CHARACTERISTICS AND FUNCTIONAL PROPERTIES OF PROTEINS FROM THAI EDIBLE INSECTS AND UTILIZATION OF INSECT POWDER IN NOODLE

NIPHATTHA CHATSUWAN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN FOOD SCIENCE
FACULTY OF AGRO-INDUSTRY
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG
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(Assoc. Prof. Dr. Praphan Pinsirodom)

Dean

Faculty of Agro-Industry

Date : Dec. 20, 2018

ชื่อวิทยานิพนธ์	คุณลักษณะและสมบัติเชิงหน้าที่ของโปรตีนจากแมลงบริโภคได้ของไทยและการใช้ประโยชน์ผงแมลงในบะหมี่
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บทคัดย่อ

สารสกัดโปรตีนที่ละลายน้ำได้ จากแมลงบริโภคได้ 3 ชนิด จากตระกูล Lepidoptera คือ ดักแด้ไหม : *Bombyx mori* Linn . (WSPB) และตระกูล Orthoptera คือ ตั๊กแตน 2 ชนิด ได้แก่ *Patanga succincta* : (WSPP) และ *Chondracris roseapbrunner* (WSPC) นำมาศึกษาสมบัติเชิงหน้าที่ และกิจกรรมการต้านอนุมูลอิสระพบว่า ผลผลิตจากการสกัดโปรตีนด้วยน้ำ ของแมลงทั้ง 3 ชนิด โดยคิดในฐานน้ำหนักเปียก อยู่ระหว่างร้อยละ 3.96 ถึง ร้อยละ 7.49 โดยพบว่า *C. roseapbrunner* ให้ผลผลิตการสกัดได้มากที่สุด นอกจากนี้พบกรดอะมิโนที่มีมากที่สุด คือ กรดกลูตามิก รองลงมา ฮิสติดีน ฟีนิลอะลานีน ไกลซีน อะลานีน ลูซีน โพรลีน และอาร์จินีน ตามลำดับ เมื่อตรวจสอบโดย SDS-PAGE พบว่าโปรตีนหลัก WSPB มีน้ำหนักโมเลกุลเท่ากับ 40, 64 และ70 กิโลดาลตัน ตามลำดับ โปรตีนหลักของ WSPP และ WSPC มีน้ำหนักโมเลกุลเท่ากับ 29, 40, 50, 70 และ 146 กิโลดาลตัน ตามลำดับ เมื่อตรวจสอบด้วยเทคนิคอินฟราเรด (FTIR) สเปกโตรสโกปี พบว่า โปรตีนในสารสกัดยังคงความสมบูรณ์ของโครงสร้างระดับทุติยะภูมิของโปรตีนจากแมลง 3 ชนิด จากการศึกษาสมบัติเชิงหน้าที่ ได้แก่ ค่าเซอร์เฟสไฮโดรโฟบิก ปริมาณหมู่ซัลไฟดริลอิสระ และหมู่ซัลไฟดริลทั้งหมด ของ WSPB คือ 3.52, 22.17 ไมโครโมลต่อกรัม และ 23.08 ไมโครโมลต่อกรัม ตามลำดับ ส่วนค่าเซอร์เฟสไฮโดรโฟบิกของตั๊กแตน WSPC มีค่าสูงกว่า WSPP แต่ปริมาณหมู่ซัลไฟดริลอิสระ และหมู่ซัลไฟดริลทั้งหมด ของตั๊กแตนทั้ง 2 ชนิด มีปริมาณไม่แตกต่างกัน ความสามารถในการละลายของสารสกัดโปรตีนที่ละลายน้ำได้ของดักแด้ไหม มีความสามารถละลายได้ดีในช่วง พีเอช 5-11 ส่วนสารสกัดโปรตีนที่ละลายน้ำได้ของตั๊กแตน มีความสามารถละลายได้ดีทั้งในช่วงพีเอชที่มีความเป็นกรด และด่าง โดยสารสกัดโปรตีนที่ละลายน้ำได้แมลงทั้ง 3 ชนิด ละลายได้น้อยที่สุดที่ พีเอชเท่ากับ 4 สมบัติการเป็นอิมัลชันของสารสกัดโปรตีนที่ละลายน้ำได้ จากแมลง 3 ชนิด (ค่าดัชนีความสามารถในการเกิดอิมัลชัน (EAI) และดัชนี

ความสามารถในการคงตัวของอิมัลชัน (ESI)) และ ความสามารถในการเกิดฟอง มีค่าต่ำ แต่มีความสามารถในการคงตัวของโฟมที่ดีเมื่อเปรียบเทียบกับโปรตีนโบวีนเซรัมอัลบูมิน ความสามารถการเป็นสารต้านอนุมูลอิสระของสารสกัดโปรตีนที่ละลายน้ำได้ จากแมลง 3 ชนิด พบว่า สารสกัดโปรตีนทั้ง 3 ชนิด มี ความสามารถการเป็นสารต้านออกซิเดชัน DPPH$^{\bullet}$, ABTS$^{\bullet+}$ และความสามารถในการให้อิเล็กตรอนแก่ Fe^{3+} ด้วยวิธี FRAP โดยเฉพาะสารสกัดโปรตีนจากตั๊กแตนทั้งสองชนิด มีความสามารถในการต้านอนุมูลอิสระ DPPH$^{\bullet}$ และ ABTS$^{\bullet+}$ สูงกว่าสารสกัดโปรตีนจากดักแด้ไหม ดังนั้น โปรตีนจากแมลงทั้ง 3 ชนิด เป็นแหล่งที่มีศักยภาพของสารต้านอนุมูลอิสระและสามารถใช้เป็นส่วนผสมในอาหารแปรรูปเพื่อเพิ่มฟังก์ชันการทำงานที่ต้องการและเพิ่มคุณค่าทางโภชนาการได้ โดยในการทดลองนี้ตั๊กแตนปาทังก้า (*P. succincta*) ซึ่งเป็นแมลงที่มีปริมาณโปรตีนมากที่สุด จึงถูกนำมาทดลองผลิตเป็นผงตั๊กแตนปาทังก้าสกัดไขมัน (DBLP) เพื่อนำไปใช้ในการผลิตบะหมี่

