HEXANE DEFATTED BOMBAY LOCUST POWDER: CHARACTERISTICS AND ITS APPLICATION AS PROTEIN SUPPLEMENT IN BAKED PRODUCTS

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Thesis	Hexane Defatted Bombay Locust Powder: Characteristics and Its Application as Protein Supplement in Baked Products
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ABSTRACT

Impacts of hexane defatting on physicochemical, oxidative stability and total volatile compounds of Bombay locust (*Patanga succincta* L.) powder 'BL' were studied. Defatted insect powder (F/BL) showed marked decrease in fat content (1.2%, in wet basis) with higher protein content (78.7%, in wet basis) significantly ($p \leq 0.05$), compared with those of BL (29.8% fat and 56.5% protein). Based on electrophoresis result, hexane defatting had no effect on peptide distribution of insect powders. F/BL had lower peroxide value with greater oxidative stability ($p \leq 0.05$), while less variety of volatiles with more off-flavor compounds, particularly lipid oxidation and insect hormone odors, were observed from BL. F/BL was confirmed as an excellent source of amino acids and minerals. F/BL fortified biscuit stick determined physicochemical and sensory properties of the final products. F/BL fortified biscuit stick showed a better sensory acceptance, compared with those from BL at the same level used. Based on sensory characteristics, biscuit stick with 10% F/BL (F/BL-10) was selected and studied on its chemical composition and microstructure. F/BL-10 possessed more porous structure with higher protein and lower energy value, compared with those from 10% BL. The results suggested that hexane defatting could effectively be used for preparing Bombay locusts powder, which could potentially use as an alternative protein source with excellent applicability to the baked product, particularly biscuit stick.

Protein-enriched brown rice flours (BRF) were developed by fortification of F/BL at different levels (10, 20, 30%). The physicochemical characteristics of the mixed flours were then investigated. The application of resulting mixed flour on preparing protein-enriched cake was also studied. The chemical composition, water activity, color as well as rheological property of the resulting mixed flours were varied at different levels of F/BL added. The protein content of resulting mixed flours was effectively improved, especially at 30% replacement provided almost 4-fold increased (11 – 42%), compared with control (BRF without F/BL fortification) ($p \leq 0.05$). Protein-enriched cakes prepared using the mixed flours, showed the differences in their physicochemical properties, texture profiles, chemical composition and sensory characteristics, which were influenced by F/BL fortification level. The 20% replacement of brown rice flour by F/BL (20-MF) provided a protein-enriched cake (20.8% of protein) with liking score ranged from 7.0 – 7.4 for all attributes tested, indicating the good acceptability. Scanning electron microscopy (SEM) of the 20-MF cake revealed that it had a denser structure with the less porosity than the control. Overall, F/BL could be a promising and an effective alternative protein source for developing protein-enriched flour and baked products.

Keywords: *Patanga succincta* L., hexane defatting, insect powder, fortification, baked product, protein-enriched food.

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CHAPTER 1

INTRODUCTION

Recently, protein content is a leading concern of food product development, where the promote of non-animal-based protein sources has brought about enthusiasm for plant- and insect-based protein (Zielińska, Karaś, & Baraniak, 2018). Alternative proteins are becoming increasingly important due to their sustainability for feeding significant numbers of the world population (Elhassan, Wendin, Olsson, & Langton, 2019; Tieland, Borgonjen-Van den Berg, Van Loon, & de Groot, 2015). Thus, edible insects have been discovered worldwide, as a nutritious food source for regular diets and emerge as a solution limited for protein production (Chen, Feng, & Chen, 2009; Megido et al., 2014; Van Huis et al., 2013). They are not only referred to as a good source of protein but also fat, minerals, calories and micronutrients (Kim, Weaver, & Choi, 2017; Köhler, Kariuki, Lambert, & Biesalski, 2019; Yhoung-aree, 2010). Generally, they contain high nutrients contents, especially protein (15 – 82% dry basis) and fat (4 – 77% dry basis) (Kim et al., 2017; Köhler et al., 2019; Kouřimská & Adámková, 2016; Paul et al., 2017). However, insect-based food product consumption is generally not accepted for human consumption in many countries, particularly in the Western world. In addition, it is affected by the unique sensory characteristics including taste, flavor, texture and appearance of insect. Thus, masked, in the form of powder, could allow insect to be introduced as food, where the insect powder or flour might have greater acceptability as an ingredient in human food (Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016). Insect powder is an interesting and effective way to tackle those psychological and palatability factors, such as disgusting and food fear of the unusual for eating insects (Attila, Ryan, Dalma, & Howard, 2017; Dobermann, Swift, & Field, 2017). Bombay locust (*Patanga succincta* L.), or grasshopper, is a common edible insect in Southeast Asia (Hanboonsong, Jamjanya, & Durst, 2013; Yen, 2015). It contains high protein content (36.3%) and both essential and non-essential amino acids (Köhler et al., 2019), in which its fat content ranges from 4.7 – 14.8%, depending on its processing (Yhoung-aree, 2010). This significant amount of lipid content governed use and characteristics of insect proteins (L'Hocine, Boye, & Arcand, 2006; Lee et al., 2016). Furthermore, it also determined the oxidation stability, as well as sensory characteristics of insect-based food ingredients, particularly insect powder (Ahmed et al., 2016; Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012). Defatting by organic solvents is simply applied, not only for removing lipid, but also for concentrating protein content and improving lipid oxidation stability (Bußler et al., 2016). Moreover, some pigments, off odor compounds, and pheromones could be co-extracted during defatting (Cheng, Huang, Li, Zhou, & Cen, 2014; Dossey, Tatum, & McGill, 2016; Mishyna, Chen, & Benjamin, 2020; Purschke, Stegmann, Schreiner, & Jäger, 2016; Ribeiro et al., 2019; Wang et al., 2019). Although many alternatives lipid extraction have been used (Talbot, 2011), hexane defatting is still common with more than 96% oil removal on average (Ricochon & Muniglia, 2010; Yousefi & Hosseini, 2017). Nevertheless, there is no information on the impact of hexane defatting on the characteristics of Bombay locust (*P. succincta* L.) powder and its potential to alleviate future food challenges as an alternative protein source.

The protein enrichment level could be then maximized by adding larger proportions of insect powder, with less offensive properties, to the final products. Several edible insect powders have been developed as alternative protein source, marketed as insect flour or insect protein-enriched wheat flour (Azzollini, Derossi, Fogliano, Lakemond, & Severini, 2018; Campbell, Euston, & Ahmed, 2016; Terry, Lupul, & Coate, 2017). Several protein sources, such as plant based (González-Montemayor, Flores-Gallegos, Contreras-Esquivel, Solanilla-Duque, & Rodríguez-Herrera, 2019; Mesías, Holgado, Márquez-Ruiz, & Morales, 2016) and edible insects (Akande, Jolayemi, Adelugba, & Akande, 2020; de Oliveira, da Silva Lucas, Cadaval, & Mellado, 2017; Delicato, Schouteten, Dewettinck, Gellynck, & Tzompa-Sosa, 2020), have been added into wheat flour for protein enrichment as well as nutritional quality improvement of its relevant baked products. Those protein sources are subjected to complement the lacking food components and booster their health benefits in BP (Beelen et al., 2018; Martins, Pinho, & Ferreira, 2017; Stelten et al., 2015). The gluten-free BP has been addresed presently to provide an alternative product for people with gluten intolerance and improve final product quality, without changing their dietary pattern. The wheat was replaced with many other resources, such as corn, potato and rice flour. Protein from other sources was then fortified to obtain the nutritional requirement of product, such as soy protein isolate and insect powder (Gularte, Gómez, & Rosell, 2012). As alternative source of flour, rice (*Oryza sativa* L.) flour flour is a known as gluten-free cereals. Brown rice or unpolished rice is rich in phytochemicals (Mohan et al., 2014). Brown rice flour (BRF) contains a higher fiber (1.2% (w/w) dry basis) but lower protein content (8.5 – 9.5% (w/w) dry basis), compared with wheat flour (12.6% protein, 0.85% fiber, (w/w) in dry basis) (Khoshgozaran-Abras, Azizi, Bagheripoor-Fallah, & Khodamoradi, 2012; Mohan et al., 2014). Improvement of protein content and quality of BRF food products, especially baked products had been reported by fortification of protein from both vegetable and animal sources, which noticeably impacted on characteristics and quality of the final product, such as color, texture, batter rheology and sensory properties (de la Hera, Martinez, Oliete, & Gómez, 2013; de Oliveira et al., 2017; Gadallah, 2017; Islam, Taneya, Shams-Ud-Din, Syduzzaman, & Hoque, 2012). The level of protein enrichment, especially in the form of both mixed flours, wheat and BRF, should be optimized to obtain good nutritional value and satisfactory baked product quality. Therefore, it is congenial to utilize Bombay locust powder for protein enrichment of BRF, in which the nutritional value, especially protein content, was improved in a practical application in baked products. Therefore, the main objective of the present study was to study the impact of hexane defatting on physicochemical characteristics, oxidative stability as well as total volatile content of Bombay locust powders. Moreover, amino acid and mineral compositions were also evaluated, where its application on fortification in baked product as biscuit stick was then investigated in both sensory and nutritional characteristics. In addition, Bombay locust powder with a good oxidative stability was selected to develop protein-enriched brown rice flour and cake. The characteristics and pasting properties of Bombay locust powder mixed flour (MFs) were then measured. The application of MFs in cake model was studied on both cake batter and cake product forms.

1.1. Objectives

The objectives of this research were:

1. To evaluate the impact of hexane defatting on physicochemical characteristics and oxidative stability of Bombay locust powders.
2. To develop and evaluate the characteristics of protein-enriched biscuit stick fortified by Bombay locust powders.
3. To develop and characterize the protein-enriched brown rice flour and cake fortified by defatted Bombay locust powder.

1.2. Scope of research

This research focused on the utilization of Bombay locust (*Patanga succincta* L.) as an alternative protein supplement for human consumption, particularly in baked products, where conventional hexane defatting was conducted to improved the characteristics, nutritional value and food applicable of defatted insect powder (F/BL). Yield, protein recovery and physicochemical characteristics, total volatile compounds as well as oxidative stability of F/BL, were then investigated comparatively with that of Bombay locust powder without defatting (BL). F/BL showed the better oxidative stability with less off-odors, was then observed on its amino acid and mineral composition. To reconfirm the impact of hexane defatting on improving the applicable of resulting insect as alternative protein source in food products, F/BL was consequently applied for preparing protein-enriched biscuit sticks at different levels of fortification (5, 10 and 15%, w/w of mixed flour), in which F/BL fortified biscuit sticks were subjected to analyse in comparison with those from BL. F/BL fortified biscuit sticks were characterized on their physicochemical and sensory properties. The 10% F/BL fortified biscuit sticks revealed a good sensory acceptance, which was then characterized on its chemical composition and energy value as well as microstructure.

For better understanding and getting indept detail on the role of F/BL in baked product, F/BL was incorporated with brown rice flour (BRF) at different levels (0, 10, 20 and 30%, w/w of mixed flour), where the resulting mixed flours (MFs) were then characterized and used for preparing insect brown rice cake. Chemical composition, water activity, color and pasting properties of MFs were evaluated in comparison with those of BRF. The rheological properties of cake batter prepared using F/BL mixed flours were investigated, while quality characteristics of the relavant cakes were also determined. Physicochemical properties, cake quality, texture profiles and energy value of cakes were measured. In addition, the sensory properties of the resulting cakes were evaluated through 9-hedonic scale, in which F/BL fortified cake obtained a good acceptability was selected and subject to study its microstructure in comparison with that of control (0% fortification level).

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CHAPTER 2

LITERATURE REVIEWS

2.1. Bombay locust

Bombay locust (*Patanga succincta* L.) is one of the most marketed insects in Thailand. It is categorized in family of Acrididae and commonly called as grasshoppers. Usually, it has larger size compares to common grasshoppers (Yen, 2015). The morphology of Bombay locust, as shown in Figure 2.1, it has green color with black spots in early instar, and during development stages it has varies color, such as orange-brown, pale brown and even green with/without black spots (Steedman, 1990). It is widespread in southwest and southeast Asia. Bombay locust seasonally occurs during August – October in Thailand (Hanboonsong et al., 2013). In India, it was the major locust pest during 18' to 19's, but it got extinct at the end of 1908. However, in other countries, it plays important role as local pest because of the deforestation for cultivation (Usmani & Usmani, 2018).

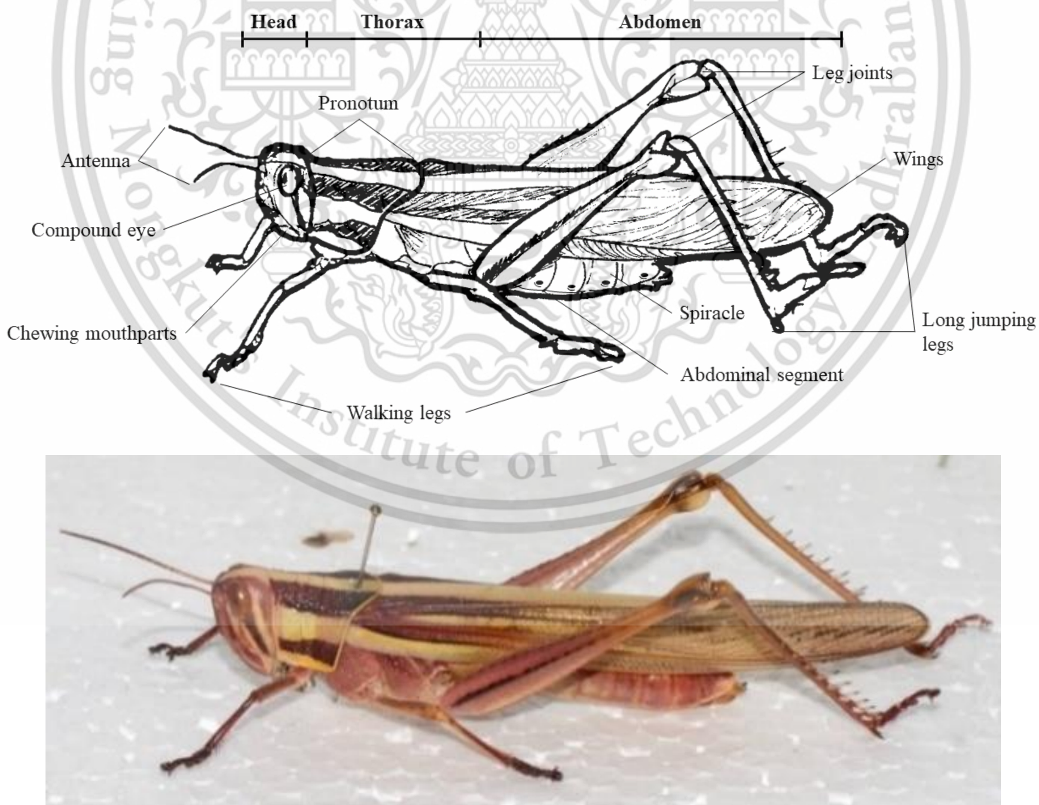


Figure 2.1 Morphology of Bombay locust (*Patanga succincta* Linn.)
Source: redrawn from ICAR (2013)

Bombay locust distributes in Saudi Arabia, Pakistan, India (throughout the plains), Sri Lanka, Myanmar, Bangladesh, China, Taiwan, Japan, Philippines, Vietnam, Indonesia, Malaysia, Thailand and Australia (Usmani & Usmani, 2018). The trend of Bombay locust as human food occurred in several Asian countries. In the 1970s, it was known as a destructive pest for maize in Thailand. Then, about 8 years later, there was a food intervention program to promote insects as an alternative food source. Nowadays, it becomes a popular food and is not considered as a pest anymore (Yen, 2015). In Thailand it is commercialized with price 220 – 250 THB per kg fresh for wholesale. Based on its high demand, it has been developed the way to increase its productivity. Entomologists from Thailand have built-up the sustainable insects farming system through technology utilization as well. It has been reported that the productivity was significantly increased, from 6,523 tons in 2006 up to 7,500 tons for the last five years (Hanboonsong et al., 2013). For the further study about *Patanga succincta* L., focusing on its fat and protein content, it has the highest protein content and the lowest fat content, compared to other protein sources as shown in Table 2.1 (Peters, 1988). Bombay locust showed the high protein content with both essential and non-essential amino acids, as shown in Table 2.2 (Yhoung-aree, 2010). Therefore, Bombay locust could be a great alternative choice to overcome future food challenge as an alternative protein source.

Table 2.1 Nutrition value of livestock compared to *Patanga succincta* L.

Animals	Protein (%)	Fat (%)
Cattle	15.8	24.3
Sheep	14.6	30.5
Pigs	13.0	33.3
Poultry	20.5	4.3
***Patanga succincta* L.**	**24.4**	**1.5**

Source: Peters (1988)

Table 2.2 Amino acid content in common species of edible insects.

Amino acids \ Edible insect		House crickets (*Acheta Testacea Walker*)	Scarab beetles (*Holotrichia* sp.)	**Bombay locusts (*Patanga succincta* L.)**	Bamboo caterpillars (*Omphisa fuscidentalis*)	Silkworm pupae (*Bombyx mori* L.)	Hornet grubs (*Vespa* sp.)
Essential amino acid (mg/gram protein)	Isoleucine	29.8	32.1	**32.7**	33.9	46.1	42.6
	Leucine	60.9	51.8	**59.5**	60.0	70.6	78.5
	Lysine	46.1	18.8	**35.7**	56.0	77.2	59.0
	Methionine & Cysteine	30.9	44.6	**20.9**	41.7	36.3	20.8
	Phenylalanine & Tyrosine	62.4	49.3	**60.0**	100.7	122.0	165.0
	Threonine	29.0	26.9	**22.3**	34.9	45.3	45.3
	Tryptophan	24.4	27.1	**17.3**	41.1	19.0	10.1
	Valine	34.4	29.3	**35.6**	38.8	52.2	53.7
Amino acid score (%)*		68.7	34.2	**55.8**	77.5	100.0	59.4
Non-essential amino acid (mg/gram protein)	Arginine	45.1	32.3	**36.0**	47.9	58.8	41.0
	Histidine	15.4	16.1	**13.5**	23.3	35.4	35.3
	Alanine	78.0	58.3	**92.7**	37.7	39.4	43.5
	Aspartic acid	69.2	61.2	**48.8**	88.2	88.9	79.6
	Glutamic acid	96.8	97.6	**76.4**	93.2	107.3	180.6
	Glycine	47.2	52.8	**48.8**	32.7	29.7	48.2
	Proline	45.2	47.0	**48.7**	40.7	44.4	56.8
	Serine	35.9	31.3	**23.9**	41.3	37.9	3.8

*Amino acid score is the lowest number for any of the essential amino acids in a protein.

Source: Yhoung-aree (2010)

2.2. Edible insect consumption

In many cultures insects are a normal part of human diet. It has been reported that around 2,000 species of insects are consumed around the world. However, there is only 1,462 species is edible. In fact, only a few of species that can be accepted by human because of its palatability, nutrition and accessibility (Lukiwati, 2010). Insects are eaten in several form, such as raw, cooked and added to other foods. Addressing entomophagy, or eating insects, is a potentially high nutritious source of protein, fat, vitamin, fiber and micro nutrients. The nutritional value of edible insects is varying because of the wide range of edible insects' species and the development stages of those. Barennes, Phimmasane, and Rajaonarivo (2015), showed that most of Lao people is frequently eat insects, about more than 90% of the total respondents. Insects have an optimal fatty acid ratio are house crickets, short-tailed crickets, Bombay locust and scarab beetles (Rumpold & Schluter, 2013).

Table 2.3 Average nutrient composition of edible insect orders (on a dry matter basis).

Insect orders	Nutrients				
	Protein (%)	Fat (%)	Fiber (%)	NFE* (%)	Ash (%)
Cockroaches (*Blattodea*)	57.30	29.90	5.31	4.53	2.94
Beetles (*Coleoptera*)	40.69	33.40	10.74	13.20	5.07
Flies (*Diptera*)	49.48	22.75	13.56	6.01	10.31
Beetles (*Hemiptera*)	48.33	30.26	12.40	6.08	5.03
Bees, wasps, ants (*Hymenoptera*)	46.47	25.09	5.71	20.25	3.51
Termites (*Isoptera*)	35.34	32.74	5.06	22.84	5.88
Caterpillars (*Lepidoptera*)	45.38	27.66	6.60	18.76	4.51
Dragonflies (*Odonata*)	55.23	19.83	11.79	4.63	8.53
Grasshoppers, locusts, crickets (*Orthoptera*)	61.32	13.41	9.55	12.98	3.85

*NFE: nitrogen-free-extract

Source: Rumpold and Schluter (2015)

The average nutrition value among insects is relatively high. Table 2.3 shows the average nutrient composition and energy contents of edible insect orders. Edible insects from Orthoptera have the highest protein content (Rumpold & Schluter, 2013). The point to be made is not that human should all start eating insects, just because other people consume them, but that they constitute an acceptably nourishing material. The food industry has made great progress in increasing the palatability of many nutrients (Peters, 1988). It had been reported on nutritive value of edible insects as good source of protein with 22.8 – 65.4% protein of dry weight (Xiaoming, Ying, & Zhiyong, 2010).

2.2.1. Edible insects and their safety

Regarding to the safety issue of entomophagy, is has been reported that there were allergies caused by some edible insects, such as crickets and grasshoppers. Nevertheless, food allergy caused by insect consumption is rarely happened despite edible insects have been consumed in many Asian, African and South American countries (Pener, 2016). Riberio, Cunha, Sousa-Pinto, and Fonseca (2018), reported that most of edible insect's allergens have cross-reactivity/co-sensitization with crustacean. In example: mealworms, crickets, grasshoppers and silkworms; had same cross-reactivity with shrimp and/or prawn allergens.

Phiriyangkul, Srinroch, Srisomsap, and Chokchaichamnankit (2015) found that allergens from Bombay locust had cross-reactivity to shrimp allergic serum. So, people with shrimp allergic would have tendency to insect allergic. SDS-PAGE assay was used for immunodetection of Ig-E binding proteins and LC-MS/MS analysis for proteins identification. Based on the result from 1D-immunobloting, there were three proteins as hexamerin (HEX) (~65kDa), enolase (~43kDa) and arginine kinase (AK) (~38kDa) were identified as allergen in raw *P. succincta*. On the other hand, four proteins, HEX (65 – 70 kDa), pyruvate kinase (52 – 55 kDa), enolase (40 – 43 kDa) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (30 – 34 kDa) were identified as allergen in *P. succincta*. Phiriyangkul et al. (2015) proven that thermal processing of Bombay locust reduced allergenicity. Linares, Hernandez, and Bartolome (2008) studied on allergy of protein extracts from three species of cricket (*G. campestris, G. bimaculatus* and *A. domesticus*). From *in vivo* prick skin test, the protein extract from three crickets showed the positive results.

In 2014, FAO with Wageningen UR organized program entitled "Insects to Feed the World". It initiated other continuous projects in order to improve insects' productivity and utilization in food sector. As the solving vehicle of food insecurity issue, regulations have been managed by government related to good manufacturing practices (GMP), Hazard Analysis and Critical Control Point (HACCP), labeling and many others. Insects contribute important role in food industrial upscaling nowadays. Greater demand of variety and quality of food by consumers, makes food industries have to remind about its ecological responsibility and sustainability (Morone, 2016).

2.2.2. Edible insects in food applications

Insects have high nutritional value, rich in protein and lipids, and can be used as nutritive supplement to human diets. In less acute food situations, various insects have been evaluated as delicacies and have even become articles of food commerce for centuries. Nowadays, it is being developed both scientific studies and food recipes (Mishyna et al., 2020). The main goal of insect utilization in food application is to enrich food product as well as improves its nutritional value, particularly protein, and improve its palatability (Figure 2.2).

(A)

(B)

Figure 2.2 Insects food-based products (A: cricket flour;
B: insect powder application in food products)
Source: Nutribug (2017)

The challenge of commercialize edible insects food-based products is providing high quality of product, which have good palatability. Studies of product development from insect powder have a good future prospect in food industry, as well as good production system is applied. The key point is to convince consumers about benefits of insects as food and food ingredients (Attila et al., 2017; Dobermann et al., 2017). Insect powder, made from *T. molitor*, had been enriched in extruded snack to improve its protein content. By addition of 10% of insect powder, protein content significantly increased from 10.3 g/100g up to 13.7 g/100g of snack (Azzollini et al., 2018). Roncolini et al. (2019) utilized the same insect on protein-enriched bread at 5 and 10% of fortification. The significant increase in protein content and essential amino acids (i.e. tyrosine, methionine, isoleucine, and leucine) were exhibited along with fortification levels. Moreover, organic cricket powder has been used as flour replacer for chocolate chip cookies. The replacement of 15.6% of wheat flour resulted in higher volume and tenderness of cookie, compared to the control. However, the increase of replacement ratio of cricket powder decreased overall acceptability in sensory evaluation (Terry et al., 2017). Osimani et al. (2018) developed bread enriched with cricket powder (*Acheta domesticus*). The addition of 10% cricket powder resulted in suitable dough for bread-making. It has been proven that edible insect powder could be used for protein enrichment, especially for leavened baked goods.

2.3. Lipid oxidation

Oxidation is the major cause of food deterioration. It becomes the most important thing to be concerned in food manufacturing. Controlling lipid oxidation has to be done to meet the acceptable oxidation stability and shelf life over certain period of storage (Frankel, 2014). Lipid oxidation results in rancidity and mainly caused by oxygen occurrence. Rancidity as the product of oxidation reduces acceptance of food product. It does not only produce rancidity but also reduce nutritional quality and safety of food products. Food with higher lipid content is riskier to oxidation. Thus, oxidation shorten food product shelf life (Kolakowska, 2010).

