for the slow digestion (Agama-

Acevedo, Islas-Hernández et al. 2012). In the current study, unripe papaya flour contained a high amount of dietary fiber that demonstrated to decrease the proportion of wheat starch in the substitution. Papaya flour was reported to have effectiveness in water holding capacity which associated with the fiber content properties which could affect to the metabolic activity of dietary fiber in the gastrointestinal tract and also change the viscosity of the product (López-Marcos, Bailina et al. 2015). The dietary fiber had ability on the viscosity of foods by trapping the food complex within the matrix and hindering the food molecules to access the digestive enzymes. Consequently, the anti-motility of the food complex might influence to delay the digestion and absorption of glucose (Blackburn, Redfern et al. 1984). Previous study of Abd El-Hay et al. (El-Hay, El-Mehiry et al. 2018) studied the effect of incorporation of different levels of papaya (*Carica papaya* L.) on the glycemic index of functional cupcake. The results showed that the incorporation of high levels of papaya exhibited the positive effect in terms of lowering the glycemic index. These might be due to the high dietary fiber content in papaya which play a role as a hurdle during the digestion process. The change of glycemic index was affected by the dietary fiber which could increase viscosity process (El-Hay, El-Mehiry et al. 2018). It could be stated that the combination of unripe papaya flour and riceberry flour that work together in the digestion process and also the dilution of wheat flour concentration might show the potential attenuation of the digestion of riceberry crackers. Similar results of the low value of glycemic index were found in cookies incorporated with the unripe banana flour due to the presence of indigestible carbohydrates (Agama-Acevedo, Islas-Hernández et al. 2012).

2.8 Scanning Electron Microscope (SEM) examination of riceberry crackers

In the current study, the microstructure properties of riceberry crackers between the surface and cross-sectional structure were determined. The riceberry

crackers microstructure was obviously affected by the high level of unripe papaya flour incorporation. The surface of riceberry cracker incorporated with 7%UPF exhibited more rougher, it might be due to the morphology of unripe papaya flour that presented the irregular particles together with the bumpy shape of riceberry flour. Surprisingly, the fortification of unripe papaya flour could improve the internal structure of riceberry crackers. The large porous structure of riceberry crackers with high level of unripe papaya flour incorporation was found. Eventually, the riceberry cracker exhibited the airy structure, then resulted to decrease hardness of crackers. These correlated with the findings in the current study of texture characteristics of riceberry crackers incorporated unripe papaya flour. Since the unripe papaya flour had the competency to absorb more water during cracker dough preparation. Consequently, the formation of gluten matrix was generated as the sufficient hydration of gluten, then developed larger air cells surrounding structures (Shewry, Halford et al. 2002). Faridi reported that the insufficient hydration of gluten in the development of gluten network might exhibit the compactly packed of the structure in the cracker production.

2.9 Sensory evaluation

The hedonic assessment was used to evaluate the riceberry cracker since the unripe papaya flour was incorporated. Hedonic test considered as the ideal and economical method to search for the influence of various factors such as ingredients or manufacturing. The appearance, color, flavor, fracturability and hardness had no different among the riceberry cracker. Although the score of fracturability and hardness showed no different as the limitation in the human sense, those scores were consistent with the texture characteristics of riceberry crackers. Fracturability scores increased as the unripe papaya flour substitution increased. The crispness or fractuability is desirable texture attributes which frequently relate to the freshness of

manufactured products (Tunick, Onwulata et al. 2013). Paranginangin et al. reported that the increasing in crispness score was found in fish crackers as the presence of a lower hardness value which the consumer exhibited more preferable in the product. The riceberry cracker with high level of unripe papaya flour incorporation showed significantly decreased in the taste and overall acceptability scores. Nevertheless, both of taste and overall acceptability scores were in the moderate acceptable (7.02-7.50). These scores are consistent with previous findings regarding the like moderately score of rice crackers with apple pomace flour which scores were in the range of 7.25-7.57. The decreasing of the preference score of taste might be affected by the bitter taste as its correlated to the polyphenols content in the ingredients (Bechoff, Cissé et al. 2014). Jaegar et al. (Jaeger, Axten et al. 2009) stated that the polyphenol-rich foods were associated with bitter flavor. The results of sensory scores together with the physical characteristics pointed out that riceberry cracker with 5% unripe papaya flour incorporation had desirable characteristics and also well accepted by consumers.

CHAPTER VI

CONCLUSION

The objective of the research was to improve the quality of wheat flour cracker product. The current findings indicated that the incorporation of riceberry flour into wheat flour-based crackers significantly increased hardness and redness together with a decrease in fracturability, lightness and yellowness of the cracker. In addition, cracker incorporated with riceberry flour significantly increased phytochemical components resulting in increasing antioxidant abilities with a low predicted glycemic index as delay the starch digestibility. Furthermore, the fortification of riceberry flour at 20% not only improved the flavor but also increased the overall acceptance of the cracker. Another objective of the research was to add the value of unripe papaya flour by using as an active ingredient to partial of wheat flour substitution in riceberry crackers. The results showed that the unripe papaya flour at 5% incorporation could be an acceptable ingredient for partial of wheat flour substitution to produce the riceberry crackers with a low starch digestion, low predicted glycemic index, high overall acceptability score and bioactive compound and also provide effectiveness antioxidant activity with desirable color and texture. Therefore, the combination between riceberry and unripe papaya flour could be recommended as the value-added natural ingredients for developing novel crackers with high nutritional value.

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