คุณภาพของบะหมี่ที่ทดแทนแป้งสาลีบางส่วนด้วย DBLP ที่ระดับต่าง ๆ ได้แก่ ร้อยละ 5, 10, 15, 20 และ 30 โดยการวิเคราะห์สมบัติทางเคมี กายภาพ คุณภาพการปรุงสุก ลักษณะเนื้อสัมผัส และการประเมินทางประสาทสัมผัส พบว่า เมื่อปริมาณ DBLP เพิ่มขึ้น ค่าความสว่างของบะหมี่ลดลง และมีสีน้ำตาลแดงเข้ม ส่วนคุณภาพการปรุงสุก ได้แก่ ร้อยละการผลิตหลังการปรุงสุก และการสูญเสียจากการปรุงสุกของบะหมี่ พบว่า การทดแทนแป้งสาลีด้วยผงแมลง ทำให้คุณภาพการปรุงสุกลดลง อย่างไรก็ตามการเติมกัวร์กัม ร้อยละ 0.5 สามารถช่วยปรับปรุงคุณภาพการปรุงสุก และลักษณะเนื้อสัมผัส โดยพบว่า บะหมี่ที่ทดแทนด้วย DBLP ร้อยละ 5 และเติมกัวร์กัม ร้อยละ 0.5 มีค่าร้อยละหลังการปรุงสุกและการสูญเสียจากการปรุงสุก เท่ากับร้อยละ 229.28 และร้อยละ 4.03 ตามลำดับ นอกจากนี้ ยังมีค่าแรงยึดขาด และค่าความยืดหยุ่นของเส้นบะหมี่ เท่ากับ 15.69 กรัม.แรง และ 30.37 มิลลิเมตร ตามลำดับ ซึ่งมีค่าใกล้เคียงกับสูตรควบคุมที่ไม่มีการเติมผงแมลง นอกจากนี้ ผลการประเมินทางประสาทสัมผัส พบว่าผู้ชิมให้การยอมรับบะหมี่ที่เติม DBLP ร้อยละ 5 โดยมีคะแนนความชอบโดยรวมเท่ากับ 6.1 จากคะแนนเต็ม 7 และผลการวิเคราะห์องค์ประกอบทางเคมีพบว่า บะหมี่ที่ทดแทนผงแมลงร้อยละ 5 มี ความชื้นร้อยละ 24.07 ไขมันร้อยละ 4.04 เถ้าร้อยละ 2.44 และโปรตีนร้อยละ 16.65 การเติม DBLP สามารถเพิ่มปริมาณโปรตีนในสูตรการผลิตบะหมี่ได้

Thesis Title	Characteristics and functional properties of proteins from Thai edible insects and utilization of insect powder in noodle
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Student ID.	55680151
Degree	Doctor of Philosophy
Program	Food Science
Year	2018
Thesis Advisor	Assoc. Prof. Dr. Praphan Pinsirodom

ABSTRACT

Water soluble proteins extracted from three species of edible insects from 2 orders including order Lepidoptera; silkworm pupae: *Bombyx mori* Linn. (WSPB) and order Orthoptera; two grasshoppers: *Patanga succincta* (WSPP) and *Chondracris roseapbrunner* (WSPC) were characterized as well as their functional properties and antioxidant activities were investigated. The extraction yield, on a wet weight basis, was ranged from 3.96% to 7.49% for the three WSPs. Protein yield extraction from *C. roseapbrunner* was the highest. The most abundant amino acid in all proteins were glutamic acid, followed by aspartic, histidine, phenylalanine, glycine, alanine and leucine, proline and arginine. The electrophoretic study revealed that protein samples with MW of 40, 64 and 70 kDa were the major protein components in WSPB. Moreover, MW of 29, 40, 50, 70 and 146 kDa were the major protein components found in WSPP and WSPC. FTIR analysis showed that those proteins remain their secondary structural integrity. The surface hydrophobicity, free and total sulfhydryl group contents for WSPB were 3.52, 22.17 µmol/g and 23.08 µmol/g, respectively. WSPC had the surface hydrophobicity higher than WSPP, but the sulfhydryl group content was not significant difference between proteins from the two species of grasshoppers. WSPB was highly solubilized in the pH range of 5-11. All grasshopper proteins were mostly soluble in both strong acidic and alkaline aqueous solutions. The three WSPs showed a minimum value of solubility at pH 4. Moreover, all WSPs exhibited poor emulsifying properties (emulsifying activity index (EAI) and emulsion stability index

(ESI)) and foaming capacity but they had greater foam stability with comparable to bovine serum albumin. All WSPs had high antioxidant potential based on DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP assay. However, WSPs from grasshoppers had antioxidant potential higher than those of WSPB. Therefore, protein from these three edible insects can be a potential source of antioxidant and served as an ingredient in processed foods to enhance its desired functionality and nutriti onal value. Moreover, *P. succincta* which contained the greatest protein content was further selected to be used in noodle preparation. Defatted bombay locust powder (DBLP) was prepared from *P. succincta*.

The quality factors of noodle with partial replacement of wheat flour by 5, 10, 15, 20, and 30% defatted bombay locusts powder (DBLP) were characterized as well as their chemical, physical, cooking quality, texture property and sensory characteristics were evaluated. The results of noodle formula test showed that as the amount of DBLP increased, the lightness of the noodles decreased and the appearance color became dark red-brown. The quality of cooking in terms of cooking yield and cooking loss were also decreased as the content of DBLP increased. However, adding 0.5% of guar gum could improve the quality of insect noodles. The resulted noodle that replaced wheat flour with DBLP 5% after adding guar gum had the cooking quality and texture properties similar to the control. The cooking yield and cooking loss of the insect noodle were 229.28% and 4.03%, respectively. The tensile strength and elasticity of the insect noodles were 15.69 g.force and 30.37 mm. The results from sensory evaluation showed that the overall liking of cooked noodles were well accepted by panelist. Moreover, the noodle with partial replacement of wheat flour by 5% DBLP were composed of 24.07% moisture content, 4.04% fat, 2.44% ash and 16.65% protein. The incorporation of DBLP at 5% in the noodle ingredients significantly increased protein content.