There are three major lipid oxidation pathways in foods: auto-oxidation, photo-oxidation and enzymatic-oxidation, as shown in Figure 2.3 (Hu & Jacobsen, 2016). Auto-oxidation is a free radical reaction consists of chain initiation, propagation and termination, that is primary pathway of lipid hydroperoxide formation. The presence of oxidation initiator, such as light, trace transition metals or free radicals, induces hydrogen atom losing of unsaturated fatty acid (LH), then forms alkyl radical ($L^{\bullet}$). Alkyl radical reacts with oxygen (O_2) and results in peroxide radical ($LOO^{\bullet}$). Then, $LOO^{\bullet}$ will react with other LH and forms hydroperoxide (LOOH), as the primary product of oxidation. This primary product is odorless and tasteless, so it has no significant effect on sensory quality. However, it can react with ferrous ion (Fe^{3+}) and generates alkoxyl radical ($LO^{\bullet}$) that leads the formation of secondary lipid oxidation products through β-scission mechanism, resulting aldehyde, ketones, organic acids, etc. generation. The secondary product of lipid oxidation affects sensory quality on food significantly (Hu & Jacobsen, 2016). Some compounds form flavor notes, called rancid.

The second pathway is photo-oxidation which occurs when there is light exposure to food containing lipid. Oxygen (O_2) becomes reactive singlet oxygen ($^1O_2$) and reacts with LH to generate LOOH. The $^1O_2$ is very reactive and reacts directly with high-electron-density double bound without activate LH. Then, it will go to the same way of auto oxidation. Oxidative rancidity occurs rapidly through the presence of light because it does not need an induction period (Hu & Jacobsen, 2016). The third pathway is enzymatic oxidation which form hydro-peroxide as intermediates. This pathway needs enzyme as catalyst, such as lipoxygenase. Lipoxygenase activity induces a single fatty acid hydro-peroxide synthesis from substrate of fatty acid (Ahmed et al., 2016). Lipid oxidation provides not only rancidity, but also off flavors, color degradation, nutrition value reduction, and may generate toxic compounds, that may danger consumer's health. In order to prolong the shelf life, lipid content, fatty acid composition of lipid used, the exposure to oxygen, light and enzyme as well as the use of antioxidants would be concerned to reduce and retard the lipid oxidation (Ahmed et al., 2016).

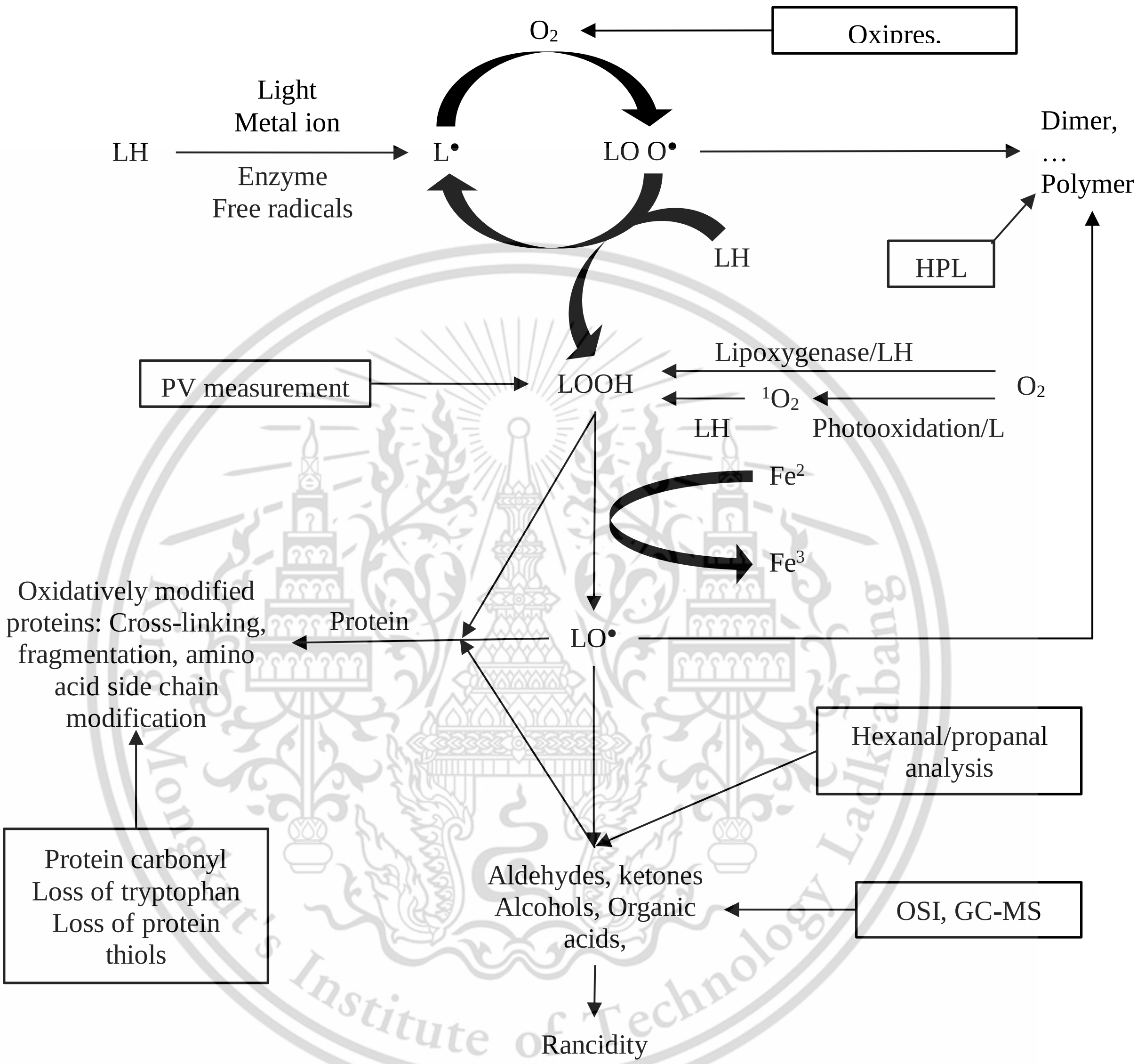


Figure 2.3 Three major reaction pathways responsible for lipid oxidation in foods
Source: Hu and Jacobsen (2016)

2.4. Retardation of lipid oxidation in food

2.4.1. Fat removal

Fat removal is the way to reduce, remove, or even change fat content, especially in foods. It becomes important issue nowadays because of the increasing number of fat-related diseases in worldwide, such as coronary heart disease (CHD) (Zock, 2006). Moreover, the higher fat content render lipid oxidation which affect food quality and shelf life (Hu &

Jacobsen, 2016). There are many fat removal techniques used in different food products, including mechanical force (Talbot, 2011), enzymatic reaction (Park, Bae, Park, Choi, & Suh, 2014) and solvent extraction (Halim, Gladman, Danquah, & Webley, 2011; Li, Naghdi, et al., 2014). Organic solvent has been widely used in food industry to extract lipid, such as isopropanol, methanol, dichloromethane and hexane. Takeungwongtrakul, Benjakul, Santoso, Trilaksani, and Nurilmala (2013) used different kind of organic solvent and its combination to extract carotenoid from hepatopancreas of pacific white shrimp. Extraction of carotenoid-containing lipid could be successfully performed using solvent mixture of hexane and isopropanol (50:50, v/v) with hepatopancreas/solvent ratio 1.0:4.5 (w/v) for three times. Carotenoid content of from six commercial high-yield corn hybrid were also extracted using hexane. Lutein and zeaxanthin showed the predominant carotenoids and present at 15-fold higher concentrations than *β*-cryptoxanthin and *β*-carotene (Kljak & Grbeša, 2015). Sandoval-Montemayor et al. (2012) extracted antimicrobial compound from Mexican lime (*C. aurantifolia*) using hexane and found 98 compounds. Lipid extraction was also used in crude biodiesel production from wet microalgae using *n*-hexane (Cheng et al., 2014). Hexane can be used for lipid extraction of food-grade sorghum. It yielded higher for ground form than whole kernel sorghum (Christiansen, Weller, Schlegel, & Dweikat, 2008). Hexane defatting has been widely applied for defatted powder production (Table 2.4). Therefore, it was safe to use hexane in food product as single and/or combined solvent for lipid extraction or fat removal.

Table 2.4 Hexane applications as solvent for preparing defatted powders.

Sample	% lipid removed (%)	Author(s)
Mealworm	85.3 – 97.9	Son, Lee, Hwang, Nho, and Kim (2019); Choi, Wong, and Auh (2017); Bußler et al. (2016)
Japanese beetle	91.0 – 93.6	Jang et al. (2018)
Cricket	94.6	Choi et al. (2017)
Silkworm pupae	98.9	Choi et al. (2017)
Black soldier fly larvae	56.0	Bußler et al. (2016)
Coffee ground	74.8 – 85.2	Efthymiopoulos et al. (2018)
Soy flour	97.2	Kang et al. (2017)

2.4.2. Antioxidant addition

Food products containing fats, oils and lipid, are easily get deteriorate during processing and/or storage. As mentioned before, lipid oxidation in food can be prevented by using food additives, namely antioxidant. Antioxidant is compound that has ability in ward off the negative effects from oxidation through electron donation. The electron will be donated to oxidants and make it more stable. The mechanisms of antioxidant are varying, depend on the class of it (Table 2.5) (Pokorny, Yanishlieva, & Gordon, 2001). There are a lot of food additives being used in food product, both natural and synthetic antioxidants.

Common natural antioxidant used in foods are vitamins (vitamin A, C, and E), carotenoids (β-carotene, lycopene, and astaxanthin), polyphenols (tea polyphenols and red wine polyphenols), and flavonoids (flavonoids, isoflavone, xanthones, and anthocyanins) (Li, Chen, et al., 2014). Synthetic antioxidants also widely used in food application for the same main goal. Popular synthetic antioxidants used are butylated hydroxy anisole (BHA), ter-butyl hydroxyquinone or t-butylhydroquinone (TBHQ), gallic acid, and butylated hydroxytoluene (BHT). In addition, not all of above are permitted in every country, it depends on the regulation. Regarding to its safety concern as food additives, there is limitation of utilization. The limitation is due to negative effects relates to its carcinogenicity (Thorata, Jagtapb, Joshib, Sutarb, & Kapdib, 2013). In example, BHA (E320) has 0-0.5 ADI mg/kg body weight and BHT (E321) has 0-0.3 ADI mg/kg body weight (Shahidi & Zhong, 2001).

Table 2.5 Mechanism of antioxidant activity.

Antioxidant class	Mechanism of antioxidant activity	Examples of antioxidants
Proper antioxidants	Inactivating lipid free radicals	Phenolic compounds
Hydroperoxide stabilizers	Preventing decomposition of hydroperoxides into free radicals	Phenolic compounds
Synergists	Promoting activity of proper antioxidants	Citric acid, ascorbic acid
Metal chelators	Binding heavy metals into inactive compounds	Phosphoric acid, Maillard compounds, citric acid
Singlet oxygen quenchers	Transforming singlet oxygen into triplet oxygen	Carotenes
Substances reducing hydroperoxides	Reducing hydroperoxides in a non-radical way	Proteins, amino acids

Source: Pokorny et al. (2001)

2.4.3. Packaging modification

Food packaging is subjected to maintain the quality and safety of foods from the time when it was packed to consumers. It gives protection from any physical, chemical, and biological hazards during storage and distribution. Other functions of packaging are providing convenience and as vehicle for marketing and communication, in order to give information regarding food products. Different kind of foods have different properties and product handling too. Packaging material is specifically used for specific form of product. It consists of polymers as constituents, such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyamide (PA), commonly named as plastic. It was widely used as packaging materials because of its availability, low cost, and mechanical characteristics (Cruz, Alves, Khmelinskii, & Vieira, 2018). On the other hand, packaging condition also play an important role on maintaining food quality. Different packaging of powder product has different shelf life. For example, milk powder can be packed in metal cans and multilayers pouches. A metal cans provide excellent barrier properties. Metal restricts exchange of moisture and O_2, so that it inhibits oxidation and deterioration (An, Lee, & Lee, 2018).

Gas-flushing method, by using nitrogen, is usually combined with various packaging material, to limit the presence of O_2. In United States, nonfat dry milk (NDM) and whey powder are packaged in no. 10 cans (157 × 178 mm with a capacity of 3,108 mL) in a reduced-O_2 atmosphere, can extend up to 54 months of shelf life. Other common practice on powder packaging is a laminated multilayer pouch. Food products in pouches have shorter shelf life compared to metal cans. Basically, this type of packaging consists of sandwich construction, with minimum two plastic layers. One on inside is low density polyethylene (LDPE), so that pouches can be sealed, and one on outside is biaxially oriented polypropylene (BOPP) or PET; in order to protect foods from mechanical hazards. Shelf life of powder packed in multilayers pouches up to 12 months (Tehrany & Sonneveld, 2009).

Modified atmosphere packaging (MAP) and controlled atmosphere storage (CAS) are common way to prolong food products' shelf life in low cost. It has been utilized widely for both fresh and processed food. MAP modifies at the initial gas composition and does not need further control. However, CAS has to maintain gas concentration during storage by constant addition or removal of gases (Kirtil & Oztop, 2016). MAP has been used for fresh food, such as meat, poultry, vegetables, and many other. Tørngren, Darré, Gunvig, and Bardenshtein (2018) reported MAP (by modify concentration of O_2 and CO_2) on meat product with 5.8 GHz microwave treatment, could extend shelf life up to ten days. The use of oxygen-free packaging (vacuum condition) or decreasing oxygen content (MAP replacement with nitrogen or CO_2) could extend meat shelf life during storage. It inhibited enzymatic reaction that correlated with product deterioration (He et al., 2018).

2.5. Baked products

Baked products entangle numerous reactions through different interaction between each ingredient during heat process, called baking. The term of baking is not only limited to the bread production but to all food products in which flour is the main material and to which heat is applied, through radiation of an oven or heating appliance. Bread, cake, pastry, biscuits, crackers, cookies and pies (Figure 2.4), are main part in baked products (BP) (Hui, Corke, De Leyn, Nip, & Cross, 2008).

Figure 2.4 Commercial baked products.
Source: Indiamart (2013)

Currently, BP, particularly bread, bakery and pastry, were one of the fastest growing products in food industry with a significant increase of annual demand. The healthy food trend has come along with BP growth in which consumers perceive many baked products as healthy nutrition supplies. The ‘low’ or ‘light’ of nutrition claim in the final product has attracted consumer that allows the guilt-free indulgence (Martínez-Monzó, García-Segovia, & Albors-Garrigos, 2013). Thus, BP innovation through adding and/or reducing some indredients, developing gluten- and allergen-free, have been addressed to tackle its future challenge. Salt, a key point in dough and batter development, has been controlled through its amount reduction, particularly in bread making (Silow, Axel, Zannini, & Arendt, 2016). Moreover, sugar reduction has been done in sweetened baked good production (including cakes, biscuits, coookies, buns, muffin, etc.) through artificial sweeteners replacement (Sahin, Zannini, Coffey, & Arendt, 2019). Many of food industry by-products (from fruits, vegetables, cereal, legumes, nuts, oilseeds, brewery, distillery, winery and marine) have been observed and developed as functional ingredients in baked goods (Benjakul & Karnjanapratum, 2018; Martins et al., 2017; Singh, Benjakul, & Karnjanapratum, 2019). As an alternative and functional ingredient, insect fat was applied as a butter partial replacer fro producing cake, cookie and waffle, which could improve their final quality at a degree differently (Delicato et al., 2020).

In example, gluten-free baked products are aimed to be eaten by those who suffer from an allergy to gluten or celiac disease (CD). There are also people who believe that the have gluten intolerance, which is explained by the occurrence of digestion problem after ingesting gluten without any full immune response to gluten found in CD. Another special nutritional needs are children, eldery, pregnant women, manual worker, atheles and those are with diabetic. Thus, in case of special dietary need issue, the ordinary food laws are set aside (Edwards, 2007). Nevertheless, wheat flour replacement with any gluten-free flours raise another technical problem during baking, particularly loaf rising due to the absence of gluten. A solution by using gum (i.e. xanthan gum and guar gum) and other polysaccharides has been investigated recently. Rice, maize and potato starch were perfectly combined with gum in order to enhance the dough development (Edwards, 2007; Hui et al., 2008). Thus, the various gluten mixed flours have been developed and commercialized to fulfill the market demands as well as providing a convenient product.

2.5.1. Biscuit

Biscuit is derived from the Latin, *panis biscoctus*, which stands for 'twice cooked bread' and referred to was ship's biscuits. Biscuits became a staple food for European sailors voyaging to Asia, Africa, Australasia and the Americas. Although the term of 'biscuits' is widely used in many countries, in the United States, the products are called 'cookies and crackers' (Davidson, 2016). The principle ingredient of biscuits is wheat flour. Biscuits were made by cooking a dough then followed by drying-out the product. Drying-out was aimed to lower the moisture content and further could effectively extend the product's shelf-life (Edwards, 2007). Then, biscuits are considered as nutritious food and available in many forms for both sweet and savory. They are also considered as a good 'vehicle' for improving human nutrition needs through fortification and/or enrichment. Biscuits have many functional forms, enriched with calcium, iron and vitamins and formulated for infants, children and the elderly and for those with special needs such as gluten-free foods (Davidson, 2016). Several studies were conducted to produce nutritious biscuits for special needs with health benefits, including low glycemix index (Delamare et al., 2020), low gluten

(Adeola & Ohizua, 2018; Islam et al., 2012), high-energy (Akande et al., 2020; Homann, Ayieko, Konyole, & Roos, 2017; Kumar et al., 2019), calcium-fortified (Benjakul & Karnjanapratum, 2018), fat-reduced (Moriano et al., 2019) and fiber-enriched (Jia et al., 2020). In general, biscuits consist of flour and water as main ingredients which result the unpleasant final product. Biscuits are categorized into five types based on the different ingredients, formulation, process making and final properties (Table 2.6). Crackers, semi-sweet biscuits, short-dough biscuits, cookies and snack cakes, describe the biscuit terminology. In example, some kind of biscuits need slow baking to let the dough rise while in contrast anothers need a quick baking in certain temperature (Davidson, 2016; Hui et al., 2008).

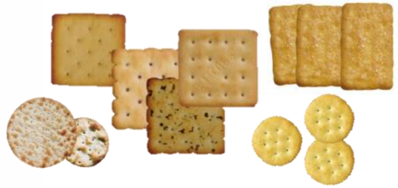
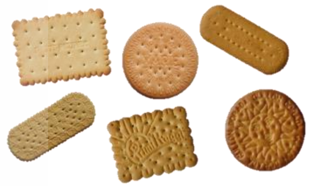
Short dough biscuits are prepared using a dough with high level of fat and sugar in which hinder hydration of the gluten. Gluten development is not desirable for making this kind of biscuits. So, soft or short biscuits are generally made with low-protein flour (7 – 9%). However, water and fat dispersion throughout the dough are the most important factor governing dough development and biscuit texture (Davidson, 2019). The dough is prepared by two-stage process: forming emulsion and flour incorporation. A high initial energy input is required in the first stage for to help the dispersion and make sure there is small chance for gluten development. The second stage mixing, where the flour is added, is very short to avoid developing the gluten. In some cases, some of the sugar is added with the flour. The problems then are making a satisfactory emulsion in the first stage but avoiding gluten development in the second (Davidson, 2016).

2.5.2. Cake

Cakes and sponge cakes are the second most consumed baked products that contribute 37% of the total commercial distribution after fresh sliced breads (Martínez-Monzó et al., 2013). Cake is known as semi-dry sponge foods which the distinguish texture is governed by entrapped air bubbles in a protein and starch network. It is resulted from the rheological behavior changes of cake batter or fluid, to final product (solid) after baking. This phenomenon is explained by the cake batter expansion during baking due to gas resulting from chemicals dissolved in there. The basic processing of cake involves mixing, depositing, baking, cooling and packaging (Cauvain & Young, 2006). Generally, cake is made of soft wheat of cake flour (7.5 – 9.2% protein) and variable levels of fat, sugar, eggs, milk, baking powders, emulsifiers and other optional ingredients, i.e. flavorings – for speciality cakes (Al-Dmoor, 2013; Cauvain, 2016). Appropriate ingredients, proper balanced formula and optimum process (mixing and baking), are the key points determining cake quality (Cauvain & Young, 2006; Cauvain & Young, 2011; Choi & Baik, 2013). Cake flour, in form of mixed flour, have been developed and formulated by composting wheat flour with other functional ingredredients, including cowpea protein (Campbell et al., 2016); whey protein isolate (Díaz-Ramírez et al., 2016); soy protein isolate (Majzoobi, Ghiasi, Habibi, Hedayati, & Farahnaky, 2014); squid ovary powder (Singh et al., 2019). In addition, dietary fiber extracted from okra was incorporated in sponge cake and perfomed the better quality, both cake batter and final product (Qasem et al., 2017a, 2017b).

A significant increase of celiac disease has forced the rapid growing of gluten-free based products (Cureton & Fasano, 2009). The gluten-free concept has been applied in cake using various flours, including rice flour (de la Hera et al., 2013; Turabi, Sumnu, & Sahin, 2008); egg white and whey flour (Sahagún, Bravo-Núñez, Báscones, & Gómez, 2018); shorgum and chickpea flour (Gadallah, 2017). The absence of gluten in cake making impacted its rheological, physical and chemical characteristic. Thus, other functional ingredients, particularly food additives, have been utilized in developing gluten-free cake with favorable quality without giving any detrimental impact on their nutrition (Bhaduri, 2013; de la Hera et al., 2013; Quiñones, Macachor, & Quiñones, 2015). Hence, as an alternative protein source, edible insect can be utilized to develop gluten-free cakes.

Table 2.6 Categories of biscuits.

Category	Characteristics	Figures
Crakers	Doughs have a high-water content (15 – 25%); cracker doughs are often laminated (the dough sheet is made up from multiple thin layers); crackers have an open with flaky texture; low moisture contents of final product (1.5 – 2.5%); i.e. Cream cracker, 'TUC' type, *Malkist* cracker, 'Ritz' type, soda cracker, water biscuits.	
Semi-sweet biscuits	Doughs have strong, developed gluten that gives an elastic dough, which is sheeted and cut (it often shrinks in the first stage of baking); doughs have low sugar and fat; doughs have water contents typically of around 12%; low moisture contents of final product (1.5 – 3.0%); i.e. *Marie*, *Petit beurre*, breakfast biscuit.	
Short-dough biscuits: Rotary moulded	Doughs have a low water content but more fat and sugar than the semisweet biscuits; relatively slow baking at comparatively low temperatures is needed; i.e. Shortbread, Italian frollini, *Digestives*, caramelized biscuit.	
Cookies	Doughs consist of high fat and sugar; long baking times with relatively low baking temperatures are needed; i.e. fruit-, oat-, chocolate-, egg-cookie, filled cookies, *Danish* butter cookies.	
Snack cakes	Cakes are produced from soft batters with relatively low viscosity; some snack cakes are baked in pans that are carried through the oven on chain tracks; i.e. Korean 'pies, snack cakes with injected filling, muffin, *saccotino*.	

Source: Davidson (2019)

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2.6. Wheat flour

Wheat (*Triticum aestivum* L.) is known as the world's most important grains due to its gluten-forming proteins – glutenin and gliadin, which are govern the extensibility and elasticitity required for bakery and pasta (Ferrari, Clerici, & Chang, 2014). It consists of bran (12%), which is the outer husk; endosperm, the white centre (85.5%); and tiny germ (2.5%). For commercial purposes, common wheat is generally classified as hard or soft, red or white, spring or winter. Of these, hard and soft wheat kurs are used in the baked goods that vary bakery products (Hui et al., 2008). Wheat flours are produced through milling and approximately obtain the 70 – 75% of extraction yield. The flour contain moisture between 13% and 15%. Carbohydrate, particularly starch; protein and fat, along side with some fiber, ash and trace minerals and vitamins, are the nutrition substantial of wheat flour (Davidson, 2019). Different protein contents of wheat flours specifically determine the suitable baked product (Table 2.7). A dough made from strong flour with a high protein content is extensible and can be machined into a continuous sheet for crackers and hard biscuits. A weak flour with a low-protein content produces a short dough that may be moulded or a soft, high-fat dough that may be deposited on the baking band and when baked gives a soft, tender cookie (Davidson, 2016). The gluten network plays role in trapping air and gas bubbles formed by yeast fermentation or by leavening agents such as sodium bicarbonate or ammonium carbonate.

Table 2.7 Wheat classes and their general characteristics and principal uses in baked products.

Class	General characteristics	General uses
Hard red winter (HRW)	High protein, strong gluten, high water absorption	Bread and related products
Soft red winter (SRW)	Low protein, weak gluten, low water absorption	Cakes, cookies, pastries, pie crusts, crackers, biscuits
Hard red spring (HRS)	Very high protein, strong gluten, high water absorption	Bread, bagels, pretzels and related products
Hard white	High protein, strong gluten, high water absorption, bran lacks pigments	Bread and related products
Soft white	Low protein, weak gluten, low water absorption, bran lacks pigments	Noodles, crackers, wafers (specks are undesirable)
Durum	High protein, strong gluten, high water absorption	Pasta

Source: Atwell and Finnie (2016)

2.7. Brown rice flour

The increasing cases of celiac diseases (CD) or gluten digestion disorder render the trend of gluten-free food products. A lot of studies on food product development have been conducted in order to substitute and/or replace wheat flour with other sources. Rice (*Oryza sativa* L.) is source of starch and protein which has ability to replace wheat as well (Gularte, Gómez, et al., 2012). In the same study, mix flour (from rice, chickpeas, lentils, and beans) was used to make gluten-free cake. However, gluten-free cake from rice flour will decrease its final quality. Thus, gums and emulsifier blend could improve its final quality (Turabi et al., 2008). There are other sources of starch and protein, such as corn, potato and legumes, that can be used for baked products (Gularte, de la Hera, Gómes, & Rosell, 2012). Based on varies alternative gluten substitute or replacers, it shows the great opportunities to develop gluten-free product. Shevkani and Singh (2014) developed gluten-free muffins from field pea and kidney bean, with addition of amaranth protein isolates.

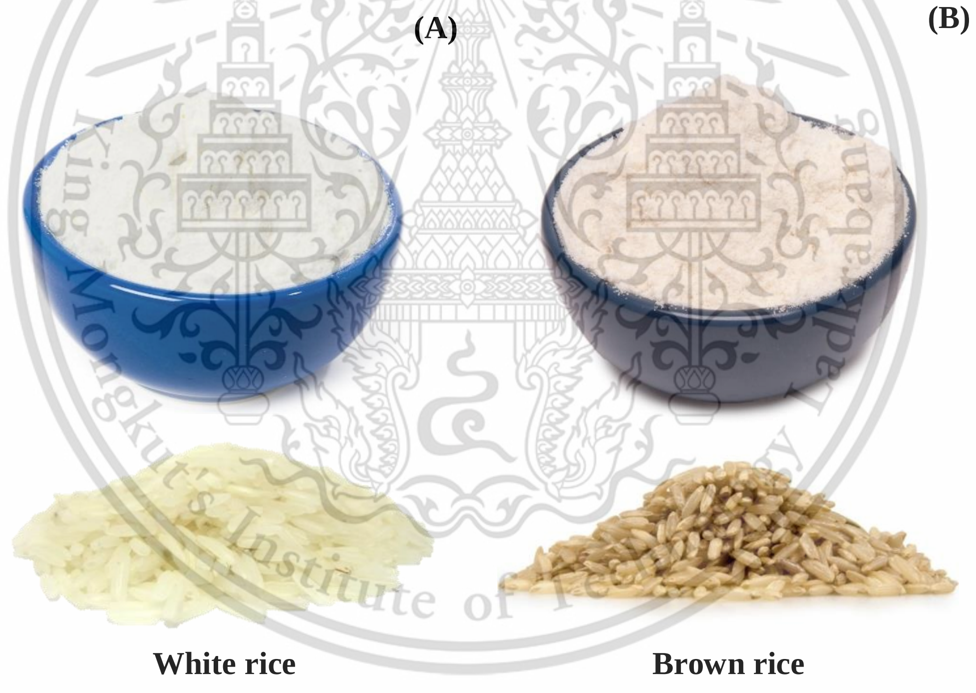

Figure 2.5 White rice (A) and brown rice (B), and their flour counterpart
Source: Nuts.com (2020)

Brown rice (BR) or unpolished rice is rich in phytochemicals such as polyphenols, oryzanol, phytosterols, tocotrienols, tocopherols and carotenoids, as well as vitamins and minerals (Mohan et al., 2014). As one of gluten-free flour, brown rice flour, can be widely used in food application. It contains 9.5% protein, 13% moisture and 1.4% ash. Renzetti and Arendt (2009) developed brown rice bread through protease treatment to improve final quality. It improved bread texture and rheological properties. Islam et al. (2012) also developed biscuit from brown rice flour substitute with wheat flour. At the level of 10% substitution (brown rice flour), could enhance acceptable sensory properties. For food application, especially on bakery products, particle size, flour source and formulation of flour, are important parameters. The finer size of rice flour resulted in low volume of batters and small-and-uniform bubbles, which produced cakes with high volume and low firmness (de la Hera et al., 2013). Khoshgozaran-Abras et al. (2012) prepared the mix flour for flat bread and wheat-based dough by using brown rice flour. The best result was 5% fortification of brown rice flour into wheat flour, which showed similar quality of final product with good consumer acceptance.

2.8. Gelatinization and pasting properties of starch

Rheological properties are the key points covering fluid dynamics, heat transfer and reaction kinetics, in which explain the material behavior changes. Food rheology, in-process intermediates and finished goods are highly dependent on the physical state of the structural matrices, i.e. glass-rubber transition and viscous flow are determined by the combination of temperature and moisture content (Kaletunc & Breslauer, 2019). Then, in baked goods making, those phenomenons affect the batter and final product microstructure that determine its texture (Faridi & Faubion, 2012; Hesso et al., 2015). In term of flour behavior changes, gelatinization is used to explain several changes that occur in granular starch at different temperature intervals. These changes include irreversible loss of birefringence, loss of x-ray diffraction pattern, absorption of water and swelling, change of shape and size of starch granule and leaching of amylose from the granule. Then, these changes resulted in the formation of a gel or paste (Shelton & Lee, 2000). Starches, from different sources, are vary on their type, shape and dimensions in which perform different pasting properties (Kaletunc & Breslauer, 2019). Pasting properties of starch are important indicators that describe how the starch will behave during processing. Pasting occurs after or simultaneously with gelatinization and expressed as recorded viscosity during measurement (Wani et al., 2012). Figure 2.6 represents a pasting curve of typical flour as its viscosity is recorded as a function od time and temperature. The diagram also represents the flour characteristics (viscosity, starch properties, starch damage, swelling, pentosans) and the enzyme activity that are considered for flour selection, blending and treatment (Cauvain & Young, 2009). In example, starch with high paste viscosity is suitable to be utilized as a thickening agent in foods and finishing agent in the textile and paper industries (Wani et al., 2012).

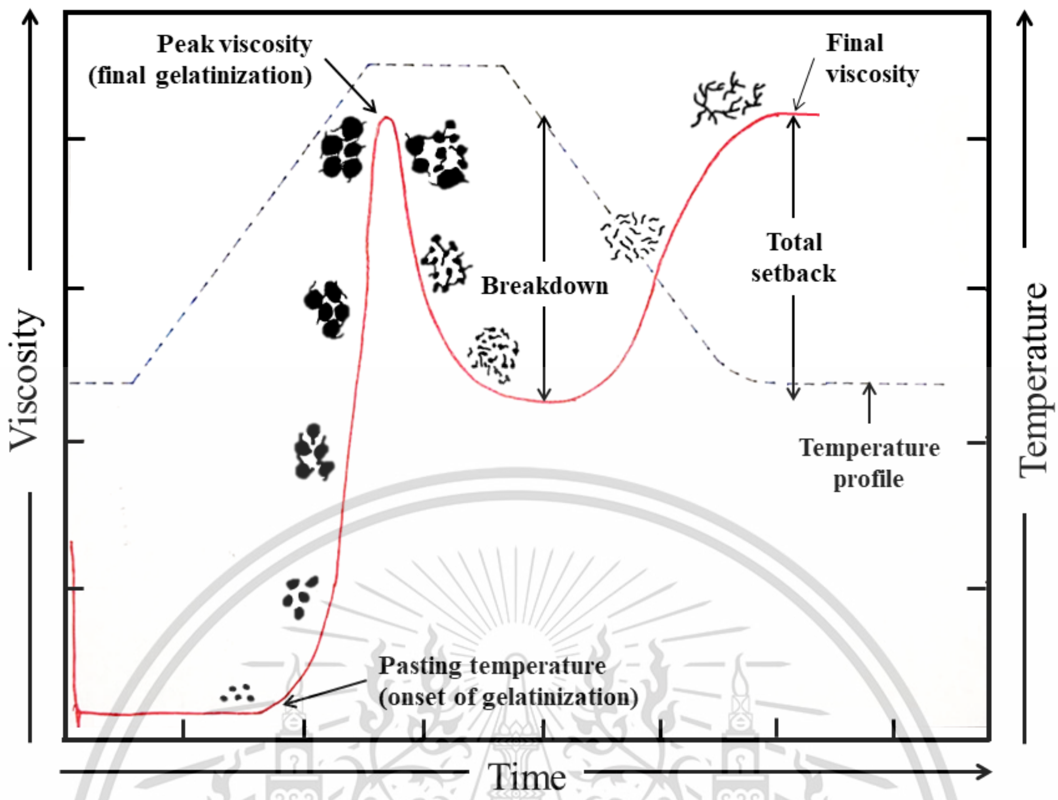

Figure 2.6 Typical starch pasting curve showing pasting parameters measured with Rapid Visco Analyzer or Visco Amylograph
Source: redrawn with modification from Schirmer, Jekle, and Becker (2015); Wani et al. (2012)

Gelatinization takes place when starch is heated and continuously agitated in excess of water. This cause the changes of the physicochemical interactions between amylose and amylopectin, which affect the starch granule structure. The granules become swelling and bursting due to the breakage of the native starch structure. The amylose leaches out and the granules disintegrate resulting in the formation of a viscous material, known as paste (Schirmer et al., 2015; Wani et al., 2012). The temperature needed to get the flour paste is known as pasting temperature. Thereafter, the viscosity continues to increase with constant heating rate until the rate of granule swelling equals to the rate of granule collapse – referred to as the peak viscosity (PV) (Kaletunc & Breslauer, 2019). PV implies the swelling extent or water-binding capacity of starch and often correlates with final product quality since the swollen and collapsed granules relate to the texture of cooked starch. Once PV is achieved, a drop-in viscosity (breakdown), is reached as a result of disintergration of granules. The breakdown viscosity also suggests the degree of stability during cooking. Then, it continues to the cooling stage and the viscosity rises again (setback) in which is caused by retrogradation of starch, particularly amylose. Setback viscosity indicates the final product texture as it is linked to syneresis or weeping during freeze-thaw cycles. Finally, it reaches the final paste viscosity as a result of the its stability. This relates with the starch capacity to form a viscous paste or gel after cooking and cooling (Schirmer et al., 2015; Wani et al., 2012). In addition, other components naturally present in the starchy material (i.e. protein, lipid and fiber) or additives, can interact with starch granule and influence the pasting behavior (Cauvain & Young, 2009; Edwards, 2007). In example, the presence of protein containing disulfide bonds, influenced shear strength and gelatinized paste rigidity of rice starch (Wani et al., 2012). It has been reported that different sources fortified in baked products revealed the changes on rheological properties of cake batter and dough, as final product quality was impacted too (Jia et al., 2020; Khoshgozaran-Abras et al., 2012; Renzetti & Arendt, 2009; Shevkani, Kaur, Kumar, & Singh, 2015; Sozer, 2009). In order to improve the baked product quality, food additives, particulary gum, had been used to work synergistically with other ingredients during processing (Noorlaila, Nor Hasanah, Yusoff, Sarijo, & Asmeda, 2017; Qasem et al., 2017a; Turabi et al., 2008).

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CHAPTER 3

Impact of hexane defatting on characteristics of *Patanga succincta* L. powder and its application on nutritional improvement of biscuit stick

3.1. Abstract

Impacts of hexane defatting on physicochemical, oxidative stability and total volatile compounds of Bombay locust (*Patanga succincta* L.) powder 'BL' were studied. Defatted insect powder (F/BL) showed marked decrease in fat content (1.2%) with higher protein content (78.7%) significantly ($p \leq 0.05$), compared with those of BL (29.8% fat and 56.5% protein). Based on electrophoresis result, hexane defatting had no effect on peptide distribution of insect powders. F/BL had lower peroxide value with greater oxidative stability ($p \leq 0.05$), while less variety of volatiles with more off-flavor compounds, particularly lipid oxidation and insect hormone odors, were observed from BL. F/BL was confirmed as an excellent source of amino acids and minerals. F/BL fortified biscuit stick determined physicochemical and sensory properties of the final products. F/BL fortified biscuit stick showed a better sensory acceptance, compared with those from BL at the same level used. Based on sensory characteristics, biscuit stick with 10% F/BL (F/BL-10) was selected and studied on its chemical composition and microstructure. F/BL-10 possessed more porous structure with higher protein and lower energy value, compared with those from 10% BL. The results suggested that hexane defatting could effectively be used for preparing Bombay locusts powder, which could potentially use as an alternative protein source with excellent applicability to the baked product, particularly biscuit stick.

3.2. Introduction

Alternative protein sources are becoming increasingly important due to their sustainability for feeding significant numbers of the world population (Elhassan, Wendin, Olsson, & Langton, 2019; Tieland, Borgonjen-Van den Berg, Van Loon, & de Groot, 2015). Edible insects have been shown to be a nutritious food source for direct consumption or as a food additive (Chen, Feng, & Chen, 2009; Megido et al., 2014; Van Huis et al., 2013). They are not only a good source of protein but they also contain fat, minerals, calories and micronutrients (Kim, Weaver, & Choi, 2017; Köhler, Kariuki, Lambert, & Biesalski, 2019; Yhoung-aree, 2010). However, consumption of intact whole insects is commonly not accepted for human consumption in many countries, particularly in the west. Thus, proceeding them into forms that may hide their form, such as powder or flour, could allow insects to be more readily acceptable as human food (Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016).

Bombay locust (*Patanga succincta* L.), also called grasshopper, is a common edible insect in Southeast Asia (Hanboonsong, Jamjanya, & Durst, 2013). It has a high protein content (36.3%) and both essential and non-essential amino acids (Köhler et al., 2019). The fat content of locusts was reported to range from 4.7 – 14.8%, depending on how it has been processed (Yhoung-aree, 2010) and this significant amount of lipid has been shown to govern its use and characteristics (L'Hocine, Boye, & Arcand, 2006; Lee et al., 2016). In addition, lipid content of the insects also determines oxidation stability, as well as sensory characteristics when used as food ingredients, particularly insect powder (Ahmed et al., 2016; Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012). Defatting insect powder by organic solvents can be simply applied, not only for removing lipid, but also for concentration the protein. Also, some pigments and pheromones were shown to be co-extracted during defatting (Cheng, Huang, Li, Zhou, & Cen, 2014; Dossey, Tatum, & McGill, 2016; Mishyna, Chen, & Benjamin, 2020; Purschke, Stegmann, Schreiner, & Jäger, 2016; Wang et al., 2019). Although alternative lipid extraction methods have been used (Talbot, 2011), hexane defatting is still common, which has been shown to remove more than 96% oil on average (Ricochon & Muniglia, 2010; Yousefi & Hosseini, 2017). Nevertheless, no information is available on the impact of hexane defatting on the characteristics of Bombay locust powder and its potential contribution to alleviate future food challenges as an alternative protein source.

Several protein sources, such as plant based (González-Montemayor, Flores-Gallegos, Contreras-Esquivel, Solanilla-Duque, & Rodríguez-Herrera, 2019; Mesías, Holgado, Márquez-Ruiz, & Morales, 2016) and edible insects (Akande, Jolayemi, Adelugba, & Akande, 2020; de Oliveira, da Silva Lucas, Cadaval, & Mellado, 2017; Delicato, Schouteten, Dewettinck, Gellynck, & Tzompa-Sosa, 2020), have been added to wheat flour in order to enrich the protein level of bread and other bakery products. These protein sources complement nutrients lacking in the food and booster their health benefits of the products (Beelen et al., 2018; Martins, Pinho, & Ferreira, 2017; Stelten et al., 2015). Therefore, the main objective of the present study was to evaluate the impact of hexane defatting on physicochemical characteristics, oxidative stability, total volatile content, amino acid composition and mineral content of Bombay locust powder. Also, the fortification of the baked product, biscuit stick, with Bombay locust powders was investigated for its effects on both sensory and nutritional characteristics.

3.3. Materials and methods

3.3.1. Materials

Frozen Bombay locust (*Patanga succincta* L.) was procured from a local supplier (Mr. BUC FOOD, Phra Nakhon Si Ayutthaya, Thailand). All-purpose flour (KITE, United Flour Mill Public Co., Ltd., Samut Prakan, Thailand), unsalted butter (Orchid, Mali Group Co., Ltd., Saphan Sung, Thailand), canola oil (Naturel, Lam Soon Public Co., Ltd, Samut Prakan, Thailand) and other ingredients were purchased from a local market in Ladkrabang, Bangkok, Thailand. Analytical grade chemicals – sodium dodecyl sulphate (SDS); Coomassie Blue R-250; *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were obtained from Bio-Rad Laboratories (Hercules, CA, USA). Bovine serum albumin (BSA) was obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and hexane was from Macron Fine Chemicals™ (Dublin, Ireland).

3.3.2. Preparation of insect powder

Frozen Bombay locust was washed and screened to remove other contaminating insects and freeze-dried (DK-3450 Lynge, Labogene ApS, Denmark) and ground into powder using a laboratory grinder (MF-800, Tefal®, France). The powder was sieved using a 2 mm (10-mesh) screen and kept in metal-foil laminated polyethylene pouches at -20 °C until use. The prepared Bombay locust powder was referred to as 'BL'.

3.3.3. Preparation of defatted insect powder

Fats were removed from BL by using hexane as a solvent following the method described by Choi, Wong, and Auh (2017), with a slight modification. Briefly, the ratio of sample:solvent 1:5 (w/v) was mixed with continuous stirring for 3 h at 25 °C, with the solvent changed hourly, three times in total, and centrifuged in a refrigerated centrifuge (Allegra®x-12r, Beckman Coulter, Inc., USA) at 3,000 rpm for 20 min at 25 °C to separate the sediment. The sample was collected and dried on an aluminum foil tray in a fume hood overnight (18 h, 25 – 28°C). The defatted insect powder was labelled 'F/BL'. Both BL and F/BL were then analyzed.

3.3.4. Calculation of yield and protein recovery

The yield of F/BL was reported as a percentage of the mass of F/BL compared to the mass of BL using Formula 1.

$$\text{yield (\%)} = \left(\frac{\text{weight of F/BL (g)}}{\text{weight of BL (g)}}\right) \times 100 \quad (1)$$

Protein recovery was determined as a percentage of the total protein content of F/BL compared to that of BL. The protein content was obtained following the Kjeldahl method (AOAC, 2000). The protein recovery was then calculated using Formula 2.

$$\text{Protein recovery (\%)} = \left(\frac{\text{total protein of F/BL (g)}}{\text{total protein of BL (g)}}\right) \times 100 \quad (2)$$

3.3.5. Characterization of F/BL

3.3.5.1. Chemical compositions

Protein, fat, moisture, ash and fiber contents (on a wet basis) were determined following AOAC methods (AOAC, 2000). Total carbohydrate was calculated by subtraction of the sum of protein, fat, moisture and ash from the total mass of the sample.

3.3.5.2. Water activity (a_w) and color

Water activity (a_w) was measured using an Aqua Lab 4TE (SN S40002336, Decagon Devices, USA). Colors of samples were measured in the CIE *L**, *a** and *b** space, using a CR-400 Chroma Meter (Konica Minolta Sensing Americas, Inc., USA). Where *L** measures 'lightness', *a** 'redness', and *b** 'yellowness'. Total differences of color (ΔE*) and chroma (ΔC*) were then calculated and compared with a white standard, following the method described by Benjakul and Karnjanapratum (2018).

3.3.5.3. Protein pattern

Protein composition of the sample was observed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). The polyacrylamide gels, contained 10% separating gel and 4% stacking gel, were used for loading the sample (15 µg protein) and subjected to electrophoresis using AE-6440 equipment (20 mA/gel). The molecular weight of each sample protein was determined based on protein standards.

3.3.5.4. Peroxide value (PV)

PV was determined using the ferric thiocyanate method following Shantha and Decker (1994) using a UV-1200 Spectrophotometer (Mapada Instrument Co., Ltd., Shanghai). Cumene hydroperoxide was used to calculate the calibration curve (0.1 – 1.0 mM).

3.3.5.5. Thiobarbituric acid reactive substances (TBARs)

Thiobarbituric acid reactive substances (TBARs) were determined following the method described by Takeungwongtrakul, Benjakul, Santoso, Trilaksani, and Nurilmala (2013) and 1,1,3,3-tetraethoxypropane (a precursor of malonaldehyde, 0 – 0.25 mM) was used for the calibration curve.

3.3.5.6. Oxidative stability of F/BL

Oxidative stability was measured as induction time (h) using a Rancimat instrument (743 Rancimat, Metrohm, Switzerland) at 80 °C with 20 L/h gas flows (Laubli & Bruttel, 1986; Metrohm, 2018).

3.3.6. Total volatile compounds

Total volatile compounds in F/BL and BL were determined using solid-phase microextraction by gas chromatography-mass spectrometry (SPME/GC-MS), following Karnjanapratum and Benjakul (2014). The volatile compounds were reported as their relative abundances ($\times10^6$).

F/BL samples showed the good oxidative stability and less off odors, and therefore they were selected for evaluation of their nutritional values, including amino acid and mineral content. F/BL was also selected in order to study its application in baked products by fortification in a "short dough biscuit stick", where their physicochemical properties, sensory properties and nutritional characteristics were investigated in comparison with those from BL.

3.3.7. Amino acid composition

Samples were hydrolyzed using 0.4 M methyl sulfonic acid under reduced pressure at 100 °C for 22 h to prevent tryptophan oxidation. The hydrolysates were neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). A 10 µL aliquot was placed in an amino acid analyzer (JLC-500/V AminoTacTM, JEOL Inc., USA). The amino acid composition was expressed as residues/1000 residues (Karnjanapratum, Benjakul, Kishimura, & Tsai, 2013).

3.3.8. Minerals content

Calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P) and zinc (Zn) were determined, following Kim et al. (2017), using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 5300 DV, PerkinElmer, MA, USA). The samples were prepared through wet digestion before being used for mineral content analysis.

3.3.9. Application of F/BL in baked product by fortification in biscuit stick

3.3.9.1. Preparation of Bombay locust powder fortified biscuit stick

F/BL was incorporated with all-purpose flour (F/BL mixed flours) at different levels of total weight replacement, 5, 10 and 15% (w/w) following Delamare et al. (2020) with slight modifications. The ingredients were prepared and consisted of F/BL mixed flours (62.5%), water (21.0%), canola oil (12.0%), unsalted butter (1.5%), pepper (1.0%), chicken flavor (0.5%), salt (0.5%), baking soda (0.5%) and baking powder (0.5%). All of the dry ingredients were sieved, then mixed with other ingredients and kneaded, then rolled and molded using sheeter (Marcato, Atlas 150 Design, Marcato S.r.l., Italy) with *Attachment Lasagnette*. The dimension of sheeted dough was expressed in width, length and thickness ($6.5 \times 100 \times 3.3$ mm^3). It was baked in a preheated electric oven at 180 °C for 7 min. The baked sticks were then removed from the oven, cooled (25 °C, 15 min) and packed in polyethylene zip-lock bags for further analyses. The resulting biscuit sticks, fortified with F/BL at 5, 10 and 15%, were referred to as 'F/BL-5, -10, and -15', respectively. The same process was used for BL and referred to as 'BL-5, -10, and -15', respectively. All F/BL samples were then subjected to analyze in comparison with those of BL.

3.3.9.2. Physicochemical characteristics

Water activity (a_w) and color of all sample were measured. Total differences of color (ΔE^*) and chroma (ΔC^*) were then calculated and compared with control (BL fortified sticks at the same replacement level) as described in section 3.3.5.2.

Baking loss percentage was determined according to AACCI (2010). The hardness (N) of each biscuit stick was also measured using a texture analyzer (TA.XT.plus®, Texture Technologist Corp., USA) with a knife edge (with slotted inserted, HDP/BS) and heavy duty platform (HDP/90) at a test speed of 10 mm/s.

3.3.9.3. Sensory evaluation

Sensory evaluation was conducted using 30 untrained panelists to evaluate appearance, color, odor, taste, texture, flavor, aftertaste and overall liking score of samples. A nine-point hedonic scale was used, in which 1 = extremely dislike, 5 = neither like or dislike and 9 = extremely like. All samples were labelled with a 3-digit number and served randomly to the panelist. They were asked to neutralize their mouth with water between testing each sample.

3.3.10. Characteristics of the selected F-BL fortified biscuit stick

All the biscuit sticks with 10% F/BL (F/BL-10) obtained good sensory characteristics with ≥7 of liking score for all attributes tested. These biscuit sticks were therefore selected and characterized for their nutritional content and microstructure in comparison with that fortified with BL at the same level.

3.3.10.1. Chemical composition and energy value

Moisture, protein, fat, ash and total carbohydrate contents were measured following AOAC methods – see section 3.3.5 (a). Cholesterol, total sugar and sodium, were determined following analytical method No. 976.26, 925.35 (B) and 984.27, respectively (AOAC, 2019). Energy value was calculated using the Atwater system (Merrill & Watt, 1973).

3.3.10.2. Scanning Electron Microscopy (SEM)

Microstructure of biscuit sticks was observed and captured using a SEM (FEI-XL30, FEI Company, Hillsboro, OR, USA) following Benjakul and Karnjanapratum (2018). An acceleration voltage of 20 kV with 5-10 Pa of pressure were used to visualize each sample at both 50× and 200× magnification.

3.3.11. Statistical analysis

The experiments were conducted in triplicate. All data were analyzed and statistical significance determined, using Independent t-Test (for two samples) and Duncan Multiple Range Test ($p \leq 0.05$), was considered as statistically significance (Field, 2000) using the Statistical Package for Social Science (IBM SPSS Statistics, IBM, New York, USA).