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Niphattha Chatsuwan

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CHAPTER 1

INTRODUCTION

1.1 Introduction

In 2050 the world population is estimated at more than 9 billion people, resulting in an additional need for food and feed outputs (FAO, 2009). Conventional sources of protein will not be sufficient for the global human population, and alternatives sources such as insects will be required (Zielińska *et al.*, 2015). Approximately 1,900 species of edible insects are traditionally consumed in many parts of the world, for example in Africa, Asia and Latin America and are considered as having potential to contribute to the world's food security (van Huis, 2013). Edible insects (dry basis) have very high crude protein content and edible insect proteins have been reported to be a good source of essential amino acids. In present time, the benefits of insect-consumption are to utilize its components as ingredients. The successful use of protein ingredients depends on their abilities to fulfill one or more functional requirements, e.g. good solubility, emulsion/foam stabilization, or gel formation (Chalamaiah *et al.*, 2012; Garcés-Rimón *et al.*, 2016). From previous report, Omotoso (2006) evaluated the functional properties of Pallid Emperor moth (*Cirina forda*) larva showing intermediate solubility and emulsion properties. Kim *et al.* (2016) determined the effects of adding pre-treated mealworm larvae (*Tenebrio molitor*) and silkworm pupae (*Bombyx mori*) flours on nutritional, physicochemical and textural properties of emulsion sausages. Edible insects could be a potential source of fat and protein.

Extracted protein groups based on their solubility in solvents produces water-soluble and water-insoluble fractions, which can be used for applications in the food industry and functional properties of proteins, including, solubility, amino acid profile, thermal stability, water and oil binding, gelling, foaming and emulsifying capacity (Bußler *et al.*, 2016). Valle *et al.* (1982) executed a protein extraction from the Mexican fruit fly *Anastrepha ludens* with the highest protein solubility at pH10 and protein precipitation at pH 5. Yi *et al.* (2013) investigated functional properties of aqueous protein extracting from

five insect species. Gelling property of insect proteins depended on concentration and pH. Moreover, Mariod and Fadul (2015) used hot water, mild acid, and distilled water for extraction of protein (gelatin) from two insects, melon bug (*Coridius viduatus*) and sorghum bug (*Agonoscelis versicoloratus*). Extraction of insect gelatin using hot water gave the highest yield. The spectra of insect's gelatin were similar to commercial gelatin.

There is a long history in rural communities of Northern and Northeastern Thailand of consuming edible insects. The most popular are silkworm pupae, grasshoppers/locusts, crickets and bamboo worms all of which are good sources of protein. Recipes have been developed, depending on the type of insect, to enhance acceptability. Currently, cricket farms have over 20,000 places and Thailand has the capacity and expertise in the commercial cultivation of insect (Hanboonsong *et al.*, 2013).

Utilizations edible insect powder as raw material or ingredient was found in varieties products from Europe such as protein powder, bars, bread, cookies, brownies, spaghetti and pasta. However, In Asia is not used varieties in processed food. Noodles are a traditional food widely consumed throughout Asia. Noodles are suitable food because of their easy consumption. Therefore, adding edible insect powder in noodles is a choice for consumer and value added for edible insects and noodles.

In this study, the characterization, functional properties and antioxidant properties of water soluble proteins from three edible insects, the effects of extract on the quality of protein edible insect powder will be elucidated in this experiment and utilization of edible insect powder in noodle will also be evaluated. All of these experiments will promote edible insects to obtain to the choice value-added product and can be used as source of food ingredient.

1.2 Objectives

1.2.1 To characterize water soluble proteins prepared from three species of edible insects.

1.2.2 To determine functional properties and antioxidant potentials of water soluble proteins prepared from three species of edible insects.

1.2.3 To study the effect of partial replacement of wheat flour with locust powder on the quality of noodle.

1.2.4 To study the effect of hydrocolloids on the quality of locust powder noodle.

CHAPTER 2

LITERATURE REVIEW

2.1 Insects

2.1.1 Insects anatomy

Insects belong to the class of the Arthropoda known as the Insecta. Insects are bilaterally symmetrical and generally elongate. The body is segmented and grouped into three distinct regions-head, thorax and abdomen. The head (cranium or caput), is the anterior capsule-like structure that bears the brain, mouthparts and sense organs like antennae and eyes. The thorax is the middle body region of an insect, usually composed of three segments, the prothorax, mesothorax and metathorax. Each segment bears one pair of legs. Winged insects have one pair of wings attached to the second segment (mesothorax) and a second pair attached to the third segment (metathorax). All segments have an internal skeleton for the attachment of muscles. The abdomen is the posterior body region of an insect. In insects (and some other animals) the skeleton is mostly on the outside (referred to as an exoskeleton).

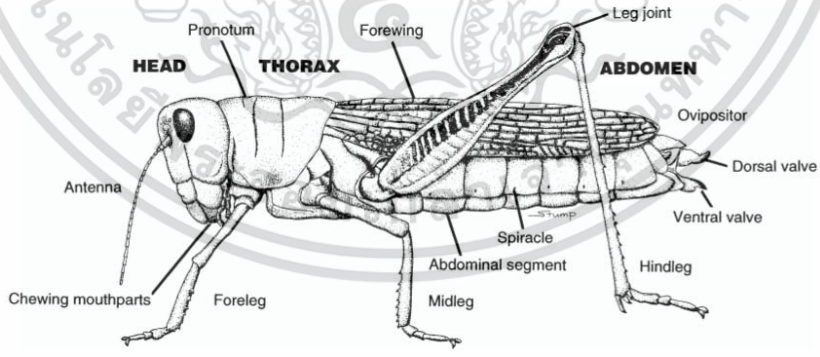


Figure 1. Insect anatomy (Orthoptera)

Source: Pfadt (2002)

2.1.2 Insects as a source of food

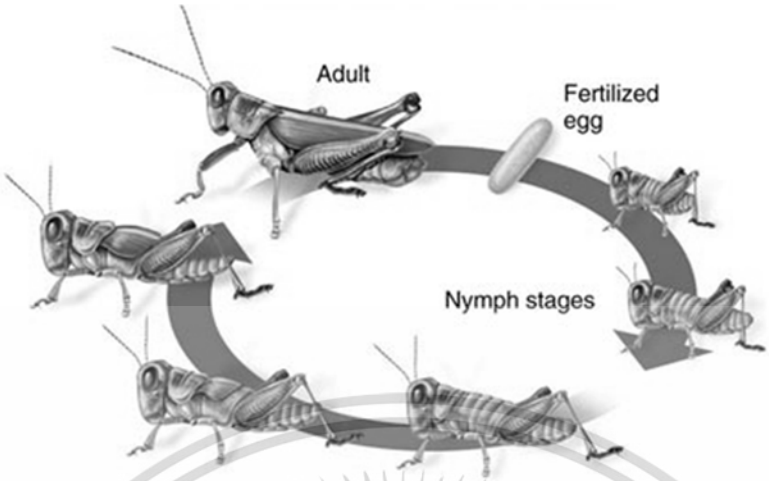
The practice of consuming insects is called entomophagy, from the Greek éntomon, insect, and phagein, to eat. It is estimated that edible insects are part of the diet of at least two billion people and more than 1900 insect species are currently used as food (FAO, 2009). The insect's acts as a good source of proteins, vitamins, minerals and energy and are more affordable to rural communities compared to animal proteins.