3.4. Results and discussion

3.4.1. Impact of hexane defatting on yield, protein recovery and characteristics

3.4.1.1. Yield and protein recovery

The resultant, defatted powder (F/BL), from conventional hexane extraction showed that 69% (w/w) of yield with 96% (w/w) of the lipid had been removed, which indicated other non-lipid compounds were removed by hexane in addition to lipids. Nevertheless, the remained lipids were might be bound with other insect compounds or matrices. The F/BL protein recovery was 84%, which is slightly higher than the reported yield of defatted *Tenebrio molitor* (83%) and *Hermetia illucens* (73%), in a three-time extraction with hexane (Bußler et al., 2016). Yield of defatted insect powder has previously been shown to be governed by several factors, including lipid content of the initial insect powder, defatting used, process conditions and solvent used (Bußler et al., 2016; Choi et al., 2017; Zhao, Vázquez-Gutiérrez, Johansson, Landberg, & Langton, 2016). Some non-lipid components, particularly nitrogenous material, were co-extracted with the defatting solvent, which consequently affected the protein content decreasing protein recovery (Efthymiopoulos et al., 2018). Inorganic nitrogen (urea, creatinine, uric acid and ammonia) from the insect intestinal tracts were possibly also washed out during defatting (Weihrauch, Donini, &

O'Donnell, 2012). Further, the very low polarity of hexane would be expected to cause protein denaturation by disrupting the intramolecular hydrogen bonding of the protein to some degree and those denatured fragments would also be dissolved in the solvent during hexane defatting (Kang et al., 2017).

3.4.1.2. Chemical compositions

The chemical analysis of BL, before and after defatting (F/BL) showed that fat removal using hexane had an impact on powder characteristics, especially the chemical composition (Table 3.1). Defatting with hexane, in this study, successfully removed more than 95% of lipid content from BL, whereas the fat content decreased from 29.8% for BL to 1.2% for F/BL. Consequently, protein content of F/BL was concentrated and increased significantly ($p \leq 0.05$) to more than 20%. This was consistent with defatted mealworm powder (Son, Lee, Hwang, Nho, & Kim, 2019) and soy flakes (Lee, Choi, & Eun, 2015; Son et al., 2019), where a higher protein fraction was obtained after defatting. The previous reports showed that protein content of Bombay locust ranged from 15 – 36%, depending on sample preparation (Chatsuwan, Nalinanon, Puechkamut, Lamsal, & Pinsirodom, 2018; Köhler et al., 2019; Yhoung-aree, 2010). Choi et al. (2017) removed fat from mealworm, cricket and silkworm pupae, with more than 90% fat removed, which consequently improved their protein content, ranging from 62 – 74% on a dry basis. Hexane was also reported to be effectively used for fat extraction from Yellow mealworm with 96% fat removal (Purschke et al., 2016; Purschke et al., 2018). Defatting, using hexane, showed the higher protein content with lower fat content than with other solvents such as ethanol and methanol (Son et al., 2019). Also, the carbohydrate content significantly ($p \leq 0.05$) decreased with increase in ash and moisture contents after defatting. On the other hand, there was no significant difference ($p \leq 0.05$) between fiber content from BL (15.4%) and F/BL (14.7%). Edible insect carbohydrate, particularly sugars, has been shown to vary between 1 and 10% (Resh & Cardé, 2009). These low molecular weight constituents could be easily eluted during defatting. Rumpold and Schluter (2013) reported the ash content of edible insects varied between Orthoptera, Coleoptera, Lepidoptera and Hemiptera. It was found that locusts contained only a small amount of ash as they had a shorter internal calcified skeleton, compared to the longer skeletons, predominantly found in many vertebrates (Rumpold & Schluter, 2015). Nevertheless, chitin polysaccharides are the main material of insect exoskeletons, which are insoluble in many solvents due to their compact structure (Rinaudo, 2006). Therefore, hexane defatting would effectively remove fat from BL, where the protein content would be concentrated. Changes in chemical composition would therefore also affect physicochemical characteristic, as well as oxidative stability, of the insect powder.

Table 3.1 Characteristics of Bombay locust powder without (BL) and with hexane defatting (F/BL)

Parameters	**BL**	**F/BL**
Chemical composition		
Protein (%)*	56.49 ± 0.65[b]	78.73 ± 0.77[a]
Fat (%)	29.84 ± 0.20[a]	1.20 ± 0.01[b]
Ash (%)	2.52 ± 0.02[b]	3.31 ± 0.05[a]
Moisture (%)	2.32 ± 0.04[b]	11.76 ± 0.07[a]
Carbohydrate (%)	8.82 ± 0.41[a]	4.99 ± 0.73[b]
Fiber content (%)	15.36 ± 1.27[a]	14.71 ± 0.34[a]
Water activity	0.087 ± 0.005[b]	0.558 ± 0.006[a]
Color		
*L**	34.82 ± 0.20[b]	53.21 ± 0.32[a]
*a**	8.78 ± 0.07[b]	11.20 ± 0.03[a]
*b**	9.31 ± 0.41[b]	14.87 ± 0.23[a]
ΔE*	58.56 ± 0.26[a]	42.48 ± 0.23[b]
ΔC*	10.05 ± 0.34[b]	16.11 ± 0.22[a]
Lipid oxidation		
PV (mg cumene/kg sample)**	54.44 ± 0.81[a]	48.49 ± 1.13[b]
TBARs (mg malonaldehyde/kg sample)***	9.08 ± 0.76[b]	13.36 ± 0.93[a]
Oxidative stability		
Induction time (h)	22.49 ± 1.12[b]	44.81 ± 0.19[a]

Data are mean ± standard deviation (n=3).
*The conversion factor used is 6.25.
**PV: peroxide value.
***TBARs: thiobarbituric acid reactive substances.
Different lowercase superscript letters in the same row indicate significant differences ($p \leq 0.05$).

3.4.1.3. Water activity (a_w) and color

The impact of hexane defatting on water activity (a_w) and color of BL and F/BL are shown in Table 3.1. F/BL had higher a_w (0.558) than BL (0.087). This matched the increased moisture content of F/BL. The increased a_w of F/BL can be attributed to polarity changes in BL with decreases in non-polar components, resulting in higher hydrophilicity and hygroscopic behavior. In addition, the moisture sorption isotherm incorporates the hygroscopic properties of numerous constituents, which sorption properties may change due to physical or chemical interactions induced by heating and other pre-treatments (Sandulachi, 2012). Notably, the low a_w indicated that both BL and F/BL were 'shelf-stable', as they had a_w<0.62, considering the microbial safety benchmark level during storage (Abdullah, Nawawi, & Othman, 2000).

Hexane defatting in the present study markedly affected the color of the powder. Significant differences ($p \leq 0.05$) were observed in lightness (L^*), redness (a^*) and yellowness (b^*), as well as differences of total color (ΔE^*) and chroma (ΔC^*) between BL and F/BL samples (Table 3.1). Fat removal increased the lightness, redness and yellowness in F/BL, which was consistent with decreased ΔE^*, where a significant decrease ($p \leq 0.05$) of the dullness was observed with increased ΔC^*. Overall, the insect powder became brighter with vivid brownish color, after defatting. Previous reports indicated that locust color was predominantly determined by insectoverdin (green), carotenoids (yellow), a bile component (blue) and melanin (brown) (Chatsuwan et al., 2018; Clarkson, Mirosa, & Birch, 2018; Goodwin, 1952; Wittkopp & Beldade, 2009). This result suggested that insectoverdin and the bile component in BL were removed during defatting, where carotenoids could be concentrated with proteins denaturation to some degree. This was confirmed by the dark green color of extracted lipids (data not shown) obtained from hexane used in defatting. Lighter colors and lower browning index of defatted *Schistocerca gregari*, *T. molitor* and *H. illucens* have been observed, due to phenolic compounds in the cuticles, which underwent oxidation and protein-phenolic interaction (Bußler et al., 2016; Mishyna, Martinez, Chen, & Benjamin, 2018). We expected less brownness of F/BL that could give more favorable appearance of final product, especially F/BL-enriched baked products.

3.4.1.4. Protein patterns

Protein patterns of BL and F/BL were analyzed using SDS-PAGE under reducing conditions in comparison with fresh Bombay locusts (F) (Figure 3.1). The fresh locust showed a wide range of protein molecular weights from 150 kDa to below 16 kDa. Six major groups of peptides that were found from F included ones with 16 – 21 kDa, 41 – 51 kDa, 60 kDa, 71 kDa, 138 kDa and 200 kDa. This was consistent with previous published work on the protein bands of several grasshopper species (*P. succincta*, *Chondracris roseapbrunner*, *Locusta migratoria* and *S. gregaria*), which ranged from 6 – 146 kDa (Chatsuwan et al., 2018; Purschke et al., 2018; Zielińska, Baraniak, & Karaś, 2017). Yi et al. (2013) reported that the bands, ranging from 14 – 32 kDa, of *T. molitor* were cuticle proteins. Tropomyosin and tubulin bands were found at 40, 50 and 100 kDa from *L. migratoria*, which showed microtubule formation (Purschke et al., 2018). Actin was observed at molecular weights of

~24 kDa and ~29 kDa from *Aleuroglyphus ovatus* (Erban, 2011). In addition, the bands ranging from 32 – 95 kDa contributed to enzymes and other proteins, e.g. myelinization-inhibiting protein, β-glycosidase, trypsin-like proteinases and myelinization-engaging types of protein (Erban, 2011; Yi et al., 2013). Different protein patterns were observed in fresh insects (F) and powders (BL and F/BL). BL and F/BL showed similar protein distributions, which included proteins in the ranges of 62 kDa, 51 – 55 kDa, 23 – 27 kDa, 19 – 16 kDa, and <15 kDa. Exposure to non-polar organic solvents, e.g. hexane, could induce protein denaturation by disrupting intramolecular hydrogen bonding of protein side chains (Kang et al., 2017). Zielińska, Karaś, and Baraniak (2018) noted that the process used for powdering insects, including physical and chemical treatments, could affect the protein patterns.

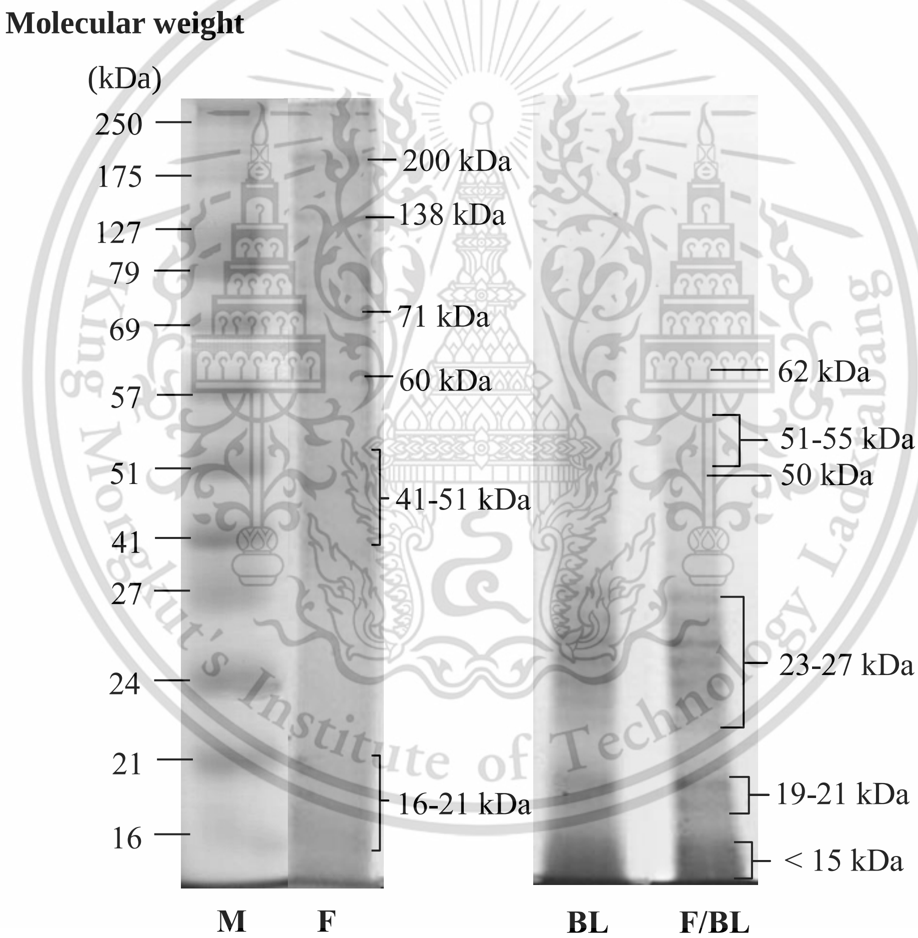

Figure 3.1 SDS-PAGE patterns of protein from Bombay locust under reducing conditions. M: protein markers, F: Fresh Bombay locust, BL: Bombay locust powder without hexane defatting, F/BL: Bombay locust powder with hexane defatting.

In the current work, heat treatments were avoided in preparing powders, but heat generated in grinding, might lead to small changes in protein patterns. Consequently, high molecular weight proteins could be degraded and small peptides then liberated. However, the result from the present study indicated that the hexane defatting method used had no effect on protein patterns of Bombay locust powder.

3.4.2. Lipid oxidation and oxidative stability

The lipid oxidation of BL and F/BL was determined by peroxide value (PV) and thiobarbituric acid reactive substances (TBARs) as shown in Table 3.1. Hydroperoxide indicates primary lipid oxidation occurrence, which is sensitive to temperature, chemical instability and affects odors and taste. It generates a complex mixture of secondary lipid oxidation products, i.e. aldehydes, ketones, alcohols and esters, which are responsible for rancidity (Hu & Jacobsen, 2016). PV in BL was higher (54 mg/kg sample) than in F/BL (49 mg/kg sample), while TBARs of BL was lower (9 mg/kg sample) than F/BL (13 mg/kg sample). The lower PV with higher TBARs indicated a secondary lipid oxidation reaction had undergone. The defatting procedures used, particularly stirring and drying, likely increased the exposure of the sample to oxygen, an essential factor contributing to lipid oxidation (Amaral, Silva, & Lannes, 2018). Gharby, Harhar, Matthäus, Bouzoubaa, and Charrouf (2016) also reported that lipid oxidation, along with formation of primary and secondary oxidation products, were generated during processing, e.g. fat extraction and heat-treatment as well as during storage. Thus, lipid oxidation reactions could take place at a higher degree, compared with those without defatting (BL).

Oxidative stability was reported as induction time (h) (Table 3.1), that is the time needed to achieve a level, in which the oxidation index (i.e. PV or carbonyl number) increased suddenly and resulted in undesirable sensory properties (Cheng et al., 2010; Schaich, 2016). BL showed a significantly lower ($p \leq 0.05$) induction time (22.5 h) than F/BL (44.8 h). The higher fat content of BL would be expected to accelerate development of oxidative rancidity. Moreover, it has been reported that grasshopper proteins, containing antioxidative amino acids or peptides, further retard oxidation (Chatsuwan et al., 2018). Thus, the more concentrated protein, after defatting, could reduce lipid oxidation (Sohaib et al., 2017) and increase stability to oxidation, which was also related to levels of oxidative enzymes inactivation, e.g. lipase, peroxidase, and lipoxygenase (Mazaheri, Torbati, Azadmard-Damirchi, & Savage, 2019), which could be denatured to some degree by hexane during defatting. Thus, defatting with hexane was shown to improve lipid oxidation stability by prolonging the induction time, which could govern the deterioration and shelf life of Bombay locust powder.

3.4.3. Total volatile compounds

The total volatile compounds (VOCs) in F/BL are listed in Table 3.2. Twenty-two VOCs were detected in F/BL, but only 7 VOCs in BL. Esters were the major VOCs, which contributed to the noticeable odor of both powders. Three of the VOCs, (3E,5E)-octa-3,5-dien-2-one, 2,3-bis(2-ethylbutanoyl)-propyl-2ethylbutanoate and methyl octadeca-11-enoate, were previously described as fatty, buttery and insect pheromone odors (Arn & Acree, 1998; Furia, 1980; Wang et al., 2019), which were notably removed from BL by hexane defatting. In previous work, the abundance of some of the VOCs have been shown to increase after defatting, e.g. methyl octanoate, methyl dodecanoate, methyl tetradecanoate and methyl hexadecanoate, which contributed to the aroma of wine (Welke, Manfroi, Zanus, Lazarotto, & Alcaraz Zini, 2012), tobacco leaves (Li et al., 2008), goat milk (Queiroga et al., 2019) and rice (Verma & Srivastav, 2018). Also, 18 VOCs were detected only in F/BL and most of these were the methyl esters of fatty acids. This suggested the insect protein could be denatured by exposure to the non-polar organic solvent, hexane, in which hydrophobic regions were destroyed and some intramolecular hydrogen bonding of protein side chains was disrupted (Bader, Oviedo, Pickardt, & Eisner, 2011; Moure, Sineiro, Domínguez, & Parajó, 2006). Consequently, small fragments of bound hydrocarbon chains would be liberated, resulting the newly generated VOCs. The major VOCs detected from F/BL, including methyl hexadecanoate, methyl octadec-9-enoate and methyl tetradecanoate, were described as the faint fatty and waxy odors detected from goat milk and rice (Queiroga et al., 2019; Verma & Srivastav, 2018). The methyl esters of fatty acids, cis-9-octadecanoic acid (oleic acid) and hexadecanoic acid (palmitic acid), which were abundant in F/BL, was consistent with the high amount of oleic and palmitic acids in another grasshopper oil extracted from *Chorthippus parallelus* and *Conocephalus discolor* (Paul et al., 2017). Several methyl esters of fatty acids were observed only in F/BL, including methyl heptadecanoate, methyl octadeca-9,12,15-trienoate, methyl octadecanoate, methyl octadeca-9,12-dienoate and methyl octadeca-9,11,13-trienoate, which were likely to have been derived from lipid oxidation and provided the faint fatty odors (Kiatbenjakul, Intarapichet, & Cadwallader, 2015). Yeo et al. (2013) noted that the esters of fatty acids were the second major volatile constituents of *Protaetia brevitarsis* larvae (beetle), after hydrocarbons. Nevertheless, the significant number of VOCs, mainly methyl esters, detected in F/BL were defined as 'pleasant' aromas, for example methyl hexanoate, methyl heptanoate, methyl octanoate, methyl 2-phenylacetate, methyl nonanoate, dimethyl octanedioate and methyl dodecanoate, which were responsible for fruity and wine-like aromas (Hadi, Zhang, Wu, Zhou, & Tao, 2013; Lasekan, Khatib, Juhari, Patiram, & Lasekan, 2013; Li et al., 2008). Those volatile esters often represented the major contribution to the odor of fruits (Hadi et al., 2013). Evans, Flore, and Frøst (2017) described

Table 3.2 Volatile compounds of Bombay locust powder without (BL) and with hexane defatting (F/BL)

Volatile compounds	**Abundance (×10^6)**	
	BL	**F/BL**
Methyl hexanoate	nd.	13.87
Methyl heptanoate	nd.	4.91
(3*E*,5*E*)-octa-3,5-dien-2-one	3.28	nd.
Methyl octanoate	6.47	23.69
2,3-bis(2-ethylbutanoyloxy)-propyl 2-ethylbutanoate	3.89	nd.
Methyl 2-phenylacetate	nd.	6.41
Methyl nonanoate	nd.	20.89
Dimethyl octanedioate	nd.	3.69
Methyl dodecanoate	23.46	66.96
Dimethyl nonanedioate	nd.	27.14
Methyl tridecanoate	nd.	4.34
Methyl tetradec-9-enoate	nd.	5.35
Methyl tetradecanoate	44.26	146.91
Methyl pentadecanoate	nd.	27.90
Methyl pentadec-8-enoate	nd.	94.15
Methyl hexadecanoate	52.82	325.60
Methyl heptadec-10-enoate	nd.	6.07
Methyl heptadecanoate	nd.	34.20
Methyl octadec-9-enoate	nd.	305.12
Methyl octadeca-9,12,15-trienoate	nd.	79.96
Methyl octadecanoate	nd.	87.60
Methyl octadeca-9,12-dienoate	nd.	11.47
Methyl octadeca-9,11,13-trienoate	nd.	6.87
Methyl octadec-11-enoate	17.76	nd.
Methyl octadeca-6,9,11-trienoate	nd.	3.70

nd.: not detected.

the taste or flavor of grasshopper (*L. migratoria*) as an intense aroma of cereal, wood and 'nuttiness'. Overall, hexane defatting reduced the insect off-odors by removing undesirable VOCs and enhancing good odors, that would positively impact their sensory characteristics and consumer acceptance. F/BL showed good oxidative stability with a higher induction time, as measured by the Rancimat system, which determines the oxidation stability of natural fats and oils, and contained fewer off-odors. It was therefore selected for amino acid composition and mineral content determination.

3.4.4. Amino acid composition

The amino acid composition of F/BL show that Alanine (154 residues/1000 residues), glutamine/glutamic acid (112/1000 residues), and glycine (103/1000 residues) were the major non-essential amino acids (Table 3.3). Leucine (81/1000 residues) was the most dominant essential amino acid in F/BL, followed by valine (59/1000 residues) and lysine (57/1000 residues). This was consistent with the total amino acid content of Bombay locust reported by Köhler et al. (2019). In F/BL there was a lower amount of essential amino acids (34%) than in longhorn grasshoppers (37 – 38%) (Fombong, Van Der Borght, & Broeck, 2017). Nevertheless, a similar ratio of essential to non-essential amino acids was measured in F/BL (0.52) and house crickets (0.53) (Köhler et al., 2019). Hydrophobic amino acids were dominant in F/BL, which is in agreement for levels in *P. succincta* and *C. roseapbrunner* (Chatsuwan et al., 2018). Previous research has shown that amino acid composition defines key properties, i.e. water- and oil-holding capacity, solubility, foaming, gelation and emulsification (Clarkson et al., 2018; Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019; Zhao et al., 2016; Zielińska et al., 2018). Differences in amino acid composition have been shown to be governed by different preparations and biological factors of insects (Kang et al., 2017; Nowak, Persijn, Rittenschober, & Charrondiere, 2016; Oibiokpa, Akanya, Jigam, Saidu, & Egwim, 2018). Sun, Shang, Liu, and Long (2010) reported that methionine and cysteine were the limiting amino acids of grasshoppers *Oedalius asiaticus*, *Angaracris rhodopa*, *Chorthippus dubius* and *Chorthippus fallax* sampled from the Tibetan plateau in China. The limiting amino acid of Bombay locust was reported to be tryptophan (Köhler et al., 2019), which could not be detected in F/BL. On the other hand, low values of cysteine, proline and hydroxyproline were detected in F/BL, but only 1 – 2/1000 residues. Similarly, four indigenous edible insect species in Nigeria had cysteine as the least abundant of amino acid (Oibiokpa et al., 2018). Furthermore, F/BL contained higher sweet amino acids, including alanine and glycine (257/1000 residues), compared with savory amino acids (aspartic and glutamic acids). This was also reported by Köhler et al. (2019), where the higher amount of sweet amino acids with a low level of savory amino acids was observed from Bombay locust.

Table 3.3 Amino acids composition of defatted Bombay locust powder (F/BL)

Amino acids	**Content (residues/1000 residues)**
Non-essential amino acids	
Alanine	154
Aspartic acid/asparagine	80
Arginine	49
Cysteine	2
Glutamine/glutamic acid	112
Glycine	103
Proline	66
Hydroxyproline	1
Serine	52
Tyrosine	37
Total non-essential amino acids	656
Essential amino acids	
Histidine	18
Isoleucine	43
Leucine	81
Lysine	57
Hydroxylysine	1
Methionine	13
Phenylalanine	27
Threonine	44
Tryptophan	1
Valine	59
Total essential amino acids	344
Total amino acids	1000

Similarly, Choi et al. (2017) found that the amino acid composition of defatted insect powders was higher after defatting (Ghosh, Lee, Jung, & Meyer-Rochow, 2017). F/BL also had a high quality amino acid profile and satisfied essential amino acid requirements for human nutrition, as do other edible insects (Choi et al., 2017; Ghosh et al., 2017; Köhler et al., 2019; Yi et al., 2013). Deficiency in some amino acids found in common diets, including cereals (Ghosh et al., 2017; Köhler et al., 2019), could be compensated by consuming F/BL, to cover some deficiencies in essential nutrients.

3.4.5. Mineral composition

Table 3.4 Minerals composition of defatted Bombay locust powder (F/BL)

Minerals	**Content (mg/100g)**
Calcium (Ca)	111.30 ± 4.79
Copper (Cu)	23.38 ± 0.56
Iron (Fe)	9.77 ± 1.04
Potassium (K)	1,265.00 ± 275.37
Magnesium (Mg)	72.40 ± 3.84
Manganese (Mn)	0.95 ± 0.17
Phosphorus (P)	78.01 ± 11.06
Zinc (Zn)	15.76 ± 1.86

Data are mean ± standard deviation (n=3).