The consumption of insects can help in reducing cases of malnutrition. The insects most commonly consumed worldwide are beetles (*Coleoptera*, 31% of all insect species consumed), caterpillars (*Lepidoptera*, 18%) and bees, wasps and ants (*Hymenoptera*, 14%). Moreover, grasshoppers, crickets and locusts (*Orthoptera*, 13%) and cicadas, leafhoppers, planthoppers, scale insects and true bugs (*Hemiptera*, 10%) are consumed. Termites (*Isoptera*), dragonflies (*Odonata*), flies (*Diptera*) and other insects each comprise less than 3% of insects consumed (FAO, 2009).

Consumption of insects is widespread especially in Africa, Asia and Latin America. Nowadays human insect-eating is traditionally practised in 113 countries around the world. Over 2000 insect species are known to be edible. Globally, the most frequently consumed species are beetles, caterpillars, bees, wasps and ants. They are followed by grasshoppers, locusts and crickets, cicadas, leafhoppers and bugs, termites, dragonflies, flies and other species.

Whole insects are normally consumed as egg, larvae, pupae and adult depending on the insect order (Table 1). The main order of edible insects is *Lepidoptera, Coleoptera, Orthoptera, Diptera, Hymenoptera and Isoptera*, accounting for 80% of edible species known (van Huis, 2013).

(A)



(B)

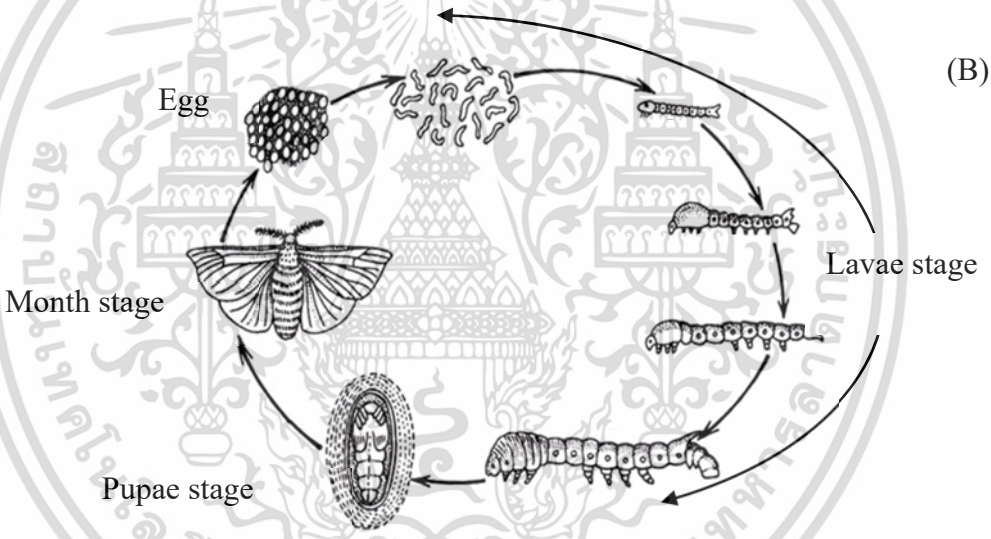


Figure 2. Insects life cycle; grasshopper/cricket (A) and silkworm month (B)

Table 1. Nutrition and Commonly eaten insect species.

Order	Common name	Consumption stage	Nutrition (%/dry weight)			
			Protein	Fat	carbohydrate	Amino acid
Orthoptera	Locusts, grasshoppers and crickets	Adults	44.10	02.20	01.20	38.87
Coleoptera	Beetles	Larvae	50.41	27.57	02.81	39.74
Lepidoptera	Butterflies and moths	Larvae, pupae	44.91	24.76	08.20	32.88
Diptera	Flies	Larvae	59.39	12.61	12.04	-
Hymenoptera	Ants,bees and waps	Larvae,adults	47.81	21.42	03.65	45.18
Isoptrea	Termites	Adults	-	-	-	44.03

Source: Modified from Kouřimská and Adámková (2016)

2.1.3 Order Lepidoptera

Other common names are: butterflies, silkworm moths, silkworm pupae, Eri silkworm pupae meal, Muga silkworm pupae meal and silkworm lavae. A number of species of silkworm are known: *Bombyx mori* Linnaeus, 1758 [Bombycidae]; *Antheraea assamensis* Helfer, 1837; *Antheraea mylitta* Drury, 1773; *Antheraea paphia* Linnaeus, 1758; *Samia cynthia ricini* [Saturniidae]. Silkworms are the caterpillars of moth species raised for the production of silk. The world's 90% production results from the cocoons of the domesticated mulberry silkmoth *Bombyx mori*, a Bombycidae moth. Silk is also produced from other domesticated or wild Saturnidae moth species, notably the Eri silkmoth *Samia cynthia ricini*, the Assam silkmoth *Antheraea assamensis*, the tussore (or tussah) moth *Antheraea mylitta* and the small tussore *Antheraea paphia* (Longvah *et al.*, 2011; Mishra *et al.*, 2003).

Spent silkworm pupae are a waste material often discarded in the open environment or used as a fertilizer (Wei *et al.*, 2009). It can be extracted to yield a valuable oil used in industrial products such as paints, varnishes, pharmaceuticals, soaps, candles,

plastic and biofuels (Trivedy *et al.*, 2008). The extracted meal is sometimes used for the production of chitin, the long-chain polymer of N-acetylglucosamine which is the main component of the exosqueleton (Suresh *et al.*, 2012). Silkworm pupae have long been part of human food in Asian silk-producing countries, and is considered as a delicacy in regions of China (Luo, 1997), Japan (Mitsuhashi, 1997), Thailand (Yhoung-Aree *et al.*, 1997) and India (Longvah *et al.*, 2011), among others. Due to its high protein content, silkworm pupae meal has been found to be suitable as a livestock feed, notably in monogastric species (poultry, pigs and fish), and also in ruminants (Trivedy *et al.*, 2008).