The mineral composition of F/BL is shown in Table 3.4. K (1,265 mg/100g) was the major mineral, followed by Ca (111 mg/100g), P (78 mg/100g), and Mg (72 mg/100g). This was consistent with levels reported by Köhler et al. (2019), in which K, P, Ca and Mg were the major minerals. Fombong et al. (2017) noted that the three major minerals of dried grasshoppers (*Ruspolia differens*) were Ca (896 – 1,124 mg/100g), K (724 – 834 mg/100g) and P (611 – 694 mg/100g). K was also reported as the major mineral of black crickets (Dobermann, Field, & Michaelson, 2019) and scarab beetles and mulberry silkworms (Köhler et al., 2019). Other research consistently showed that insects were low in Ca due to the absence of an internal skeleton (Finke, 2013). Trace minerals were also present in significant amounts, especially Cu, Zn and Fe. The Recommended Daily Intake (RDI) of Cu, Zn and Fe for adults is 0.9 mg, 11 mg, and 18 mg respectively (FAO, 2001). This suggests that F/BL is a significant source of trace minerals, particularly Cu, Zn and Fe, which had levels above their relative RDI. Mn was another trace mineral, that was detected in F/BL at 0.95 mg/100g, but the Mn content of F/BL was lower than in other insects (Fombong et al., 2017; Köhler et al., 2019). However, F/BL there was significantly ($p \leq 0.05$) higher Mn levels than in meat (0.012 – 0.029 mg/100g) (Cobos & Díaz, 2015). Therefore, F/BL would be an excellent source of minerals in both macro and trace minerals and could be consumed to increase human dietary mineral intake.

3.4.6. Fortification of biscuit stick with Bombay locust powders

3.4.6.1. Characteristics of biscuit stick

The appearance and characteristics of biscuit stick fortified with Bombay locust powders (BL and F/BL) at different levels (5, 10 and 15%) are shown in Figure 3.2 and Table 3.5, respectively. Higher intensity of brownness with darker color was observed as an increase of insect powder fortification level for both BL and F/BL, where biscuit stick fortified with F/BL had more strength in brownness, compared with that of BL at the same level added (Figure 3.2). This result was in consistent with the color values of the biscuit stick (Table 3.5). Lightness significantly decreased with decreasing of L^* by adding insect powders in a dose-dependent manner ($p \leq 0.05$). In general, increasing of redness and yellowness occurred (as indicated by increasing of a^* and b^*) were observed, when a higher level of insect powder was incorporated. Moreover, BL addition gave biscuit sticks with significantly ($p \leq 0.05$) less redness and blueness, compared with those from F/BL at the same level of fortification, especially at 10 and 15% of insect powder added. Nevertheless, the difference of color between BL and F/BL fortified biscuit sticks tended to decrease with increasing insect powder added, indicated by decreasing the total differences of color (ΔE^*) and chroma (ΔC^*) of F/BL fortified samples. This was consistent with color of Bombay locust powders used (Table 3.1), in which F/BL had the lighter color with higher intensity in redness and yellowness, compared with those from BL (Table 3.1). In addition, difference of protein content of insect powder used could govern the generation of the browning color via Maillard reaction. Higher protein content of F/BL (Table 3.1) could favor the browning Maillard reaction during baking more than that of BL. In addition, insect pigments, and hydroperoxides-protein interaction might also contribute to the color of the final product (Sikorski, Pokorny, & Damodaran, 2008).

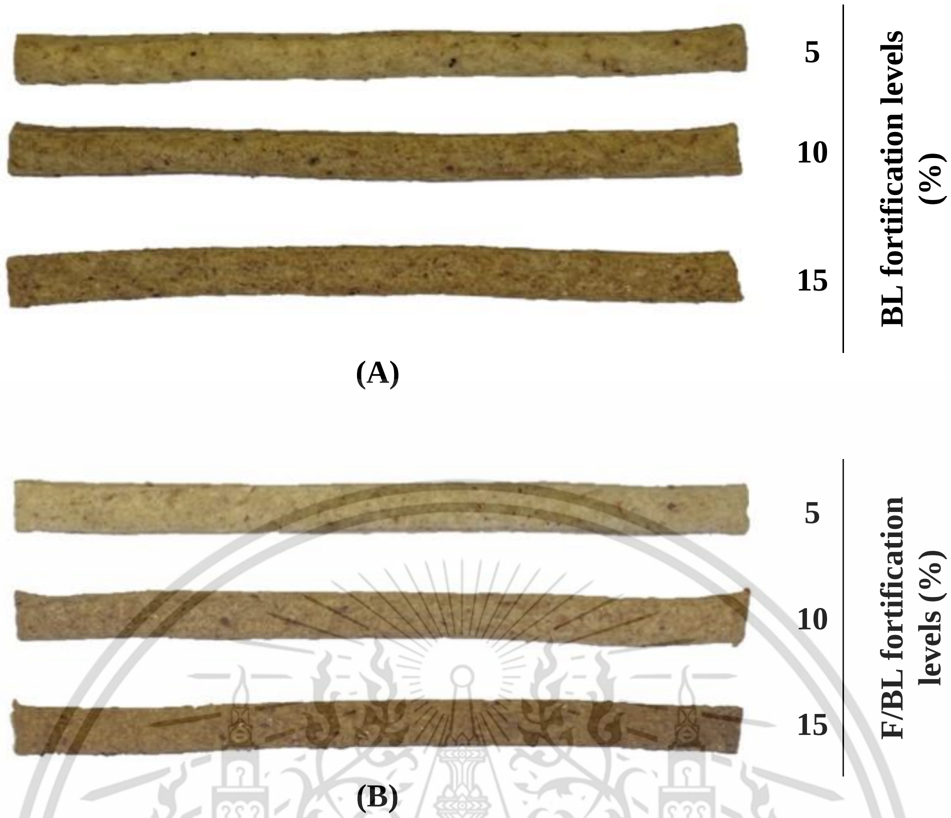

Figure 3.2 Appearance of protein-enriched short dough biscuit stick prepared using wheat flour fortified with Bombay locust powder at different levels. BL: Bombay locust powder without hexane defatting, F/BL: Bombay locust powder with hexane defatting, 5, 10, 15: Fortification levels with Bombay locust powder at 5, 10, 15%, respectively.

Fortification of the biscuits sticks with different levels of BL and F/BL affected their water activity (a_w) differently (Table 3.5). Decreasing a_w was observed with increasing level of insect powder used and BL fortified sample had significantly ($p \leq 0.05$) lower a_w levels compared to those from F/BL at the same level of fortification. This effect could indicate the dilution effect by replacement of wheat flour by Bombay locust powder, which reduced starch content and increased water insoluble chitin from the exoskeletons of the insect in biscuit dough. Less water could be then displaced by the remained starch granules during gelatinization, while the comparatively lower water-holding capacity of insect powder, particularly chitin fiber, could not bind or interact with water (Sun, Xing, Qiu, & Xiong, 2014). The lower protein level with a higher fat content of BL (Table 3.1) could hold and bind the water with lower capacity, compared with those of F/BL. Consequently, more water could easily escape during baking resulting a lower water content in the final product (Davidson, 2016). These differences on hydration capability also shown to contribute to the baking loss of biscuit differently (Table 3.1). Baking loss is measured as weight loss of the dough or batter during baking, especially the amount of evaporated water (Heo, Kim, Lee,

& Moon, 2019) The lowest baking loss was found from the lowest fortification level tested (5%) ($p \leq 0.05$), for both insect powders used. Baking loss tended to increase with increasing of insect powder levels. This observation was in line with the hysteresis phenomenon of baked product processing, where interaction of moisture content and other food components governed the amount of water loss during baking (Heo et al., 2019; van Nieuwenhuijzen et al., 2008). Increase of insect powder fortification level could increase the unbound water portion in biscuit dough resulting the higher water loss during baking.

3.4.6.2. Hardness

Hardness of biscuit sticks fortified with Bombay locust powders (BL and F/BL) at different levels (5, 10, 15%) is presented in Table 3.5. Hardness, or breaking force, is an important parameter affecting the eating quality and consumer acceptance of baked products, particularly biscuit (Adeola & Ohizua, 2018; Delicato et al., 2020). Specifically, higher BL fortification level significantly ($p \leq 0.05$) lowered hardness of biscuit sticks, while F/BL fortification provided contrary results. F/BL biscuit sticks showed significantly ($p \leq 0.05$) increasing hardness in a dose-dependent manner. Generally, a higher protein content in flour used in biscuits could lower the hardness of resulting biscuit (Adeola & Ohizua, 2018). Moreover, the reduction of the gluten network through its proportional replacement by insect powder, could also reduce the biscuit hardness (Bouazizi, Montevecchi, Antonelli, & Hamdi, 2020). In addition, several complex reactions involved in baked products, such as lipids content and oil droplet dispersion in biscuit structure, could govern the texture property of biscuit (Manley et al., 2011). The higher fat content of BL could work as a lubricant and rendered the loose texture by lowering the internal cohesion in the biscuit matrix (Moriano, Cappa, & Alamprese, 2018; Moriano et al., 2019). On the other hand, biscuits with a crumbly and crisp texture could be obtained from F/BL that contained a lower fat content (Table 3.1). Thus, fortification of biscuit sticks with Bombay locust powders having different chemical compositions, particularly fat and protein, impacted the texture property of the final product, particularly the hardness, differently.

3.4.7. Sensory characteristics

The sensory characteristics of biscuit sticks fortified with Bombay locust powder at different levels are shown in Figure 3.3. Increase of fortification level caused the decrease of liking score for all attributes tested, especially at the highest fortification level used, for both BL and F/BL added. It was noted that BL added biscuit sticks had less liking score than those of F/BL, compared at the same fortification level, particularly at 5 and 10% ($p \leq 0.05$)

(Figure 3.3). Nevertheless, there were no significant ($p>0.05$) differences in the liking score for appearance and color between BL and F/BL fortified biscuit sticks at the same level used. Delicato et al. (2020) reported that insect fats significantly ($p\leq0.05$) affected the sensory quality, when applying in baked products due to its bad and long last aftertaste, off-flavor and rancid aroma. The higher liking score for odor, taste, flavor and aftertaste attributes, of biscuit stick enriched with F/BL, reconfirm the improvement of volatile contents with less off-flavor of Bombay locust powder by hexane defatting (Table 3.2). However, panelists could not detect any differences between BL and F/BL added biscuit sticks at the highest fortification level used (15%) ($p>0.05$). This implied that the insect fortification level had reached the panelist's threshold for fortification application in biscuit stick. The present result showed that F/BL fortification at 5% (F/BL-5) and 10% (F/BL-10) provided the biscuit sticks with a good acceptance and obtained the liking score ≥7 for all attribute tested. This suggested 10% F/BL should be the maximum level of fortification to improve the nutrition value of biscuit sticks with satisfied sensory property. Therefore, F/BL-10 was selected for further studies on its chemical composition and microstructure in comparison with BL fortification at 10% (BL-10).

Table 3.5 Characteristics of biscuit stick fortified with Bombay locust powders at different levels.

Sample		Color					Water activity	Baking loss (%)	Hardness (N)
		L*	***a****	***b****	**ΔE***	**ΔC***			
BL	-5	67.33 ± 0.06^{aA}	7.55 ± 0.12^{cA}	31.15 ± 0.17^{bA}	-	-	0.321 ± 0.005^{aB}	23.27 ± 2.21^{bA}	12.15 ± 0.21^{aA}
	-10	60.89 ± 0.07^{bA}	9.42 ± 0.25^{bB}	31.42 ± 0.30^{bA}	-	-	0.161 ± 0.003^{bB}	28.38 ± 1.00^{aA}	10.75 ± 0.40^{bA}
	-15	55.47 ± 0.33^{cA}	10.79 ± 0.32^{aB}	32.55 ± 0.48^{aA}	-	-	0.072 ± 0.003^{cB}	29.44 ± 0.49^{aA}	9.46 ± 0.41^{cB}
F/BL	-5	67.97 ± 0.63^{aA}	6.91 ± 0.30^{cB}	27.05 ± 0.38^{cB}	4.23 ± 0.22^{a}	4.15 ± 0.29^{a}	0.501 ± 0.001^{aA}	23.15 ± 1.76^{bA}	8.38 ± 0.09^{cB}
	-10	58.72 ± 0.44^{bB}	10.52 ± 0.29^{bA}	29.40 ± 0.13^{bB}	3.21 ± 0.23^{b}	2.34 ± 0.10^{b}	0.266 ± 0.000^{bA}	29.29 ± 1.25^{aA}	8.93 ± 0.24^{bB}
	-15	53.63 ± 0.29^{cB}	11.57 ± 0.32^{aA}	30.29 ± 0.26^{aB}	3.03 ± 0.13^{b}	2.40 ± 0.24^{b}	0.137 ± 0.000^{cA}	29.43 ± 2.60^{aA}	12.51 ± 0.01^{aA}

Data are mean ± standard deviation (n=3).
BL: Bombay locust powder without hexane defatting.
F/BL: Bombay locust powder with hexane defatting.
5, 10, 15: Fortification levels with Bombay locust powder at 5, 10, 15%, respectively.
Different lowercase superscript letters in the same column indicate significant differences comparing between fortification levels using the same insect powder used ($p \leq 0.05$).
Different uppercase superscript letters in the same column indicate significant differences comparing between BL and F/BL at the same fortification level ($p \leq 0.05$).

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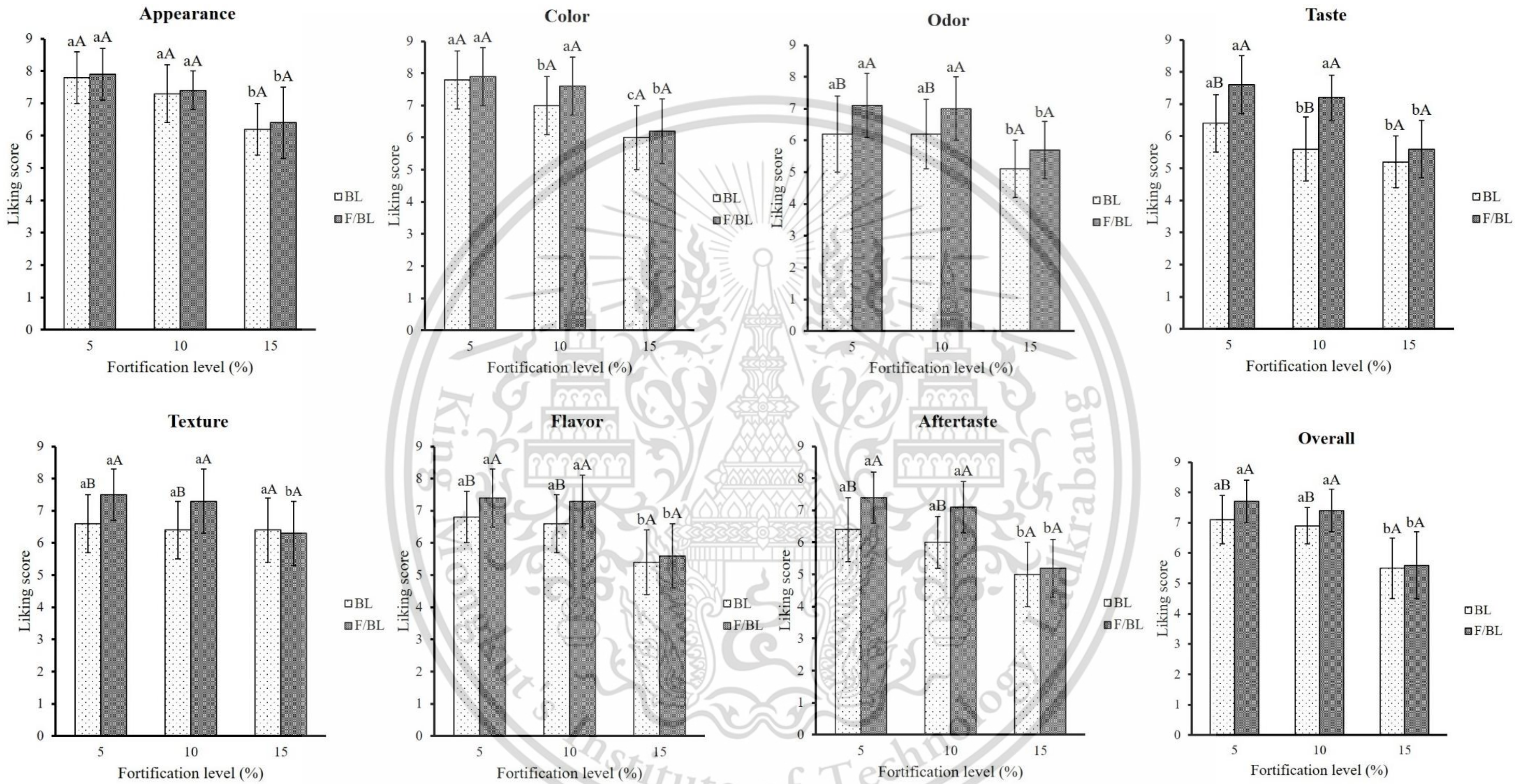

Figure 3.3 Sensory characteristics presented as liking score of biscuit stick prepared using wheat flour fortified with Bombay locust powder at different levels. BL: Bombay locust powder without hexane defatting, F/BL: Bombay locust powder with hexane defatting, 5, 10, 15: Fortification levels with Bombay locust powder at 5, 10, 15%, respectively. Different lowercase letters indicate significant difference comparing between fortification levels using the same insect powder used ($p \leq 0.05$). Different uppercase letters indicate significant difference comparing between insect powders used at the same fortification level ($p \leq 0.05$).

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3.4.8. Chemical composition and energy value

Table 3.6 Nutritional value of biscuit stick fortified with 10% Bombay locust powders.

Parameter	**BL-10**	**F/BL-10**
Protein (g/100g)*	14.72 ± 0.74[b]	16.34 ± 0.66[a]
Total fat (g/100g)	15.90 ± 0.84[a]	10.20 ± 0.81[b]
Total carbohydrate (g/100g)	60.46 ± 2.11[a]	64.03 ± 1.73[a]
Total sugar (g/100g)	2.21 ± 0.14[a]	1.60 ± 0.07[b]
Ash (g/100g)	1.65 ± 0.09[a]	1.72 ± 0.09[a]
Sodium (mg/100g)	428.07 ± 10.44[a]	446.25 ± 10.25[a]
Moisture (g/100g)	7.27 ± 0.73[a]	7.71 ± 0.67[a]
Cholesterol (mg/100g)	24.23 ± 1.63[a]	8.03 ± 0.46[b]
Energy value (kcal/100g)	443.82 ± 7.41[a]	413.28 ± 6.44[b]

Data are mean ± standard deviation (n=3).
*The conversion factor used is 6.25.
BL-10: Short dough biscuit stick fortified with 10% Bombay locust powder without hexane defatting.
F/BL-10: Short dough biscuit stick fortified with 10% Bombay locust powder with hexane defatting.
Different lowercase superscript letters in the same row indicate significant differences ($p \leq 0.05$).

Table 3.6 shows the chemical composition and energy value of biscuit stick fortified with 10% F/BL (F/BL-10) in comparison with those of 10% BL (BL-10). The higher protein level (16.3 g/100g) with a lower fat (10.2 g/100g) level were found for F/BL-10, and were significantly higher ($p \leq 0.05$) than BL-10, which was in consistent with the chemical compositions of the relevant insect powder (Table 3.1). Hexane defatting produced the insect powder (F/BL) with the higher protein and lower fat content. In addition, it reconfirmed that F/BL contained chitin form exoskeletons as the main polysaccharides, where sugar constituents would be lost during defatting. F/BL-10 had significantly ($p \leq 0.05$) lower total sugar with higher total carbohydrate content, compared with those of BL-10. However, there was no significant difference ($p \leq 0.05$) for ash (1.6 – 1.7 g/100g), sodium (428.1 – 446.2 g/100g) and moisture (7.3 – 7.7 g/100g) contents comparing between BL-10 and F/BL-10. Cholesterol and energy value of F/BL-10 were lower than BL-10 significantly ($p \leq 0.05$). The lower amount of sodium and cholesterol in food product would be beneficial for consumer (WHO, 2012). The Daily Recommended Value (DRV) for sodium is 2,300 mg/day for adults. Also, it was suggested that the sodium consumption < 2 g/day (equivalent to 5 g salt/day) for adults would lower the risk of chronic diseases. By consuming 100 g of enriched biscuit sticks, it could fulfill 18.6 – 19.4% DRV of sodium. Some biscuit ingredients contributed to the sodium content of enriched biscuit sticks, i.e. baking soda, baking powder, flavoring. Moreover, the cholesterol was found lower in F/BL-10 than BL-10. This implied that the removed lipids by hexane defatting included with cholesterol. The biscuit sticks fortified with Bombay locust powders from the present study could be claimed as 'high source' of protein, which could fulfill over 20% of the Nutrient Reference Values (NRV). Furthermore, they were 'low cholesterol' with less than 20 mg/100g sample (CAC, 1997). Overall, hexane defatting could not only augment nutritional value, oxidation stability and volatile content of Bombay locust powder but also enhance the efficiency of its application for improving nutritional value in baked products, particularly biscuit stick.

3.4.9. Microstructure

The cross-sectional microstructure of biscuit sticks fortified with 10% BL (Figure 3.4 (A and B)) and F/BL (Figure 3.4 (C and D)) show the void and network distributed in biscuit stick matrices. In BL-10, there was a continuous structure with a uniform and tight network, while F/BL-10 had a wider void and more porosity than BL-10. Gluten network formation of baked products is governed by the proportion of chemical components particularly protein, starch, water and fat, where the distribution of the voids in the biscuit structure determine towards texture and bite properties (Kumar et al., 2019). Mixing insect powder with wheat flour could interrupt gluten development and dough expansion during baking due to its water-soluble protein and exoskeleton chitin particles. The higher protein of F/BL could limit the swelling and water absorption capability of starch granules, where gelatinized starch molecules could be incorporated difficulty within a stiff protein network (Dauda, Abiodun, Arise, & Oyeyinka, 2018). In addition, chitin fibers could interfere with the network formation to form uniform strands within biscuit matrices. This result was in accordance with the hardness of fortified biscuits sticks (Table 3.5). F/BL-10, with less interconnected structure between the strands and higher pore areas, had significantly ($p \leq 0.05$) lower hardness than BL-10, which had a denser structure. Therefore, F/BL fortification impacted the microstructure of the resulting biscuit sticks, which determined the texture and eating quality properties of the final product.

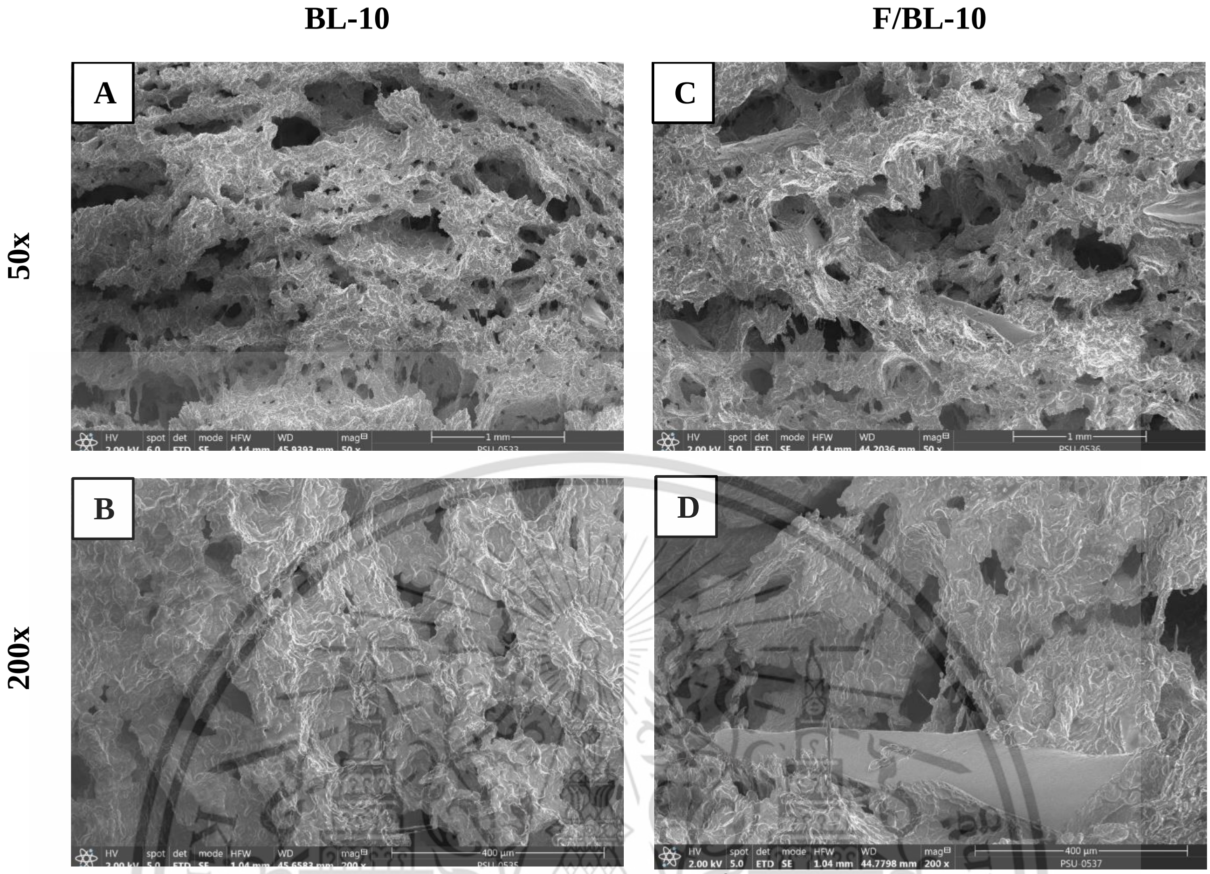

Figure 3.4 Scanning electron microscopic images of biscuit stick prepared using wheat flour fortified with 10% Bombay locust powder. BL-10: Biscuit stick fortified with 10% Bombay locust powder without hexane defatting, F/BL-10: Biscuit stick fortified with 10% Bombay locust powder with hexane defatting, A and C; 50× magnification, B and D; 200× magnification

3.5. Conclusion

Hexane defatting impacted on the physicochemical and nutritional properties of Bombay locust powder (F/BL) by successfully removing lipids, providing concentrated protein content with high protein recovery and giving better oxidative stability. Moreover, most off-odors were effectively removed from the BL by hexane defatting. F/BL was a good source of amino acids and minerals. Biscuit sticks fortified with F/BL obtained better sensory properties than those of BL, where 10% F/BL could improve its nutritional level without detrimental effects on their acceptability.

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CHAPTER 4

Quality characteristics of protein-enriched brown rice flour and cake affected by Bombay locust (*Patanga succincta* L.) powder fortification

4.1. Abstract

Protein-enriched brown rice flours were developed by fortification of defatted Bombay locust powder (F/BL) at different levels (10, 20, 30%). The physicochemical characteristics of the mixed flours were then investigated. The application of resulting mixed flour on preparing protein-enriched cake was also studied. The chemical composition, water activity, color as well as rheological property of the resulting mixed flours were varied at different levels of F/BL added. The protein content of resulting mixed flours was effectively improved, especially at 30% replacement provided almost 4-fold increased (11 – 42%), compared with control (BRF without F/BL fortification) ($p \leq 0.05$). Protein-enriched cakes prepared using the mixed flours, showed the differences in their physicochemical properties, texture profiles, chemical composition and sensory characteristics, which were influenced by BL fortification level. The 20% replacement of brown rice flour by F/BL (20-MF) provided a protein-enriched cake (20.8% of protein) with liking score ranged from 7.0 to 7.4 for all attributes tested, indicating the good acceptability. Scanning electron microscopy (SEM) of the 20-MF cake revealed that it had a denser structure with the less porosity than the control. Therefore, F/BL might be a promising alternative protein source for developing protein-enriched flour and baked product, particularly cakes.

4.2. Introduction

Recently, protein content is a leading concern of food product development, where the promote of non-animal-based protein sources has brought about enthusiasm for plant- and insect-based protein (Zielińska, Karaś, & Baraniak, 2018). Insects are becoming one of the most important alternative protein sources and they emerge as a solution limited for protein production. More than 1,900 insect species have been used as food and ~2 billion people eat insects regularly (Van Huis et al., 2013). Moreover, they contain high nutrients contents, especially protein (15 – 82% dry basis) and fat (4 – 77% dry basis) (Kim, Weaver, & Choi, 2017; Köhler, Kariuki, Lambert, & Biesalski, 2019; Kouřimská & Adámková, 2016; Paul et al., 2017).

Bombay locust (*Patanga succincta* L.) is commonly marketed insects in the southwest and southeast Asia (Yen, 2015). Köhler et al. (2019) reported that Bombay locust contains the highest protein content (36% wet basis) with both essential and non-essential amino acids compared to scarab beetle, house cricket and mulberry silkworm. However, insect-based food products acceptance is a great challenge due to the unique sensory characteristics including taste, flavour, texture and appearance of insect. Insect powder is an interesting and effective way to tackle those psychological and palatability factors, such as disgusting and food fear of the unusual for eating insects (Attila, Ryan, Dalma, & Howard, 2017; Dobermann, Swift, & Field, 2017). Fat removal, by using various type of solvents, is one optional in insect powder processing that has been applied for concentrating protein content and improving lipid oxidation stability (Ahmed et al., 2016; Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016). Moreover, some pigments and off-odor compounds could be eliminated, during solvent defatting (Ribeiro et al., 2019). It was reported that fat removal by hexane led to a less brownish insect powder (Bußler et al., 2016; Mishyna, Martinez, Chen, & Benjamin, 2019). In addition, some pheromones, which was responsible for the taste and odor of insects, could also be removed (Vogt, 2005). The protein enrichment level could be then maximized by adding larger proportions of insect powder, with less offensive properties, to the final products. Several edible insect powders have been developed as alternative protein source, marketed as insect flour or insect protein-enriched wheat flour (Azzollini, Derossi, Fogliano, Lakemond, & Severini, 2018; Campbell, Euston, & Ahmed, 2016; Terry, Lupul, & Coate, 2017).

Rice (*Oryza sativa* L.) flour is a commonly consumed gluten-free cereals. Brown rice or unpolished rice is rich in phytochemicals (Mohan et al., 2014). Brown rice flour (BRF) contains a higher fiber (1.2% (w/w) dry basis) but lower protein content (8.5 – 9.5% (w/w) dry basis), compared with wheat flour (12.6% protein, 0.85% fiber, (w/w) in dry basis) (Khoshgozaran-Abras, Azizi, Bagheripoor-Fallah, & Khodamoradi, 2012; Mohan et al., 2014). Fortification improved protein content and quality of BRF food products, especially baked products (de la Hera, Martinez, Oliete, & Gómez, 2013; de Oliveira, da Silva Lucas, Cadaval, & Mellado, 2017; Gadallah, 2017; Islam, Taneya, Shams-Ud-Din, Syduzzaman, & Hoque, 2012). Protein enrichment, from both vegetable and animal sources, noticeably impacted on characteristics and quality of the final product, such as color, texture, batter rheology and sensory properties (Gadallah, 2017; González, Garzón, & Rosell, 2019). The level of protein enrichment, especially in the form of mixed flour, should be optimized to obtain good nutritional value and satisfactory product quality. Therefore, it is congenial to utilize Bombay locust powder for protein enrichment of BRF, in which the nutritional value, especially protein content, was improved in a practical application in baked products. The aim of the present work is to develop and characterize a protein-enriched brown rice flour and cake, fortified of defatted Bombay locust powder (F/BL), where the enrichment level was optimized. The characteristics and pasting properties of resulting mixed flour were then measured. The application of mixed flour in cake model was studied in terms of cake batters and cake products were also analyzed.

4.3. Materials and methods

4.3.1. Materials

Brown Rice Flour (BRF) was procured from Bangyai Supply Limited, Nonthaburi, Thailand. Corn flour (Kitch, King Milling Co. Ltd, Samut Prakan, Thailand), canola oil (Naturel, Lam Soon Public Co., Ltd, Samut Prakan, Thailand), baking powder (Imperial, KCG Corporation Co., Ltd, Bangkok, Thailand) and other ingredients were purchased from a local market in Latkrabang, Bangkok, Thailand.

4.3.2. Preparation of defatted Bombay locusts powder

Frozen Bombay locusts (*Patanga succincta* L.) were obtained from a commercial supplier (Mr. BUC FOOD, Phra Nakhon Si Ayutthaya, Thailand). The samples were thawed by submerging under cold tap water until the core temperature of sample reached to 4 – 5°C. The insects were then washed with running tap water and drained using a plastic sieve. Then, it was packed in polyethylene (PE) bag (1 kg/pack) and kept in a deep freezer (DW-40L262, Haier, Qingdao, China, -40°C, 24 h) before freeze-drying (CoolSafe 55 Freeze dryer, ScanLaf A/S, Lynge, Denmark) under vacuum (2.1 mPa, -50°C, 48 h). The dried sample was ground into powder using a laboratory grinder (MF-800, Tefal®, France) and sieved using a 10-mesh (2 mm) screen. The obtained powder (29.8% fat) was then subjected to fat removal following Bußler et al. (2016) with slight modification. Briefly, sample powder was mixed with hexane with a ratio of 1:5 (w/v) and continuously stirred for 3 h. The solvent was changed every hour, three times in total. After draining, the sample was dried on an aluminium foil tray under a fume hood. The resulting Bombay locust powder was referred to as ‘F/BL’. Composition of F/BL was included 78.7% protein, 5.0% carbohydrate, 11.8% moisture, 3.3% ash, and 1.2% fat, in wet basis.

4.3.3. Preparation of protein-enriched brown rice flour by Bombay locust powder fortification

Different levels of BL were mixed with BRF to obtain final concentrations at 10%, 20% and 30% of mixed flour (w/w). After mixing, the mixed flours were ground using a pin mill (ZM-200, Retsch®, Germany, 6,000 min^{-1}, 0.25 mm sieve), resultant particles were < 40 μm in diameter. The resulting mixed flours with 10%, 20% and 30% BL replacement were referred to as '10-MF', '20-MF', and '30-MF', respectively. The BRF with 0% F/BL was used as the control, 'Control'.

4.3.4. Impact of Bombay locust powder fortification on characteristics of brown rice flour

4.3.4.1. Chemical compositions and fiber content

Protein, fat, moisture, ash and fiber content (% wet basis) were determined according to AOAC methods (AOAC, 2000). Total carbohydrate (% wet basis) content was calculated by subtracting the sum of protein, fat, moisture and ash from the total sample weight.

4.3.4.2. Water activity (a_w) and color

Water activity (a_w) of sample was measured using a water activity meter (Aqua Lab 4TE, SN S40002336, Decagon Devices, USA). The color of sample was measured as L^* (lightness), a^* (redness) and b^* (yellowness), in triplicates, using a colourimeter (CR-400 Chroma Meter, Konica Minolta Sensing Americas, Inc., USA). Total differences of color (ΔE^*) and chroma (ΔC^*) was then calculated following Benjakul and Karnjanapratum (2018).

4.3.4.3. Pasting properties

The samples were subjected to microvisco-amylograph tests (UNI 10872:2000), according to international standard methods, using the Brabender microvisco-amylograph (Viscograph-E 803301, Brabender Measurement & Control Systems, Germany) analyzed their pasting properties (Taccari et al., 2016).

4.3.5. Application of protein-enriched brown rice flour by Bombay locust powder fortification for preparing cake product

4.3.5.1. Preparation of brown rice cake batter

Mixed flours with different replacement levels of F/BL (10-MF, 20-MF and 30-MF) were used for preparing the cake. The formulation used for cake preparation followed the traditional rice flour cake recipe and the procedure of Qasem et al. (2017b) with slight modification (Table 4.1). Cake formulation had of two main parts, including pre-mix batter and egg white foam. The protein-enriched cakes were prepared in the same manner but the mixed flour was used instead of BRF. The batter was subjected to analyze on the rheological properties.

a. *Rheological property*

The viscoelastic properties of cake batters were investigated by measure elastic modulus (G′) using a rheometer (HAAKE RheoStress 1, Thermo Fisher Scientific, Karlsruhe, Germany), following Hesso et al. (2015).

Table 4.1 Brown rice cake recipe.

Ingredients	Baker's percentage (%)*	Amount (g)**
Pre-mix batter		
Rice flour	100	108
Corn flour		36
Salt	0.3	0.4
Baking powder	0.1	0.2
Egg yolks	62.5	90
Milk	68.8	99
Canola oil	43.8	63
Vanilla extract	3.5	5
Cinnamon powder	3.5	5
Egg white foam		
Egg white	125	180
Sugar	78.5	113
Cream of tartar	0.3	0.4

*based on total weight of flours.
**based on total weight of batter weight.

4.3.5.2. Preparation of brown rice cake

The cake batter (70 g) was poured into rectangular baking pan (166×35×29 mm^3) and baked (180°C, 40 min) in a preheated oven (Model YXD-10A, South Star®, Canada). After baking, the cakes were removed from the baking pan and allowed to cool at room temperature (25 – 28°C) for 3 h. Then, they were packed in a zip lock bags and kept at 25°C until used for characterization and sensory property analysis within 24 h.

4.3.5.3. Characterization of Bombay locust protein-enriched brown rice cakes

a. Water activity (a_w) and color

Determination of water activity and color measurement of crust and crumb was determined as described in section 4.3.4.2.

b. Cake quality

Cake qualities including baking loss, dimensions (specific volume, cake volume and density) and physical properties (volume index, uniformity and symmetry), were determined according to the AACCI method 10-91 (AACC, 2010).

4.3.5.4. Texture profile analysis

Texture profiles of cake crumbs were determined, using a texture analyzer (TA.XT.plus®, Texture Technologist Corp., USA), following AACCI method 74-09 (AACC, 2010).

4.3.6. Chemical compositions and energy value

Protein, fat, moisture, ash and total carbohydrate contents (% wet basis) were determined according to AOAC methods - see section 4.3.4.1. Energy value was calculated according to the Atwater system (Merrill & Watt, 1973).

4.3.7. Sensory evaluation

Sensory properties were evaluated on a hedonic 9-point scale with 30 untrained panelists for all protein-enriched and control cakes. Cakes properties were appearance, color (crust and crumb), aroma, taste, texture, flavour, after-taste and overall acceptability. All of the cakes were evaluated in random orders to the panelists. During the evaluation, panelists rinsed their mouths with water before-and-after sample testing.

4.3.8. Scanning Electron Microscopy (SEM)

Based on sensory evaluation of cakes, a brown rice cake made from 20-MF was selected for analysis of its microstructure, in comparison with control (without F/BL). The cake microstructure was captured using a scanning electron microscopy (SEM) with low-vacuum mode and SED detector. The specimens were visualized with a scanning electron microscope (Quanta 250, FEI, Eindhoven, Netherlands) at an acceleration voltage of 10 kV.

4.3.9. Statistical analysis

Experiments were run in triplicate. Resulting data were subjected to analysis of variance. Comparison of means was analyzed using Duncan's multiple range tests at confidence level of 0.05 (Steel & Torrie, 1986). Statistical analysis was performed using

Statistical Package for Social Science (IBM SPSS Statistics 25, IBM, New York, USA).

4.4. Results and discussion

4.4.1. Impact of F/BL fortification on characteristics of protein-enriched brown rice flour

4.4.1.1. Chemical composition and fiber content

Chemical composition and fiber content of brown rice flour, fortified with defatted Bombay locust powder (F/BL) at different replacement levels (10, 20 and 30%), are shown in Table 4.2 in comparison with BRF without fortification (control). Carbohydrate (72.4%) was the major component of BRF, followed by moisture (12.8%) and protein (11.5%) with minor amounts of fat and ash content. Fortification of F/BL markedly increased the protein content of BRF in a dose-dependent manner ($p \leq 0.05$) and the highest protein content was obtained from 30-MF (42.1%) by a 4-fold increase the control. Apart from protein content (78.7%) of F/BL, the significant amounts of fat (1.2%) and ash (3.3%) contents were also observed. Thus, fat and ash contents notably increased in the mixed flours. The lower carbohydrate and moisture contents were found in F/BL fortified flour, which indicated the dilution effect of BRF content with increasing of dry matter from insect powder. As an alternative gluten-free mixed flour provision, flour quality should be considered, as well as a food safety concerns and consumer acceptance. Moisture content is one of the quality parameters that has important roles in some reactions, especially for microbial growth. A high moisture of rice grain and flour leads to food spoilage, generally due to fungal growth, with shortened shelf life (Abdullah, Nawawi, & Othman, 2000). According to CAC (1985), the resulting mixed flours met the standard of flours for moisture content, which did not exceed 15.5% (w/w). The current international Recommended Dietary Allowance (RDA) for protein is 0.8 g/kg of body weight, regardless of age (WHO, 2007). The Daily Reference Values (DRVs) of protein for adult is 50 g based on the reference of caloric intake of 2,000 calories, while the reference nutrients intake is set at 0.75 g protein per kg body weight per day for adult (FDA, 2016b). The consumption of F/BL fortified brown rice flour at the reference amount customarily consumed (30 g) is sufficient to meet 10% of DRV of protein (FDA, 2016a, 2016b). Therefore, the resulting mixed fours meet the FDA requirement for protein-enriched flour (FDA, 2016b). Gluten-free composite flour blends have been developed by using rice flour, potato starch, cassava starch, millet flour and corn flour (Quiñones, Macachor, & Quiñones, 2015). Nevertheless, those composite flours had low protein content, compared with commercial wheat flour (12.58%). The protein has been enriched in flours by adding different protein sources, e.g. egg white, soy and beans as well as insect powder (Campbell et al., 2016; da Rosa Machado & Thys, 2019; de Oliveira et al., 2017; Majzoobi, Ghiasi, Habibi, Hedayati, & Farahnaky, 2014; Osimani et al., 2018; Singh, Benjakul, & Karnjanapratum, 2019). da Rosa Machado and Thys (2019) developed protein-enriched gluten-free breads, using cricket powder, buckwheat and lentil flours, added to rice flour. Cricket powder fortification provided the bread with higher protein content (12.5%), compared to others (6.2 – 7.1%). In addition, the fiber content of mixed flours was gradually increased with F/BL level ($p \leq 0.05$) (Table 4.2). This result was consistent with the composition of F/BL, a good source of insoluble fiber from chitin exoskeletons (9.6%) (Rumpold & Schluter, 2015). Osimani et al. (2018) found that enriched breads, incorporated with cricket powder, were considerably richer in fiber (2.6%), especially at 30% of wheat flour substitution, compared to control breads. Therefore, F/BL was an effective protein source for improving the nutritional value of brown rice flour, particularly protein content.

4.4.1.2. Water activity (a_w) and color

Table 4.2 shows water activity and color of protein-enriched brown rice flours fortified with different levels of F/BL. Water activity of all mixed flours, ranging from 0.491 to 0.525, was comparable ($p > 0.05$). Generally, microbes, especially fungi, could not grow at a_w less than 0.62 – 0.70 and the safe storage level is considered at 0.65 of a_w could maintain the moisture content for long-term storage (Abdullah et al., 2000). F/BL notably contributed to change in color of mixed flours ($p \leq 0.05$). F/BL fortification decreased L^* (lightness), but increased a^* (redness) and b^* (yellowness) of mixed flour, with increase in F/BL replacement. This indicated the effect of the natural F/BL brown color (L^*: 54.38, a^*: 7.68, b^*: 18.37). Color differences between the mixed flours and the control (BRF) were evaluated through a total difference of color (ΔE^*) and chroma difference (ΔC^*). Both ΔE^* and ΔC^* increased with F/BL concentration in a dose-dependent manner ($p \leq 0.05$). In general, the color of baked products was directly related to the color of the raw materials used. The presence of mealworm and black soldier fly larvae and cricket in the wheat flour led to brownish bread crumbs, consistent with decreased L^* and increased b^* observed in mixed flour by González et al. (2019). Therefore, adding F/BL contributed into the color changes of the protein-enriched brown rice flour, which could influence the color of the final product prepared by using these mixed flours.

Table 4.2 Characteristics of protein-enriched brown rice flours fortified with defatted Bombay locust powder at different levels.

Characteristics	Control	10-MF	20-MF	30-MF
Mixed flour appearance				
				
Chemical composition				
Protein (%)*	11.47 ± 0.10^{d}	21.00 ± 0.30^{c}	31.11 ± 0.31^{b}	42.11 ± 0.46^{a}
Fat (%)	1.81 ± 0.16^{c}	2.78 ± 0.21^{b}	3.06 ± 0.02^{ab}	3.29 ± 0.08^{a}
Ash (%)	1.47 ± 0.01^{d}	1.67 ± 0.01^{c}	1.85 ± 0.01^{b}	2.04 ± 0.01^{a}
Moisture (%)	12.85 ± 0.06^{a}	12.44 ± 0.02^{b}	12.38 ± 0.02^{b}	11.84 ± 0.10^{c}
Carbohydrate (%)	72.39 ± 0.25^{a}	62.10 ± 0.44^{b}	51.60 ± 0.63^{c}	40.72 ± 0.66^{d}
Fiber content (%)	1.59 ± 0.07^{d}	2.62 ± 0.20^{c}	3.57 ± 0.15^{b}	5.04 ± 0.09^{a}
Water activity	0.525 ± 0.008^{a}	0.502 ± 0.009^{b}	0.509 ± 0.010^{ab}	0.491 ± 0.014^{b}
Color				
*L**	88.29 ± 0.14^{a}	74.04 ± 0.03^{b}	68.28 ± 0.08^{c}	65.32 ± 0.08^{d}
*a**	0.87 ± 0.02^{d}	5.02 ± 0.07^{c}	6.39 ± 0.03^{b}	7.20 ± 0.11^{a}
*b**	12.44 ± 0.14^{d}	14.72 ± 0.09^{c}	16.06 ± 0.16^{b}	17.30 ± 0.18^{a}
ΔE*	-	15.01 ± 0.16^{c}	21.06 ± 0.12^{b}	24.32 ± 0.20^{a}
ΔC*	-	4.74 ± 0.07^{c}	6.60 ± 0.04^{b}	7.98 ± 0.19^{a}
Pasting properties				
Pasting temperature (°C)	72.9 ± 0.1^{b}	87.2 ± 0.5^{a}	50.2 ± 0.0^{c}	50.3 ± 0.0^{c}
Peak viscosity (BU)	927.5 ± 15.5^{a}	22.0 ± 0.0^{b}	12.0 ± 0.0^{b}	12.0 ± 0.0^{b}
Breakdown viscosity (BU)	478.0 ± 9.0^{a}	7.0 ± 0.0^{b}	2.0 ± 0.0^{b}	2.0 ± 0.0^{b}
Setback viscosity (BU)	507.0 ± 6.0^{a}	15.0 ± 0.0^{b}	5.0 ± 0.0^{bc}	2.0 ± 0.0^{c}
Final viscosity (BU)	958.0 ± 0.0^{a}	30.5 ± 1.5^{b}	15.0 ± 0.0^{c}	12.0 ± 0.0^{d}

Data are expressed as mean ± standard deviation (n=3).
*The conversion factor used is 6.25.
Control: Brown rice flour (BRF) without defatted Bombay locust powder (F/BL) fortification.
10-, 20-, 30-MF: Mixed brown rice flour fortified with F/BL at 10, 20, 30%, respectively.
Different lowercase superscripts in the same row indicate significant difference ($p \leq 0.05$).

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4.4.1.3. Pasting properties

Pasting properties of flours describe the behaviour changes of flour paste viscosity along with a change in temperature, which varies mainly with their composition and characteristics of starches and proteins (Shevkani, Kaur, Kumar, & Singh, 2015; Singh et al., 2014). The effect of F/BL on pasting properties of protein-enriched brown rice flour is shown in Table 4.2. Pasting temperature indicates the minimum temperature required for flour cooking and starch gelatinization temperature (Iwe, Onyeukwu, & Agiriga, 2016). The highest PT was found for 10% of F/BL replacement (10-MF) ($p \leq 0.05$). The control (BRF) had 72.9°C of PT, which was lower than that of 10-MF (87.2°C). PT could be increased as the moisture content decreased (Sun, Xing, Qiu, & Xiong, 2014), where water-soluble proteins from F/BL, especially at 10% replacement, could displace water proportion during gelatinization. On the other hand, significant decrease of PT was observed from 20 – 30% of F/BL addition, which might be caused by decreased starch content in mixed flour. The similar result was reported by Shevkani et al. (2015) who studied the impact of cowpea (*Vigna unguiculate*) protein enrichment in rice flour. PT was also governed by binding efficiency of starch pastes, related to released amylose/amylopectin from starch grains, and its chain length as well as other molecular components (Kumar & Khatkar, 2017). BL increment markedly changed behavior of flour paste viscosity with decreased peak viscosity (PV), breakdown viscosity (BV), setback viscosity (SV) and final viscosity (FV). PV defines the swelling level or water-binding capacity of starch during the heating process. Thus, BV will be reached when the viscosity is dropped afterward, which indicates starch stability degree during cooking (Wani et al., 2012). SV measures the tendency of starch retrogradation upon cooling of cooked starch pastes (Gadallah, 2017). FV indicates the viscous paste formation ability of flours upon cooling (Shevkani et al., 2015). Remarkable decrease of PV and SV of mixed flours was observed, compared with the control ($p > 0.05$). In addition, FV and SV were drastically reduced with F/BL addition as dose dependent manner. Jane et al. (1999) found that a high value of PV and BV was caused by the presence of high amylose content and high proportions of very long amylopectin branch chains. Complete gelatinized starch granules in rice flour paste, had not only increased viscosity due to the leached-out linear molecules but also the lost crystallinity (Kim & Shin, 2014). Lower SV and FV, indicated lower retrogradation and staling tendency of rice flour-based products during storage, *vice versa* (Gani, Wani, Masoodi, & Salim, 2013; Thiranusornkij, Thamnarathip, Chandrachai, Kuakpetoon, & Adisakwattana, 2018). The control flour (100% BRF) obtained the highest SV and FV which was 958 BU and 507 BU, respectively.

The increment of protein addition level caused the reduction of PV, BV, SV and FV of rice flour (Shevkani et al., 2015). Protein, lipid and insoluble chitin from exoskeleton contents, inhibit water permeation into starch granules, so that results in lowering swelling capability of starch granules (Li & Zhu, 2017). Decreasing of BV, might reveal more proteins from BL fortification, provided the starch granules with higher resistance to disintegrate at high temperature (Shevkani et al., 2015). F/BL addition might inhibit starch retrogradation of BRF. The paste viscosities of flours are mainly influenced by their starch properties. Apart of starch dilution effect from F/BL replacement, not only protein content but also fat, ash and fiber contents (Table 4.2), could govern the amount of water absorbed by rice flour granules, consequently, demonstrated their pasting properties (Martin & Fitzgerald, 2002). Thus, pasting properties of BRF was greatly affected with extensively declined by F/BL addition, which could contribute to the feasibility of resulting mix flours for preparing the baked products.