The world production of reelable silkworm cocoons was about 485,000 tonnes in 2011. By subtracting the amount of raw silk (161,000 tonnes), it can be assumed that 324,000 tonnes of fresh pupae (65,000 tonnes dry pupae) were produced in 2011 (FAO, 2012). Note that this estimate is much lower than the official statistics from China, where a figure of 440,000 tonnes of dry pupae in 2009 has been cited (Wei *et al.*, 2009). The main silk producers are China, India, Uzbekistan, Brazil, Thailand and Vietnam (FAO, 2012). Spent silkworm pupae are a highly degradable product. In silk production areas, the disposal of large quantities of pupae can cause serious environmental problems (Wang *et al.*, 2010). The utilization of this resource for feed and food or for the production of valuable biological substances such as chitin, protein, oil and fatty acids (linolenic acid) is a way to reduce the environmental impact of silk production.

Figure 3. Silkworm pupae (*Bombyx mori* Linn.)

2.1.4 Order Orthoptera

The common names are: Locust meal, locusts, desert locust, migratory locust, red locust, grasshoppers, grasshopper meal, katydids, crickets, cricket meal, house cricket, field cricket, Mormon cricket, *Orthoptera, Acridids, Acrididae, Gryllidae, Tettigoniidae*. Locusts, grasshoppers (mostly Acrididae and Pyrgomorphidae), crickets (*Gryllidae*) and katydids (*Tettigoniidae*) are insects of the order Orthoptera. They are generally edible and more than 80 species of locusts, grasshoppers and crickets are consumed worldwide for human food in Africa, South America and Asia. They may be part of the usual diets or delicacies sold by street vendors. They are eaten at home or in restaurants, both in rural and urban areas (van Huis *et al.*, 2013). Locusts are a group of grasshopper species that become gregarious and migratory when their populations are dense enough. During the swarming phase, locusts destroy or severely damage crops. They are a major pest of historical importance, notably in Africa (North, West, Sahel and Madagascar), Australia and the Middle-East. A locust swarm can represent a considerable amount of biomass. A single swarm can contain up to 10 billion insects and weigh approximately 30,000 tonnes (van Huis *et al.*, 2013). The swarming behavior makes locusts relatively easy to harvest for food. In Africa, the desert locust (*Schistocerca gregaria*), the migratory locust (*Locusta migratoria*), the red locust (*Nomadacris septemfasciata*) and the brown locust (*Locustana pardalina*) are commonly eaten (van Huis *et al.*, 2013). In Japan, China and Korea, rice field grasshoppers (including *Oxya yezoensis*, *O. velox*, *O. sinuosa*, and *Acrida lata*) are harvested for food (van Huis *et al.*, 2013). In Mexico, chapulines, which are grasshoppers of the Sphenarium genus, and notably *Sphenarium purpurascens*, a pest of alfalfa, are popular edible insects (Cohen *et al.*, 2009). The grasshopper Ruspolia differens, which is actually a katydid, is a common food source in many parts of eastern and southern Africa (van Huis *et al.*, 2013). Crickets are a common food in South East Asia, particularly in Thailand. The house cricket *Acheta domestica*, *Gryllus bimaculatus*, *Teleogryllus occipitalis*, *Teleogryllus mitratus*, the short-tail cricket *Brachytrupes portentosus* and *Tarbinskiellus portentosus* are edible cricket species (van Huis *et al.*, 2013). Grasshoppers, locusts and crickets are usually collected in the wild, preferably at night (using artificial light) or in the morning when the temperature is cooler and the insects are less active and easier to catch. Commercial farming of locusts, grasshopper and crickets for the food and

feed market is developing in South East Asia. In 2012, there were about 20,000 cricket farmers in Thailand, raising the species *A. domestica* and *G. bimaculatus*. *Orthoptera*, and particularly locusts, are commonly raised to feed pets and zoo animals (van Huis *et al.*, 2013).

The house cricket *A. domestica* is easy to farm and can produce from 6 to 7 generations per year (Hanboonsong *et al.*, 2013). It is omnivorous and can eat a large range of organic materials. Production is feasible at temperatures higher than 20 ◦C and the ideal temperature is 28–30 ◦C. Approximately 2000 insects can be bred in 1 m^2. Cricket population is self-regulated by cannibalism. The harvesting of locusts and other pest grasshoppers for food and feed is a means to biological control them, and the harvesting may help to reduce the application of chemical pesticides and thereby environmental pollution (Khusro *et al.*, 2012). For instance, the outbreak of patanga locust (*Patanga succincta*) in maize in Thailand in late 1970s led to a campaign to promote the eating of this locust, which is now farmed for food purposes (van Huis *et al.*, 2013). In Mexico, hand-picking of chapulines grasshoppers infesting alfalfa fields decreased environmental damage while generating an extra source of nutrition and income from the consumption and sale of grasshoppers (Cerritos & Cano-Santana, 2008). The presence of livestock can also help to control locust populations.

Table 2. Chemical composition of edible insects.

Chemical composition	Locust or grasshopper	House cricket	Field cricket	Silkworm pupae
Crude protein (% in DM)	57.3 ± 11.8	63.3 ± 5.7	58.1	60.7 ± 7.0
Crude fibre	8.5 ± 4.1	-	-	3.9 ± 1.1
Ether extract (% in DM)	8.5 ± 3.1	17.3 ± 6.3	10.3	25.7 ± 9.0
Ash (% in DM)	6.6 ± 2.5	5.6 ± 2.4	3.0	5.8 ± 2.4

(-) is not analysis.

Source: Modified from Makkar *et al.* (2014)

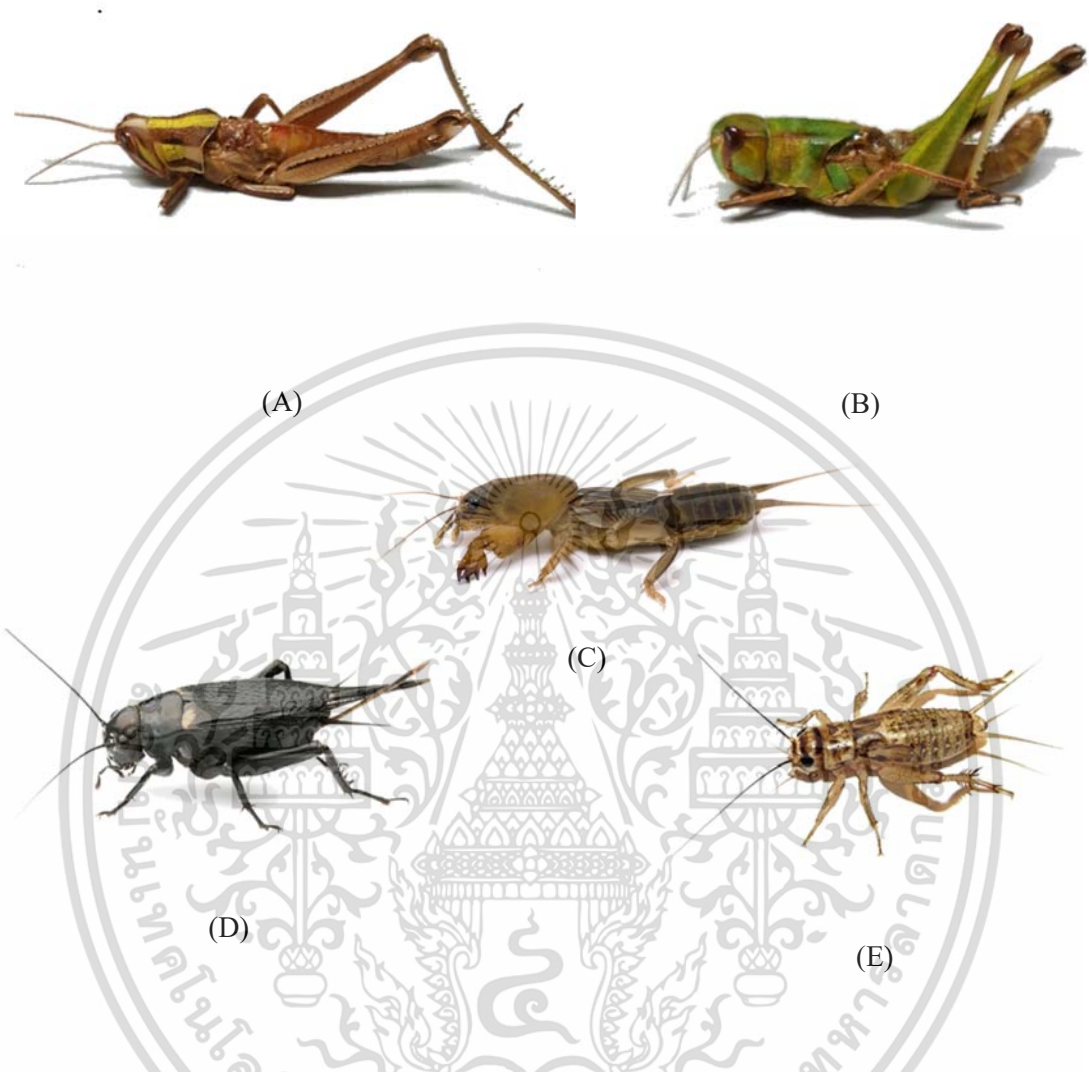


Figure 4. grasshoppers *Patanga succincta* (A) and *Chondracris roseapbrunner* (B), mole crickets (*Gryllotalpa orientalis*) (C), two-spotted cricket (*Gryllus bimaculatus*) (D) and house cricket (*Acheta domesticus*) (E)

Table 3. Amino acid composition of edible insects.

Amino acids	Amino acid content (g/16 g nitrogen)			
	Locust or grasshopper	House cricket	Field cricket	Silkworm pupae
Alanine	4.6	8.8	5.6	5.8
Arginine	5.6	6.1	3.7	5.6
Aspartic acid	9.4	7.7	6.3	10.4
Cystine	1.1	0.8	1.0	1.0
Methionine	2.3	1.4	1.9	3.5
Lysine	4.7	5.4	4.8	7.0
Isoleucine	4	4.4	3.1	5.1
Leucine	5.8	9.8	5.5	7.5
Phenylalanine	3.4	3.0	2.9	5.2
Threonine	3.5	3.6	2.8	5.1
Tryptophan	0.8	0.6	-	0.9
Glutamic acid	15.4	10.4	9.1	13.9
Histidine	3.0	2.3	1.9	2.6
Proline	2.9	5.6	4.5	5.2
Serine	5.0	4.6	3.7	5.0
Glycine	4.8	5.2	3.6	4.8
Tyrosine	3.3	5.2	3.9	5.9
Valine	4.0	5.1	4.4	5.5

(-) is not analysis.

Source: Modified from Makkar *et al.* (2014)

Table 4. Fatty acid composition of insect lipids.

Constituents in (% fatty acids)	Housefly maggot meal	silkworm pupae	Mealworm	House cricket
Saturated fatty acids (%)				
Lauric, 12:0	-	0.4	0.5	-
Myristic, 14:0	5.5	2.8	4	0.7
Palmitic, 16:0	31.1	29.6	21.1	23.4
Stearic, 18:0	3.4	3.2	2.7	9.8
Monosaturated fatty acids (%)				
Palmitoleic, 16:1 *n* − 7	13.4	13.3	4	1.3
Oleic, 18: 1 *n* − 9	24.8	18.7	37.7	23.8
Polyunsaturated fatty acids (%)				
Linoleic, 18:2 *n* − 6	19.8	16.4	27.4	38
Linolenic, 18:3 *n* − 3	2	2.1	1.2	1.2

(-) is not analysis.

Source: Modified from Makkar *et al.* (2014)

2.2 Nutritional value of edible insect

The nutritional value of edible insects is very diverse mainly because of the large number and variability of species. Nutritional values can vary considerably even within a group of insects depending on the stage of metamorphosis, origin of the insect and its diet. Similarly, the nutritional value changes according to the preparation and processing before consumption (drying, cooking, frying etc. (Kouřimská & Adámková, 2016).