4.4.2. Application of protein-enriched brown rice flours by F/BL fortification on the preparation of cake products

4.4.2.1. Rheological property of protein-enriched brown rice cake batter

Elastic modulus (G′) refers to elastic behavior and reflects deformation energy stored in the sample while it is sheared. This viscoelastic property predominantly contributes to structure changes, especially during baking, which could define the structure formation and final texture of baked products (Hesso et al., 2015; Shevkani et al., 2015). Viscoelastic properties of brown rice cake batter without and with F/BL fortification at various substitution levels (10 – 30%) during heating from 25 – 95°C, are illustrated in Figure 4.1. The batters with different viscosity were obtained by adding different levels of F/BL. Increasing of G′ was observed with increased F/BL replacement level, especially at 20% (20-MF) and 30% (30-MF) replacement. The greater viscoelasticity of the batters might be attributed to the higher water-binding/absorption capacity that reduced the availability of free water to facilitate the particles movement in the batter (Hesso et al., 2015; Shevkani et al., 2015). The constant G′ was observed for all batters tested at the first region of heating (25 – 40°C). The G′ was then increased continuously as increased temperature. The control (BRF) and mixed flours had a similar trend of G′ changed during heating. Nevertheless, the sharp increase of G′ was found from the control at 45 – 75°C, which was mainly affected by the presence of a higher amount of carbohydrate or starch content. This was consistent with its pasting properties, which showed the highest peak viscosity (Table 4.2) at 72.9°C, compared with others. The lower changing rate of G′ could be observed from mixed flours, especially 20-MF and 30-MF, which related with their lower pasting viscosity. Although 30-MF batter showed the highest G′ from the first region, the highest G′ was reached for all sample tested at the end of the heating process (80 – 88°C) with similar viscoelasticity. These characteristics revealed the protein denaturation, starch gelatinization as well as structure fixation (Lee, Inglett, & Carriere, 2004). This viscoelasticity difference was determined by an interaction between ingredient used in cake batter. Singh et al. (2019) studied different level egg white powder (EWP) replacement using ultrasonicated squid ovary powder (USOP) in cake batter. They found that USOP inclusion improved the viscoelastic properties of cake batter as well as strengthened its structure during baking. Therefore, the F/BL fortification to brown rice flour markedly affected the viscoelasticity of resulting cake batter, which mediated the structure formation of cake during baking as well as the final cake properties.

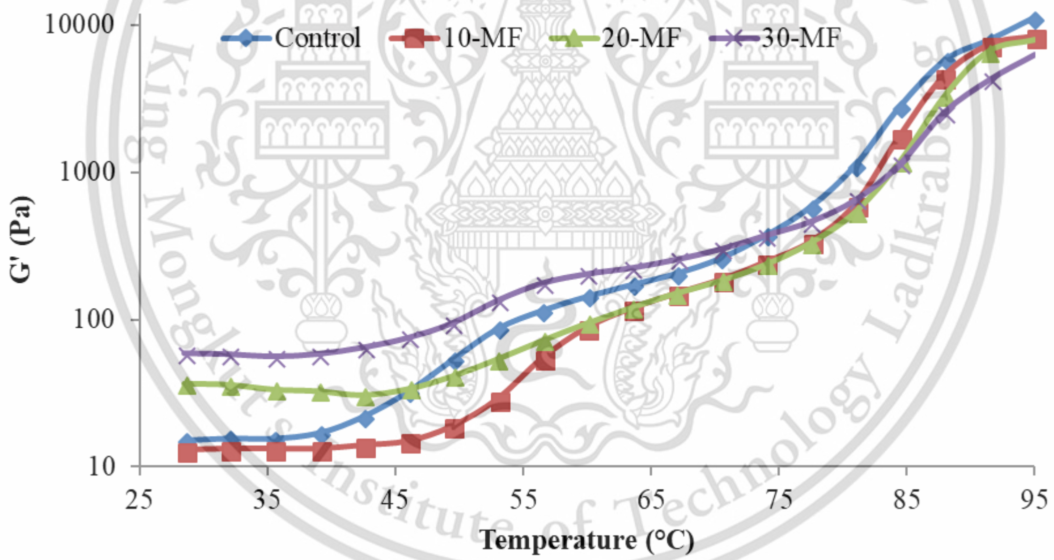

Figure 4.1 Elastic modulus (G′) of protein-enriched brown rice cake batter fortified with defatted Bombay locust powder (F/BL) at different levels during heating from 25 to 95°C. Control; brown rice cake prepared using brown rice flour without F/BL fortification, 10-, 20-, 30-MF; brown rice cake prepared using brown rice flour fortified with F/BL at 10, 20, 30%, respectively.

4.4.2.2. Characteristics of protein-enriched cake prepared using F/BL mixed brown rice flour

a. Water activity (a_w) and color

The baking process involves several chemical reactions, i.e. Maillard reactions, protein denaturation, moisture content reduction and physical transformations, such as a change in color, volume, and texture (Sun, Zhou, Yan, Huang, & Lin, 2018). Table 4.3 shows water activity and color of crust and crumb of protein-enriched cakes prepared using F/BL fortified brown rice flour at various levels. The slight decrease of water activity was observed for all resulting cakes (0.843 – 0.860) from F/BL mixed flour ($p \leq 0.05$), compared with that of control (0.873). This result might be caused by the lower moisture content and water activity of the mixed flour used for preparing the cake (Table 4.2). Moreover, the lower water activity implied rapid loss of moisture, occurred within crumb during baking (Cauvain & Young, 2011).

Table 4.3 Water activity and color of protein-enriched cakes prepared using brown rice flours fortified with defatted Bombay locust powder at different levels.

Parameters	Control	10-MF	20-MF	30-MF
Water activity	0.873 ± 0.006^{a}	0.860 ± 0.007^{b}	0.843 ± 0.005^{c}	0.844 ± 0.005^{c}
Color of crust				
*L**	56.29 ± 0.12^{a}	48.90 ± 0.58^{b}	46.39 ± 0.44^{c}	42.31 ± 0.49^{d}
*a**	15.46 ± 0.26^{a}	15.04 ± 0.13^{a}	13.96 ± 0.10^{b}	13.16 ± 0.36^{c}
*b**	28.97 ± 0.49^{a}	23.20 ± 0.68^{b}	20.01 ± 0.12^{c}	16.26 ± 0.69^{d}
ΔE*	-	10.04 ± 0.29^{b}	13.45 ± 0.08^{b}	19.05 ± 0.62^{a}
ΔC*	-	6.34 ± 0.33^{c}	9.24 ± 0.30^{b}	12.93 ± 0.52^{a}
Color of crumb				
*L**	61.56 ± 1.03^{a}	51.46 ± 0.88^{b}	46.67 ± 0.51^{c}	44.14 ± 0.56^{d}
*a**	4.91 ± 0.39^{d}	6.46 ± 0.29^{c}	6.99 ± 0.14^{b}	7.77 ± 0.14^{a}
*b**	18.87 ± 0.53^{a}	19.29 ± 0.28^{a}	17.91 ± 0.66^{b}	17.18 ± 0.33^{b}
ΔE*	-	10.24 ± 0.39^{c}	15.04 ± 0.59^{b}	17.70 ± 0.99^{a}
ΔC*	-	1.64 ± 0.06^{c}	2.09 ± 0.07^{b}	3.12 ± 0.13^{a}

Data are expressed as mean ± standard deviation (n=3).
Control: Brown rice flour (BRF) without defatted Bombay locust powder (F/BL) fortification.
10-, 20-, 30-MF: Mixed brown rice flour fortified with F/BL at 10, 20, 30%, respectively.
Different lowercase superscripts in the same row indicate significant difference ($p \leq 0.05$).

The F/BL fortification to BRF could distinctively determine the color of the resulting cake (Figure 4.2). Reducing of lightness with decreased *L** was obtained, in which increasing of greenness and blueness with lowered *a** and *b** were found from both crust and crumb as increased F/BL addition level. Consequently, increasing of the total differences of color (ΔE*) and chroma (ΔC*) of F/BL enriched brown rice cakes, was observed when F/BL content increased. Overall, cake became darker in color, when F/BL was mixed with BRF (Figure 4.2). These results were in line with the color of mixed flours from F/BL fortification

(Table 4.2). The major content of F/BL was protein, which might enhance the color changes via Maillard reaction, especially color of crust (Premi & Sharma, 2018). Similar trend of crumb color was found in the fortified gluten-free cake with germinated chickpea flour (Gadallah, 2017). Varies protein sources color was defined by the presence of different coloring constituents in flours, e.g. polyphenols which could react with proteins (Xu & Diosady, 2000). Also, the crumb color was determined by ingredients used in cake formulation as temperature inside of cakes could not facilitate Maillard reactions (Premi & Sharma, 2018). Thus, the color and protein content of F/BL influenced the color of resulting protein-enriched brown rice cake.

b. *Cake quality*

Quality of protein-enriched cakes, prepared using F/BL fortified brown rice flours, at different levels, were evaluated as baking loss and dimensions of cake (Table 4.4). Increasing of baking loss (%) was observed with increased F/BL level, and the lowest baking loss was obtained from 20-MF (22.0%) and 30-MF (21.8%) ($p \leq 0.05$). Baking loss difference was directly attributed in cake dimensions difference. The lower baking loss, resulted in the higher weight and density, with lower cake volume and specific volume. This result was consistent with the greater batter viscosity (Figure 4.1) and the higher water activity of cakes (Table 4.3). The batter with a higher G′ would obstruct the incorporation and expansion of air bubbles during the first stage of baking (Sahagún, Bravo-Núñez, Báscones, & Gómez, 2018). Replacement a part of the flour with F/BL, caused the starch content reduction, as well as the present of non-soluble exoskeleton chitin from F/BL, in the phase surrounding the bubbles, which could decrease the film layer consistency (Sahagún et al., 2018). Thus, the greater collapse and escape out of bubbles during baking would be occurred. Besides, the higher water-holding capacity of proteins due to chemical structure and their interaction with the food ingredients, could reduce water loss, increase final moisture content and water activity of cake, compared with starch (Nammakuna, Barringer, & Ratanatriwong, 2015; Shevkani & Singh, 2014). Then, protein-enriched brown rice cake with moist and dense crumb was obtained by F/BL addition.

Physical properties of final cakes were also evaluated as volume, uniformity and symmetry index as shown in Table 4.4. There was no difference in volume index of all samples tested. Replacement brown rice flour with F/BL affected symmetry and uniformity index of final cakes. Generally, the symmetry index is an indicator of surface contours, while uniformity index is a measure of cake symmetry. Significant decrease of symmetry index was obtained when F/BL was added ($p \leq 0.05$), which indicated the flat surface of cake.

Increasing uniformity index was observed from the protein-enriched cake, especially at replacement level higher than 10% ($p \leq 0.05$), compared with the control. This was in line with the rheological property of cake batter (Figure 4.1). Qasem et al. (2017a) reported that viscosity of cake batter demonstrated the cake quality parameters, particularly cake symmetry.

Figure 4.2 Appearance of top surface (A) and cross-sectional (B) views of protein-enriched brown rice cakes fortified with defatted Bombay locust powder (F/BL) at different levels. Control; brown rice cake prepared using brown rice flour without F/BL fortification, 10-, 20-, 30-MF; brown rice cake prepared using brown rice flour fortified with F/BL at 10, 20, 30%, respectively.

Table 4.4 Quality characteristics and texture properties of protein-enriched cakes prepared using brown rice flours fortified with defatted Bombay locust powder at different levels.

Parameters	**Control**	**10-MF**	**20-MF**	**30-MF**
Baking loss (% w/w)	29.46 ± 1.70[a]	26.18 ± 1.78[b]	21.99 ± 0.88[c]	21.70 ± 0.79[c]
Dimensions				
Cake volume (cm^3)	332.64 ± 19.80[a]	237.60 ± 7.48[b]	198.72 ± 7.48[c]	149.04 ± 11.22[d]
Specific volume (cm^3/g)	6.82 ± 0.31[a]	4.66 ± 0.04[b]	3.69 ± 0.10[c]	2.76 ± 0.18[d]
Density (g/cm^3)	0.14 ± 0.00[d]	0.21 ± 0.00[c]	0.27 ± 0.01[b]	0.36 ± 0.02[a]
Physical properties				
Volume index (cm)	8.10 ± 0.17[a]	8.00 ± 0.00[a]	8.00 ± 0.20[a]	7.87 ± 0.06[a]
Uniformity index (cm)	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.10 ± 0.00[a]	0.07 ± 0.06[a]
Symmetry index (cm)	-0.20 ± 0.00[a]	-0.50 ± 0.00[b]	-0.60 ± 0.20[b]	-0.67 ± 0.06[b]
Texture properties				
Hardness (N)	1.40 ± 0.28[a]	1.00 ± 0.01[b]	0.79 ± 0.03[b]	0.66 ± 0.01[b]
Springiness (mm)	0.94 ± 0.03[a]	0.86 ± 0.06[ab]	0.80 ± 0.08[ab]	0.80 ± 0.01[b]
Cohesiveness	0.80 ± 0.01[a]	0.69 ± 0.03[b]	0.69 ± 0.01[b]	0.80 ± 0.01[a]
Gumminess (N)	1.12 ± 0.21[a]	0.69 ± 0.04[b]	0.54 ± 0.01[b]	0.44 ± 0.00[b]
Chewiness (N)	1.05 ± 0.17[a]	0.59 ± 0.01[b]	0.44 ± 0.05[b]	0.36 ± 0.01[b]
Resilience	0.40 ± 0.01[a]	0.28 ± 0.02[b]	0.28 ± 0.01[b]	0.28 ± 0.01[b]

Data are expressed as mean ± standard deviation (n=3).
Control: Brown rice flour (BRF) without defatted Bombay locust powder (F/BL) fortification.
10-, 20-, 30-MF: Mixed brown rice flour fortified with F/BL at 10, 20, 30%, respectively.
Different lowercase superscripts in the same row indicate significant difference ($p \leq 0.05$).

c. *Texture profiles*

Texture profile of protein-enriched brown rice cakes is shown in Table 4.4 in comparison with the control (without F/BL fortification). The control cake required the highest compression force to deform the cake, which indicated the highest hardness ($p \leq 0.05$), compared to other cake samples. The lower hardness of fortified cake was observed, while no significant difference between fortification levels tested was observed ($p > 0.05$). This decrease in hardness of enriched cake might due to the decrease of starch content, which played a crucial role on cake structure formation baked from rice flour (Sozer, 2009). It was reported that grasshoppers contain chitin ranging from 9.6 – 14.9% dry matter basis (Fombong, Van Der Borght, & Broeck, 2017). Insect protein and insoluble fiber of chitin particle from exoskeleton, interfered the development of gelatinized starch network (de Oliveira et al., 2017), while interaction between insect protein and other components in batter was not strong as those generated in the control cake, rendering cake structure with weak backbone (Finke, 2007; Premi & Sharma, 2018; Rumpold & Schluter, 2015). Moreover, a crumbling matrix could be obtained with a low internal cohesion within crumb that might lead to the less compression resistance of cake. This result was in line with decreasing of springiness and cohesiveness of F/BL fortified cakes, which indicated the less elasticity with the weak adhesion between components, particularly F/BL protein and gelatinized starch, in cake matrix (Premi & Sharma, 2018). However, F/BL-fortified cake with 30% replacement (30-MF) showed similar cohesiveness with the control cake (p>0.05). It might relate to the lowest specific volume with the highest density of 30-MF cake (Table 4.4), which provided the moist and dense cake structure. A similar result was found for gumminess, chewiness and resilience of protein-enriched cake prepared using F/BL mixed brown rice flour, which was lower than control (p≤0.05), regardless of F/BL proportion added. The decrease in gumminess and chewiness indicated lower energy needed to disintegrate and masticate a solid food for swallowing, respectively. Additionally, decreased resilience reflected the lack of ability to recover their original shape after deformation, which caused by non-uniformity with a dense and crumby matrix of cake (Cornejo & Rosell, 2015; Phattanakulkaewmorie, Paseephol, & Moongngarm, 2011). Nevertheless, no significant difference in those parameters was detected when different F/BL proportions were added (p>0.05). There were many factors, including component, moisture content, cake volume, batter viscosity, which directly contributed the texture properties of final baked products (Arora & Saini, 2016; Miranda-Villa, Mufari, Bergesse, & Calandri, 2018; Moore, Schober, Dockery, & Arendt, 2004).

4.4.3. Chemical composition and energy value

Chemical compositions and energy value of F/BL fortified brown rice cake are shown in Table 4.5, in comparison with control. Significant increase of protein content was obtained as increased F/BL level added (p≤0.05), compared with that of the control. According to CAC (1997), the cakes could be claimed as ‘source of protein’, when their protein content was ranging from 14.9 – 24.9%. Therefore, F/BL-fortified cakes could be claimed as ‘high protein source’, especially at more than 10% F/BL added level. It was noted that F/BL addition increased protein content, while fat and ash contents were not affected. It was reported that insect powder inclusion increased protein content as well as fat and ash content of bread product (de Oliveira et al., 2017; Osimani et al., 2018). This might indicate the impact of fat removal process, implemented in F/BL production process, which could concentrate protein content and reduce fat content in the resulting F/BL. Decreasing moisture content was found from F/BL-fortified cakes, compared with that of the control ($p \leq 0.05$). This result was under the higher water activity (0.873) (Table 4.3) of the control cake compared with F/BL fortified cakes ($p \leq 0.05$), where water activity and moisture content had a positive correlation on cake making (Noorlaila, Nor Hasanah, Yusoff, Sarijo, & Asmeda, 2017). On the other hand, decreased carbohydrate content was found for F/BL fortified cakes, especially at the highest F/BL level added ($p \leq 0.05$). This result reconfirmed dilution effect of carbohydrate and starch contents of mixed flour (Table 4.2), and the corresponding cake due to F/BL replacement. However, there were no significant differences in energy value of the resulting cakes. This result was in line with energy value of protein-enriched bread using mealworm powder (Roncolini et al., 2019).

Table 4.5 Chemical compositions and energy value of protein-enriched brown rice cakes prepared using brown rice flours fortified with defatted Bombay locust powder at different levels.

Parameters	Control	10-MF	20-MF	30-MF
Protein (%)*	14.94 ± 0.58^d	18.40 ± 0.59^c	20.83 ± 0.15^b	24.94 ± 0.30^a
Fat (%)	21.55 ± 0.57^a	22.17 ± 0.36^a	21.49 ± 0.41^a	21.90 ± 0.64^a
Ash (%)	1.26 ± 0.08^a	1.29 ± 0.06^a	1.33 ± 0.04^a	1.35 ± 0.02^a
Moisture (%)	41.18 ± 0.92^a	38.72 ± 1.15^b	37.26 ± 1.03^b	37.44 ± 0.68^b
Carbohydrate (%)	21.08 ± 1.84^a	19.42 ± 1.36^a	19.10 ± 0.89^a	14.26 ± 0.48^b
Fiber content (%)	1.88 ± 0.12^d	2.50 ± 0.05^c	3.07 ± 0.16^b	4.85 ± 0.10^a
Energy value (kcal/100 g)	337.94 ± 3.62^a	350.83 ± 6.32^a	353.13 ± 6.06^a	354.33 ± 5.86^a

Data are expressed as mean ± standard deviation (n=3).
*The conversion factor used is 6.25.
Control: Brown rice flour (BRF) without defatted Bombay locust powder (F/BL) fortification.
10-, 20-, 30-MF: Mixed brown rice flour fortified with F/BL at 10, 20, 30%, respectively.
Different lowercase superscripts in the same row indicate significant difference ($p \leq 0.05$).

4.4.4. Sensory properties

Sensory properties of protein-enriched brown rice cake, prepared using mixed brown rice flour, with different levels of F/BL, were evaluated and presented as likeness score, in comparison with the control cake (without F/BL fortification) (Table 4.6). The control showed good acceptability with the highest likeness score ($p \leq 0.05$) for all attributes tested.

It was found that F/BL addition affected sensory properties of the resulting cakes, especially at the highest level used (30-MF), where the significant lower score was obtained for all attributes tested, compared with those of the control ($p>0.05$). This was related to the dark color (Table 4.3), dense and crumbly texture (Table 4.3) with a distinct change of appearance (Figure 4.1) for the cake incorporated with 30% F/BL. Appearance, color of crumb and aroma of cake were changed by F/BL addition at the lowest level used (10-MF), which directly demonstrated panelist's acceptance with lowered overall likeness score, compared with the control ($p \leq 0.05$). The results indicated the adverse effect of F/BL addition on organoleptic properties due to dark color with a unique odor of F/BL. However, the fortified brown rice cake with F/BL up to 20%, had similar likeness score on texture, flavor and aftertaste, to the control cake ($p>0.05$), while the acceptability for other attributes was in the desirable range (7-9 likeness score) (Bhaduri, 2013).

Table 4.6 Sensory properties of protein-enriched brown rice cakes prepared using brown rice flours fortified with defatted Bombay locust powder at different levels.

Sensory properties	**Likeness score**			
	Control	**10-MF**	**20-MF**	**30-MF**
Appearance	8.3 ± 0.6^a	7.8 ± 0.6^b	7.4 ± 0.8^b	6.9 ± 0.9^c
Color of crust	8.2 ± 0.8^a	8.0 ± 0.7^a	7.2 ± 0.7^b	6.7 ± 0.9^c
Color of crumb	8.4 ± 0.5^a	7.7 ± 0.7^b	7.3 ± 0.8^b	6.7 ± 1.0^c
Aroma	7.6 ± 1.0^a	7.0 ± 0.8^b	7.0 ± 0.8^b	6.4 ± 1.0^c
Taste	7.7 ± 0.8^a	7.6 ± 1.0^a	7.0 ± 1.0^b	6.2 ± 0.9^c
Texture	7.5 ± 0.6^a	7.3 ± 0.8^a	7.2 ± 1.0^a	6.2 ± 1.0^b
Flavor	7.4 ± 0.5^a	7.0 ± 0.9^a	7.0 ± 0.8^a	6.3 ± 1.0^b
Aftertaste*	7.4 ± 0.9^a	7.2 ± 1.0^a	7.0 ± 0.6^a	5.9 ± 0.8^b
Overall	8.0 ± 0.7^a	7.6 ± 0.7^b	7.1 ± 0.8^b	6.2 ± 0.9^c

*the any left-over flavor or sensations that occur after the sample has been swallowed.
Control: Brown rice flour (BRF) without defatted Bombay locust powder (F/BL) fortification.
10-, 20-, 30-MF: Mixed brown rice flour fortified with F/BL at 10, 20, 30%, respectively.
Different lowercase superscripts in the same row indicate significant difference ($p \leq 0.05$).

Majzoobi et al. (2014) reported that the appropriate level of the soy protein isolate had crucial roles on texture, sensory properties as well as consumer acceptability of cakes, where the SPI addition of less than 20%, provided a protein-enriched cake with acceptable quality. Osimani et al. (2018) found that the maximum level of cricket powder addition on bread was 10% (w/w) with acceptable liking score. The result suggested that protein-enriched cake, with good consumer acceptance, could be successfully prepared using F/BL mixed brown rice flour at 20% (20-MF), without a baneful effect on sensory properties. The 20-MF cake was then selected and subjected to study for microstructure in comparison with the control.

4.4.5. Microstructure

The inner surface of cakes was captured and observed using a scanning electron microscope (SEM). Microstructures of the selected protein-enriched cake prepared using 20% BRF replacement by F/BL (20-MF) are presented in Figure 4.3 in comparison with the control. Both samples showed a sponge-like structure, where the control cake (Figure 4.3 (A)) had a rough crumb and open structure with more porosity from the gap and air cells, compared with 20-MF cake (Figure 4.3 (B)). Besides, the denser structure with less porosity was obtained from protein-enriched (20-MF) cake. Similar structure was also observed from gluten-free cake prepared using gums mixed rice flour.

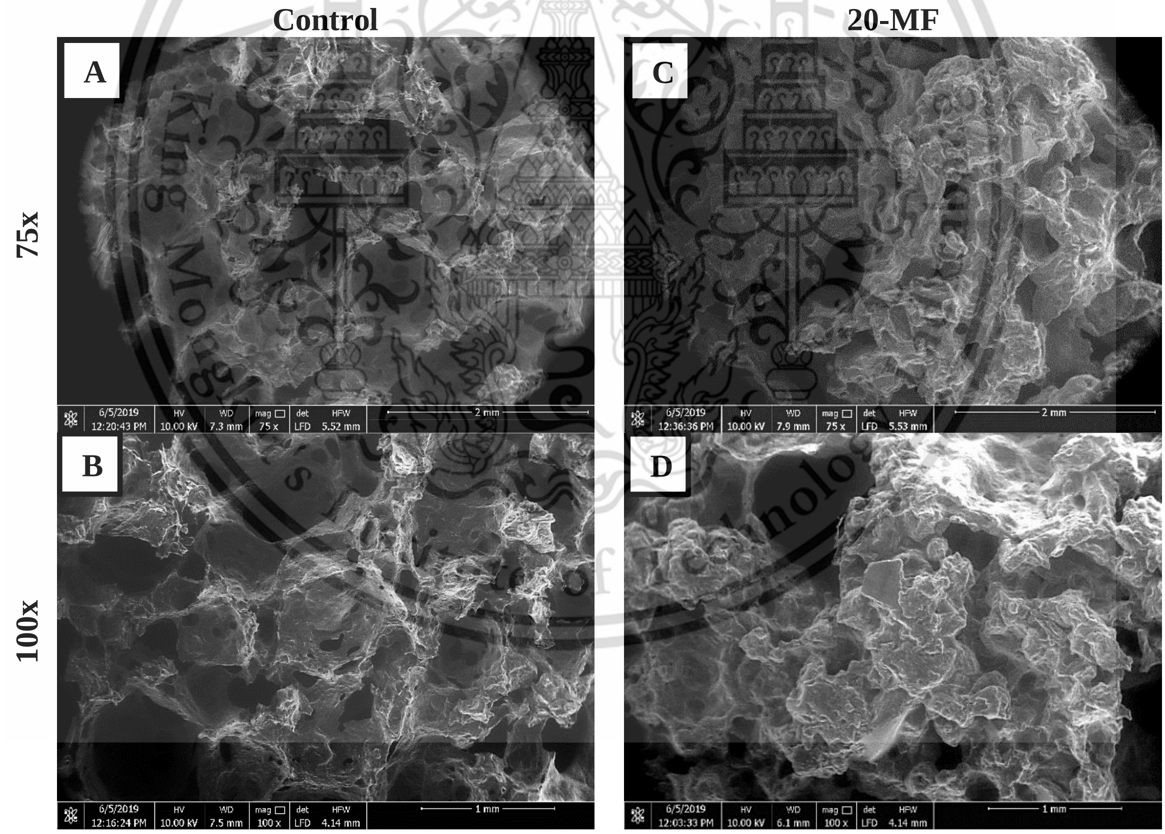

Figure 4.3 Scanning electron microscopic images of protein-enriched brown rice cake without (Control) (A and B) and with defatted Bombay locust powder at 20% of fortification level (20-MF) (C and D). A and C; 75× magnification, B and D; 100× magnification.