2.2.1 Proteins (Kouřimská & Adámková, 2016)

Kouřimská and Adámková (2016) assessed protein content in various insect species. Protein content was in the range of 44 to 59% by dry matter (Table 1), reflecting the large variability of tested species. These values are on average only a little smaller than values for egg protein or beef and even higher than in the case of many plant proteins (Finke, 2002). Measured amounts of nitrogenous substances of insects may be higher than

their actual protein content since some nitrogen is also bound in the exoskeleton (Klunder *et al.*, 2012).

Considering the amino acid composition of edible insects, they contain a number of nutritionally valuable amino acids including high levels of phenylalanine and tyrosine. Some insects contain large amounts of lysine, tryptophan and threonine, which is deficient in certain cereal proteins. For example, in Angola the intake of these nutrients may be supplemented by eating termites of the genus *Macrotermes subhyalinus* (Sogbesan & Ugwumba, 2008). The native people of Papua New Guinea normally eat tubers, where the content of lysine and leucine is low. The resulting nutritional gap could therefore be compensated by the consumption of larvae of the *Rhynchophorus* family beetle that have high amounts of lysine. On the contrary tubers contain a high proportion of tryptophan, and aromatic amino acids which are present in limited quantities in these larvae.

2.2.2 Lipids (Kouřimská & Adámková, 2016)

Edible insects contain on average 10 to 60% of fat in dry matter (Table 1). This is higher in the larval stages than in adults (Xiaoming *et al.*, 2010). Caterpillars belong among insects with the highest fat content. Tzompa-Sosa *et al.* (2014) determined the total fat content in caterpillars (*Lepidoptera*) from8.6 to 15.2 g per 100 g of insects. In contrast, the fat content ranges from 3.8 g to 5.3 g per 100 g of insects in grasshoppers and related *Orthoptera* species.

Fat is present in several forms in the insect. Triacylglycerols constitute about 80% of fat. They serve as an energy reserve for periods of high energy intensity, such as longer flights. Phospholipids are the second most important group. Their role in the structure of cell membranes has been studied (Tzompa-Sosa *et al.*, 2014). The content of phospholipids in fat is usually less than 20%, but it varies according to the life stage and insect species (Tzompa-Sosa *et al.*, 2014). There is a relatively high content of C18 fatty acids including oleic, linoleic and linolenic acids in the fat of insects (Tzompa-Sosa *et al.*, 2014). Palmitic acid content is also relatively high.

Cholesterol is the most abundant sterol in insects. Ekpo *et al.* (2009) studied the content of cholesterol in the fat of the termite *Macrotermes bellicosus* and the caterpillar *Imbrasia belina*, which are commonly consumed in Nigeria. They found that the average cholesterol content in the lipid fraction was 3.6%. Apart from cholesterol,

campesterol, stigmasterol, β-sitosterol and other sterols may be also present in edible insects (Sabolová *et al.*, 2016).

2.2.3 Fibre (Kouřimská & Adámková, 2016)

Edible insects contain a significant amount of fibre. Insoluble chitin is the most common form of fibre in the body of insects contained mainly in their exoskeleton (van Huis, 2013). Chitin in commercially farmed insects ranged from 2.7 to 49.8 mg per kg of fresh weight (from11.6 to 137.2 mg per kg of dry matter) (Finke, 2007). Chitin is considered as an indigestible fibre, even though the enzyme chitinase is found in human gastric juices (Paoletti *et al.*, 2007). However, it was found that this enzyme may be inactive. Active chitinase response in the body prevails among people from tropical countries where the consumption of insects has a long-term tradition (Muzzarelli *et al.*, 1994). Removal of chitin improves the digestibility of insect protein (Finke, 2007). Chitin is also associated with the defence of the organisms against some parasitic infections and allergic states (Finke, 2007). Lee *et al.* (2008) reported that chitin was antivirally active against tumorigenesis. Chitin and its derivative chitosan have properties that could improve the immune response of specific groups of people. They helped some individuals to be more resistant against pathogenic bacteria and viruses. There are also indications that chitin could reduce allergic reactions to certain individuals (Muzzarelli, 2010).

2.2.4 Chemical constituents of *Order Lepidoptera* (Makkar *et al*., 2014)

Silkworm pupae meal is a protein-rich feed ingredient with a high nutritional value. Its crude protein content is 60.7% (Table 2). The lysine (6–7% in 100 g crude protein) and methionine plus cystine levels of approximately 4% (Table 3) are particularly high. However, the true protein (calculated as the sum of amino acids) in silkworms was found to correspond to only 73% of the crude protein content (Finke, 2002), which was explained by the presence of chitin, since this component includes nitrogen. On the other hand, the chitin content of pupae meal is relatively low, about 3–4% DM (Finke, 2002). The presence of chitin and insoluble protein may also explain the ADF values of 6–12% in DM (Finke, 2002). Non-defatted pupae meal is rich in fat, up to 37%. Silkworm oil contains a high percentage of polyunsaturated fatty acids, notably linolenic acid (18:3), with values ranging from 11 to 45% of the total fatty acids (Rao, 1994). Silkworm litter appears to have

an extremely variable composition, with crude protein values of between 15 and 58% in dry matter (Patil *et al.*, 2013).

2.2.5 Chemical constituents of *Order Orthoptera*

Locusts and other Orthoptera species are generally rich in crude protein (50–65%), though some lower values (20%). Essential amino acid composition is reasonably good. The crude protein content of house cricket (*A. domesticus*) is also very high (Table 2). Both lysine, and methionine plus cystine contents in house cricket are lower than in locust meal (Tables 3). The palmitoleic acid level in house cricket is approximately 15-fold lower than that in housefly maggot and 4-fold lower than in mealworm. On the other hand, the level of linoleic acid (Table 4) is higher in house cricket. As for other insects, crude protein contents of field cricket (*Gryllus testaceus*) is high (ca 60%) and they contain 10–13% lipids (Table 2). Lysine content is lower in field cricket, while the level of sulphur-containing amino acids (methionine plus cystine) is higher in field cricket (Table 3).