Different types of gums affected the pore area fraction and number of pores in a cake crumb

(Turabi, Sumnu, & Sahin, 2010). More uniform structures were obtained from the control cake, in which distribution of air bubbles was more homogenous, compared to a 20-MF cake. Shevkani and Singh (2014) reported that cake batter viscosity influenced the ability of batter to entrap and retain air bubble during mixing and baking. The too high or too low viscosity of batter rendered the final cake with low volume. This result was consistent with the different viscoelasticity (Figure 4.1), cake batter and volume of cake (Table 4.4), obtained from 20-MF and the control. Protein from F/BL might enhance the formation of interconnected structure between the strands and fill the void, thus yielding a denser structure. Moreover, exoskeleton insoluble chitin in F/BL distributed throughout cake matrix, might interrupt the aeration property of cake batter during baking (Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019). Similar result was also found for baked product enriched with squid ovary powder (Singh et al., 2019), cricket (*Acheta domesticus*) (Osimani et al., 2018), cinereous cockroach (*Nauphoeta cinerea*) powders (de Oliveira et al., 2017).

4.5. Conclusion

Quality characteristics of protein-enriched brown rice flour and cake were influenced by different levels of F/BL addition. Characteristics and pasting properties of mixed brown rice flour varied with different F/BL levels added. Application of mixed flour for preparing cake confirmed the impact of F/BL addition on the change of rheological properties of cake batter. Mixed flours with different levels of F/BL added affected physicochemical and sensory properties of final cakes differently. The 20% replacement levels of F/BL could be an optimum level, rendering brown rice cake with high nutrient composition, especially protein content, and good acceptability. Therefore, F/BL could be an alternative protein source for developing high protein flour and baked product, particularly brown rice flour-based product.

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CHAPTER 5

SUMMARY AND RECOMMENDATIONS

5.1. Summary

Hexane defatting impacted the physicochemical properties and chemical composition of Bombay locust powder, particularly protein and fat contents. Defatted Bombay locust powder (F/BL) had better oxidative stability with palatable characteristics, compared with those of without defatting (BL). The off-odors were effectively removed by hexane defatting, in which F/BL was also a good source of amino acids and minerals. F/BL was successfully used as a protein supplement for preparing biscuit sticks, especially at 10% F/BL. Moreover, F/BL effectively enhanced the nutrition value of gluten-free brown rice flour that showed good applicability in preparing protein-enriched brown rice cake. F/BL fortification by replacement of brown rice flour governed rheological properties of mixed flours and cake batters as well as determined the qualities of their relevant cake products. Replacement level at 20% of F/BL was the optimum level providing nutritive cake, especially protein content, with good sensory acceptance.

Therefore, hexane defatting could effectively be used for preparing the Bombay locust powder with rich in protein and other nutrients and also improved palatability and applicability of Bombay locust powder in baked products, particularly biscuits and brown rice cake.

5.2. Recommendations

To further explore the possibility of Bombay locust powder application as protein supplement in food products, future study should be focused on:

5.2.1. Preparation of Bombay locust protein isolates and its functional properties as an alternative protein supplement

5.2.3. The shelf life evaluation of Bombay locust powders using different conditions of packaging and storage

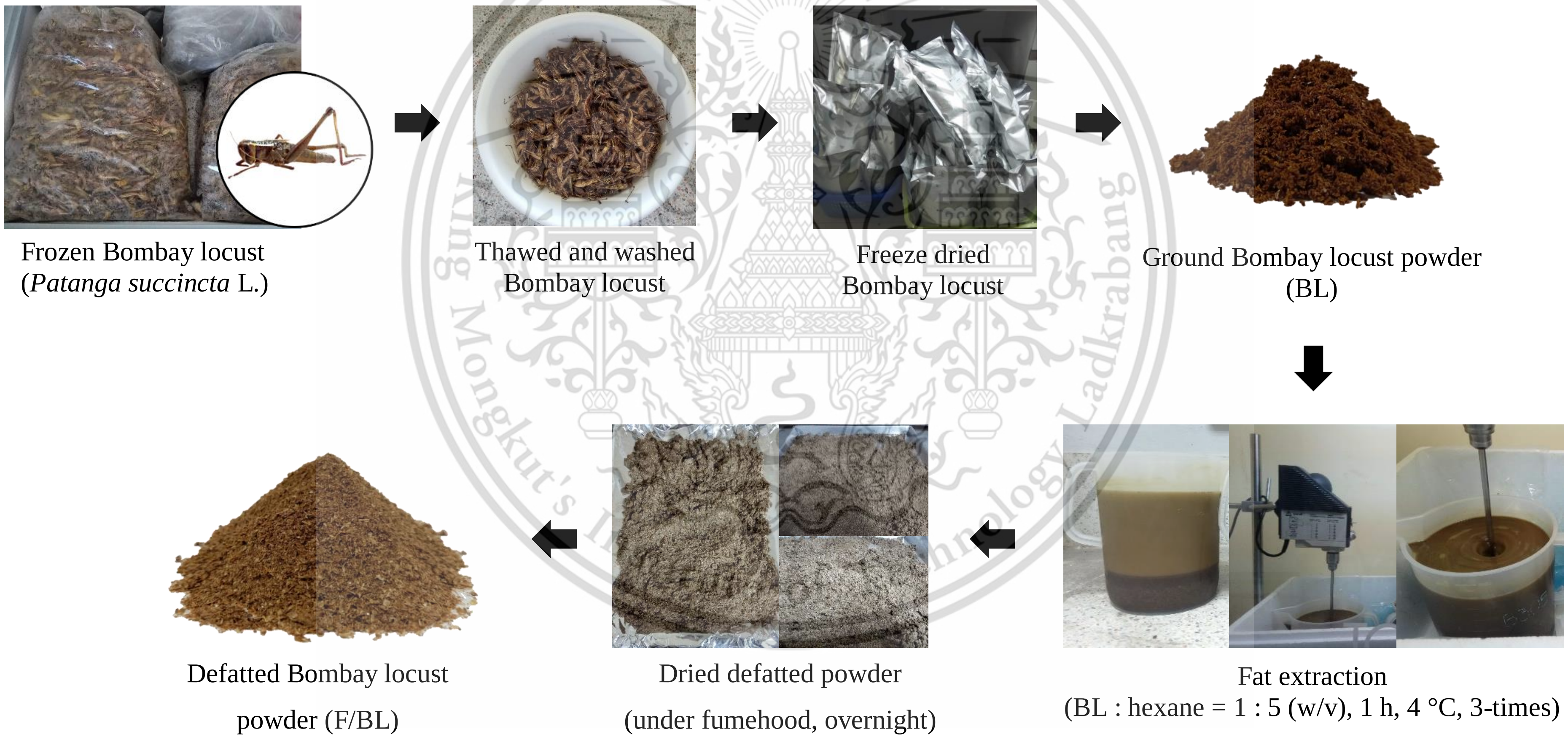
5.2.4. The application of defatted Bombay locust powder in another baked products, i.e. bread, cookies or pastry

APPENDIX

Appendix A

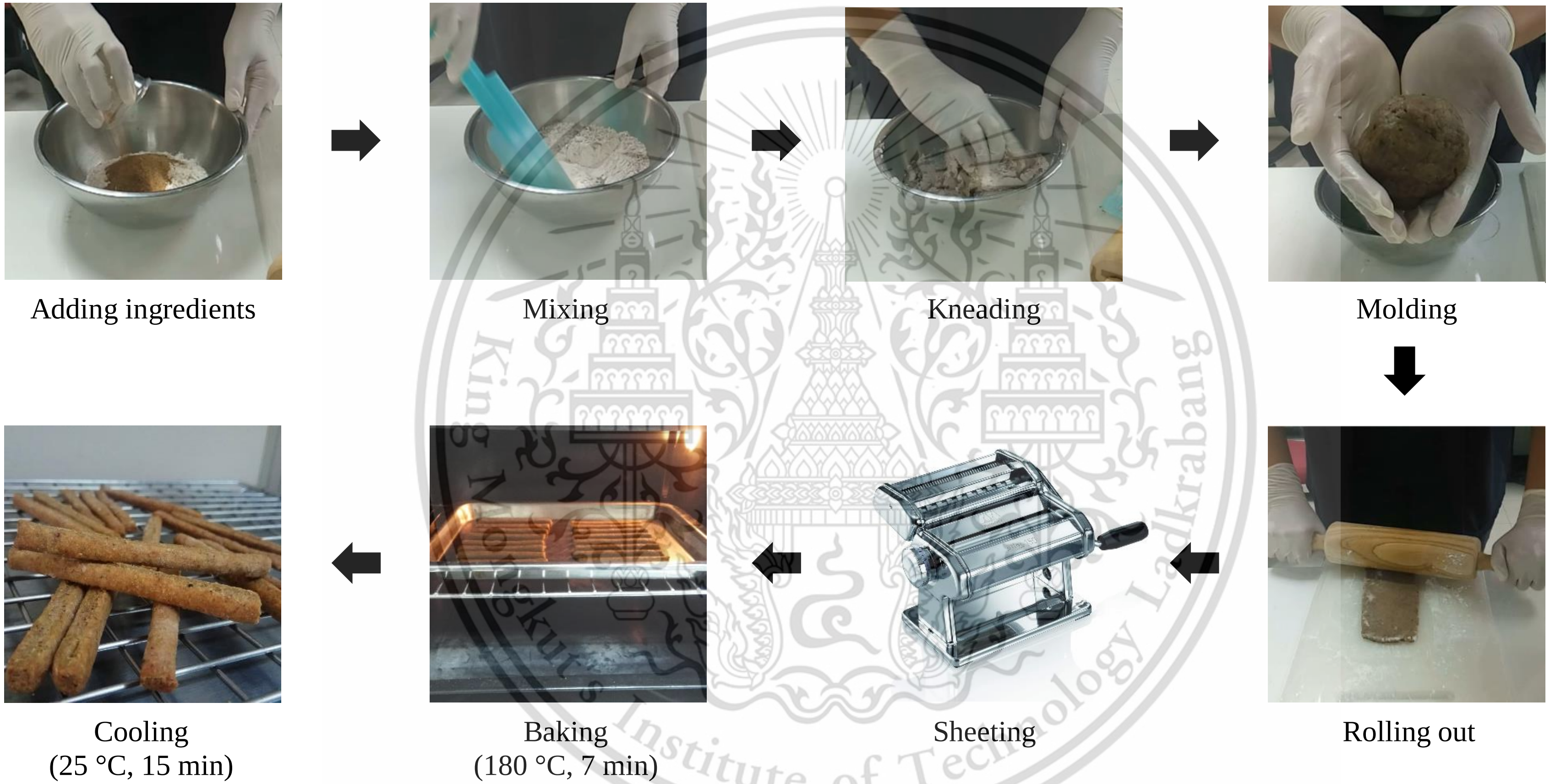
Sample preparation

Appendix A1: Bombay locust powder s preparation



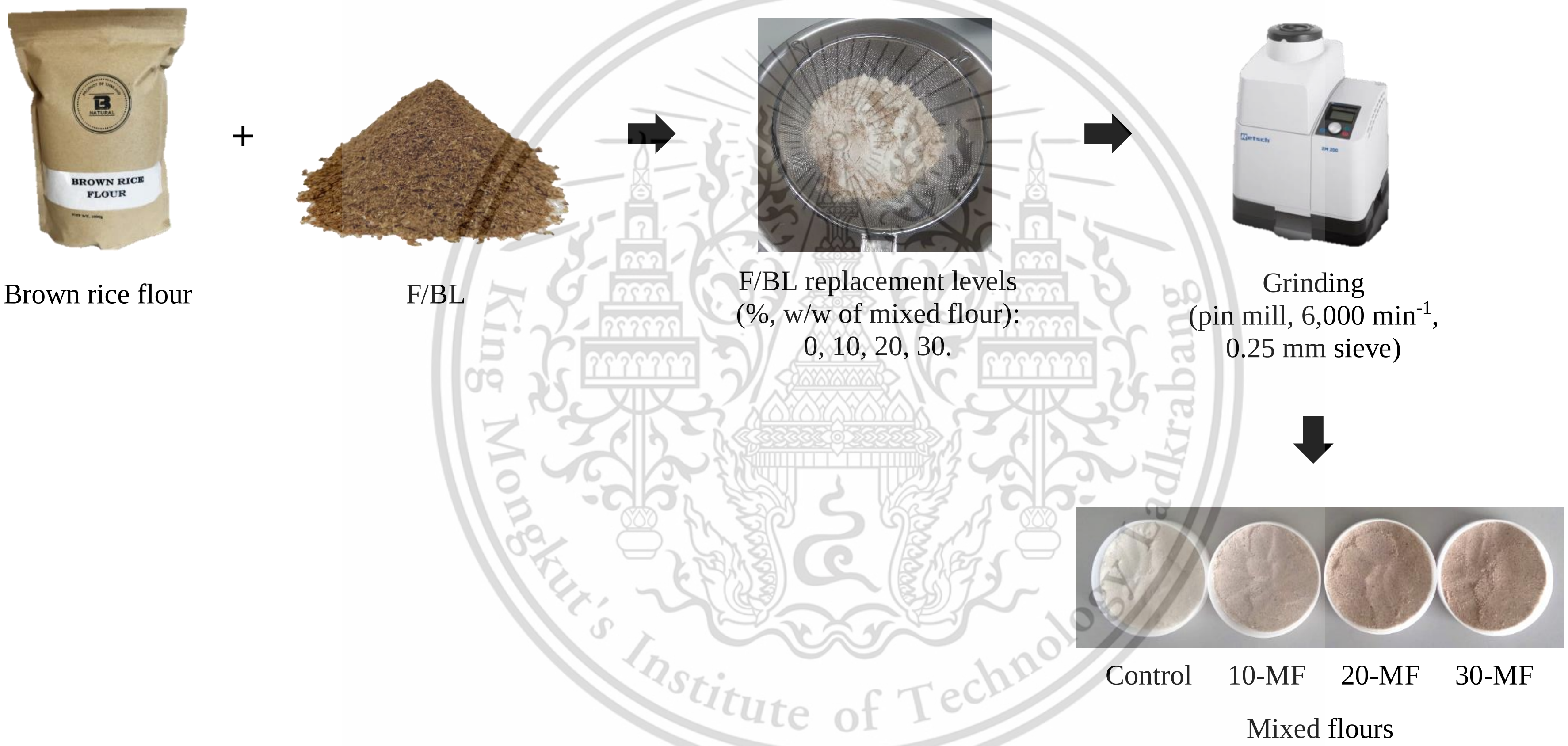
110

Appendix A2: Short dough biscuit stick preparation



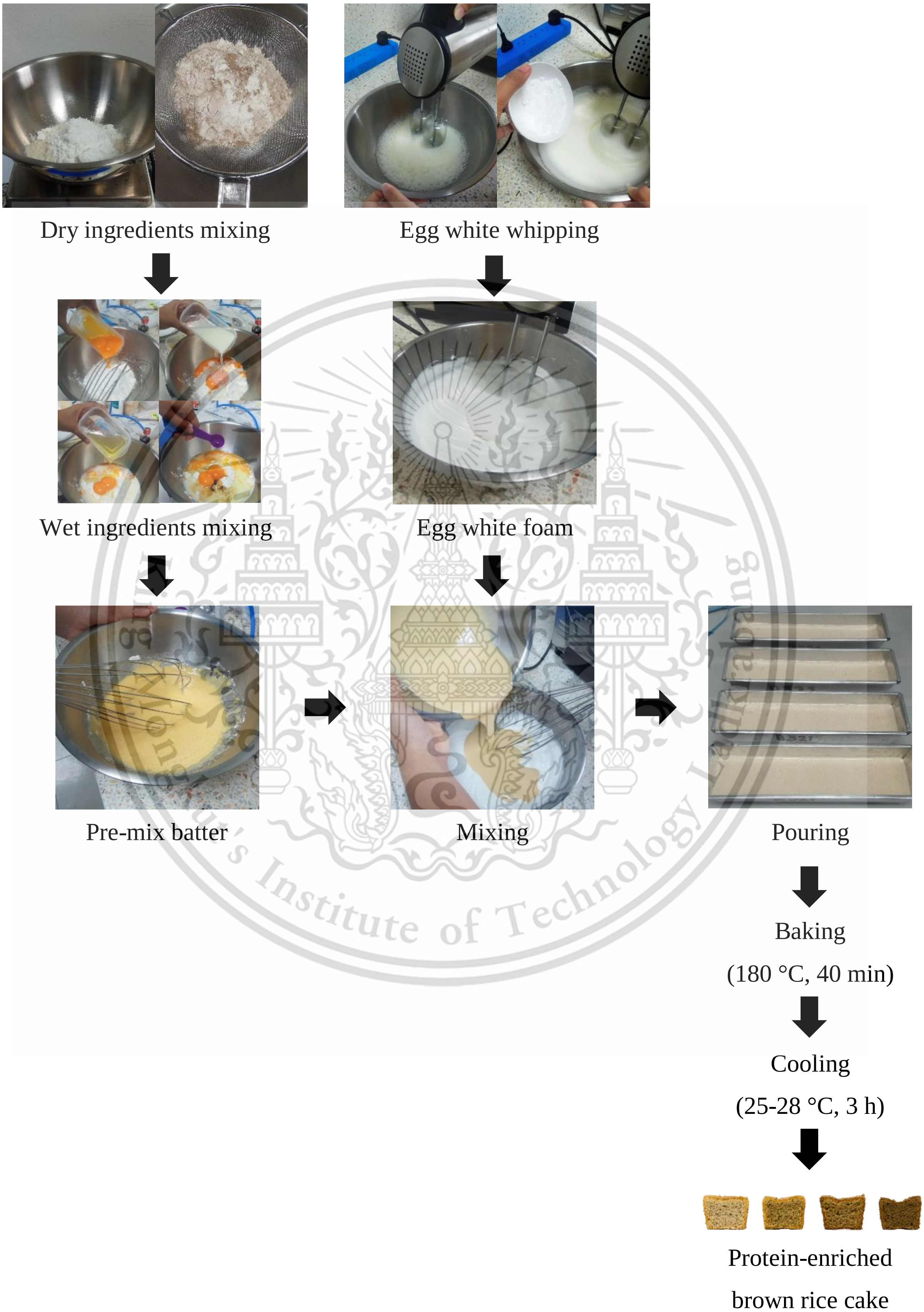
111

Appendix A3: Protein-enriched brown rice flour preparation



112

Appendix A4: Brown rice cake preparation



Appendix B

Sensory evaluation forms

Appendix B1: Sensory evaluation form of protein-enriched biscuit stick

1/2

Sensory Evaluation **No….**

Product : Protein enriched stick

Name ………………………………………………………… Date ……………. Time …………….

Instruction: You are given coded samples. Please evaluate them from left to right and give a score for each attribute below.

9 = ชอบมากที่สุด (Extremely like) 6 = ชอบเล็กน้อย (Slightly like) 3 = ไม่ชอบปานกลาง (Moderately dislike)

8 = ชอบมาก (Very much like) 5 = ไม่สามารถบอกได้ว่าชอบหรือไม่ชอบ (Neither like or dislike) 2 = ไม่ชอบมาก (Very much dislike)

7 = ชอบปานกลาง (Moderately like) 4 = ไม่ชอบเล็กน้อย (Slightly dislike) 1 =ไม่ชอบมากที่สุด (Extremely dislike)

Please do not compare between given samples.

Attributes	Sample code			
	………	………	………	………
ลักษณะปรากฏ (Appearance)				
สี (Color)				
กลิ่น (Aroma)				
รสชาติ (Taste)				
เนื้อสัมผัส (Texture)				
กลิ่นรส (Flavor)				
กลิ่นรสที่ค้างในปาก (After taste)				
ความชอบโดยรวม (Overall)				

ข้อเสนอแนะ……………………………………………………………………………………………………

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Notes:
You will be given 7 samples that served in two-rounds of testing. After the first round, you will be given 5-min rest before testing the second round. Please neutralize your mouth with water before and after sample testing.

2/2

Sensory Evaluation **No….**

Product : Protein enriched stick

Name ……………………………………………………… Date ……………. Time …………….

Instruction: You are given coded samples. Please evaluate them from left to right and give a score for each attribute below.

9 = ชอบมากที่สุด (Extremely like) 6 = ชอบเล็กน้อย (Slightly like) 3 = ไม่ชอบปานกลาง (Moderately dislike)

8 = ชอบมาก (Very much like) 5 = ไม่สามารถบอกได้ว่าชอบหรือไม่ชอบ (Neither like or dislike) 2 = ไม่ชอบมาก (Very much dislike)

7 = ชอบปานกลาง (Moderately like) 4 = ไม่ชอบเล็กน้อย (Slightly dislike) 1 =ไม่ชอบมากที่สุด (Extremely dislike)

Please do not compare between given samples.

Attributes	**Sample code**			
	………	………	………	………
ลักษณะปรากฏ (Appearance)				
สี (Color)				
กลิ่น (Aroma)				
รสชาติ (Taste)				
เนื้อสัมผัส (Texture)				
กลิ่นรส (Flavor)				
กลิ่นรสที่ค้างในปาก (After taste)				
ความชอบโดยรวม (Overall)				

ข้อเสนอแนะ……………………………………………………………………………………………………
………
………

Notes:
Please neutralize your mouth with water before and after sample testing.

Appendix B2: Sensory evaluation form of protein-enriched brown rice cake

Sensory Evaluation No….

Product : Protein enriched brown rice cake

Name …………………………………………………… Date …………… Time ……………

Instruction: You are given coded samples. Please evaluate them from left to right and give a score for each attribute below.

9 = ชอบมากที่สุด (Extremely like) 6 = ชอบเล็กน้อย (Slightly like) 3 = ไม่ชอบปานกลาง (Moderately dislike)

8 = ชอบมาก (Very much like) 5 = ไม่สามารถบอกได้ว่าชอบหรือไม่ชอบ (Neither like or dislike) 2 = ไม่ชอบมาก (Very much dislike)

7 = ชอบปานกลาง (Moderately like) 4 = ไม่ชอบเล็กน้อย (Slightly dislike) 1 =ไม่ชอบมากที่สุด (Extremely dislike)

Attributes	Sample code			
	………	………	………	………
ลักษณะปรากฏ (Appearance)				
สีของผิว (Color of crust)				
สีของเนื้อ (Color of crumb)				
กลิ่น (Aroma)				
รสชาติ (Taste)				
เนื้อสัมผัส (Texture)				
กลิ่นรส (Flavor)				
กลิ่นรสที่ค้างในปาก (After taste)				
ความชอบโดยรวม (Overall)				

ข้อเสนอแนะ……………………………………………………………………………………………………

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AUTHOR BIOGRAPHY

Sylvia Indriani was born in Madiun, Indonesia, and grew up there as well. She graduated with her Bachelor of Science degree (majoring in Food Technology – with Honors) in 2018 from IPB University, Indonesia, and was fully supported by *Rabobank Indonesia Scholarship 2013*. During her study, she joined an exchange student program in Universiti Putra Malaysia (UPM) for five months, under *ASEAN International Mobility for Students (AIMS) Scholarship 2016*. Her strong passion for food product development had brought her to pursue higher education levels in Thailand, supported by *KMITL - ASEAN Postgraduate Scholarship 2018*. This program was intended to build collaboration between ASEAN universities. She obtained her Master of Science degree (majoring in Food Science) in 2020 from King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. During her study in Thailand, her research had been granted under *KMITL Research and Innovation Services (KRIS), [KREF126201]*, as per 2019, with the project of edible insect powder preparation and its application in baked products. Also, she has gained a lot of knowledge, lab-skills, experiences, and networks. She studied the physicochemical, nutritional, and oxidative stability characteristics of insect powders and their food application, under the supervision of Assist. Prof. Dr. Supatra Karnjanapratum and Assist. Prof. Dr. Sitthipong Nalinanon at the Faculty of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

'*Finishing well: It does not matter how great we start our battle, but how will we finish it'*.

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List of publications

Indriani, S., Bin Ab Karim, M.S., Nalinanon, S., & Karnjanapratum, S. 2020. Quality characteristics of protein-enriched brown rice flour and cake affected by Bombay locust (*Patanga succincta* L.) powder fortification. *LWT – Food Science and Technology,* 119, 108876. https://doi.org/10.1016/j.lwt.2019.108876

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