2.3 The functional properties of protein (Söderberg, 2013)

The functional properties of a protein are affected by both intrinsic and extrinsic factors. The intrinsic factors are: shape, size, amino acid composition and sequence, the distribution of net charges, the ration between hydrophobicity/hydrophilicity, secondary, tertiary and quaternary structures of the protein as well as the protein's capacity to interact with other components in the food system. The extrinsic factors that affect the functionality of proteins are: pH, temperature, moisture, chemical additives, mechanical processing, enzymes and ionic strength (Kinsella, 1982). There are proteins that are associated with specific functional properties, such as egg proteins with coagulation, or soy proteins for their use in forming food gels. Some example of functional properties can be seen in Table 5 (Kinsella, 1982).

Table 5. Functional properties of proteins in food applications

General property	Functional criteria
Organoeptic	Color, flavor and ordor
Kinesthetic	Texture, mouthfeel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening Gelling, syneresis, viscosity
Surface	Emulsification, foaming (aeration, whipping), film formation
Binding	Lipid-binding, flavor-binding
Structural	Elasticity, cohesiveness, chewiness, adhesion, network crossbinding, aggregation, dough formation, textruisability, fiber formation, extrudability
Rheological	Viscosity, gelation
Enzymatic	Coagulation (rennet), tenderization (papain), mellowing (proteinases)
Blendability	Complementarity (wheat-soy, gluten-casein)
Antioxidant	Off-flavor prevention (fluid emulsion)

Source: (Kinsella, 1982)

In order to evaluate if a protein is applicable and suitable in certain food systems and food products, it is important to characterize the functionalities of the protein (Kinsella, 1982). For the proteins to be used in foods they must possess or contribute characteristics that are appropriate in interaction with other food components (e.g. water and lipids) or be suitable for processing. The functional properties that are required from a protein vary with different food applications and food systems. The three most important functional properties of food proteins in general are solubility, emulsification and foaming (Kinsella, 1982).

Proteins must show good and multiple functionalities in order to perform well in food systems. This requires a deeper understanding of the structure-function relationship, which sometimes can be hard to determine. One reason why proteins possess such different functional properties is the fact that all proteins are built up by different amino acids. The

amino acid composition affects the functional properties of a protein according to how they are disposed in the polypeptide chain, as well as what type and how many of those amino acids that are present (Kinsella, 1981).

2.3.1 Solubility (Söderberg, 2013)

The solubility of a protein is the most important functional property since the protein needs to be soluble in order to be applicable in food systems. Other functional properties like emulsification, foaming, and gelation are dependent on the solubility of proteins. Solubility can be described as when equilibrium exists between hydrophilic and hydrophobic interactions. The solubility of a protein is related to the pH, where it is minimal at the isoelectric point, making the environmental pH the most important factor when it comes to the degree of protein solubility. The solubility is also influenced by temperature and ionic strength. Freezing, heating, drying and shearing are also factors that have an influence of protein solubility in food systems. Insoluble proteins are not good for food applications and thus it is important that denaturation caused by e.g. heat is controlled so that the protein solubility not will be affected in a negative way (Raikos *et al.*, 2007).

2.3.2 Emulsions (Söderberg, 2013)

Emulsions consist of two liquids that are immiscible, where one of the liquids is dispersed in the other in form of small droplets. Emulsions can be classified according to the distribution of the oil and the aqueous phase. A system where the oil droplets are dispersed in the aqueous phase is called oil-in-water emulsion (O/W). Food systems like this are mayonnaise, milk, cream, soups and sauces. The opposite of an O/W emulsion is water-in-oil (W/O) but there are also water free emulsions and multiple emulsions (O/W/O or W/O/W). The droplets in an emulsion are called the dispersed (or internal) phase, whereas the surrounding liquid is referred to the continuous (or external) phase (McClements, 2015).

When water and oil are homogenized they rapidly separate into two layers, one layer of oil, which has high density, and one layer with water that has low density. This is called phase separation and has to do with the fact that the droplets fuse together with adjacent droplets that are similar to themselves. To get a stable emulsion (both in a short and long term perspective) it is of great importance to add an emulsifier. An emulsifier is a surface-active molecule that allows the two phases to homogenize. Surface-active

molecules are mostly amphiphilic i.e. they have both hydrophobic and hydrophilic parts, which allow the two liquids to blend together.

2.3.3 Foaming (Söderberg, 2013)

Foams consist of a gas phase, a liquid phase and a surfactant (e.g. proteins) and whipping or shaking form foams. Foods made up by foams are e.g. whipped toppings, meringues, ice creams, chiffon desserts and angel cakes (Kinsella, 1981). Angel cakes and other baked goods are solid foams. Foams are formed through unfolding and absorption of the protein, at the air-water interface, as well as film formation around the air bubbles. Different proteins have different abilities to form and stabilize foams, and just as in the case of proteins and their different emulsifying properties, this is related to different physical properties of the proteins. For a protein to have superior foaming properties, it must possess high solubility in the liquid phase as well as the ability of quickly forming a film around the air bubbles in the food system (Kinsella, 1981). The extrinsic factors that affect the foaming properties are e.g. pH, temperature and ionic strength. Foam stability and the proteins ability to form foams are also of big importance. In order for a protein to form stable foams the interfacial film should be rigid and not let the entrapped air escape (i.e. it should be almost impermeable). The protein should also have the ability to form strong bonds like hydrogen bonding and hydrophobic interactions. The protein should also possess limited denaturation at the surface to keep viscosity and rigidity (Kinsella, 1981).

2.3.4 Gelling / coagulation (Söderberg, 2013)

The globular proteins' gelling properties are of big importance in foods (Van Kleef, 1986). According to Ikeda and Nishinari (2001) is protein gelation one of the most important functional properties when it comes to modify the structure and texture of foods. One example is the importance of the gelation properties of egg in foods like cakes, omelets and confectionary. The texture of foods and thus, the gelation properties of a protein, affect consumer acceptability (Kiosseoglou & Paraskevopoulou, 2005).

Globular proteins, such as egg white and soybean protein, are able to form gels upon heating (Doi, 1993). For a gel to form it is important that the functional groups (e.g. hydrophobic groups) within the protein are exposed. This makes it easier for the groups to interact and form a three dimensional network. Gel formation is complicated, and affected by the concentration of protein, amount of water, ionic strength, time and

temperature as well as pH and interaction with other components in the food system (Raikos *et al.*, 2007). The process for gelation in short, is:

$$\text{Native protein} \xrightarrow[\text{Heat}]{} \text{Protein denaturation} \xrightarrow[\text{Heat}]{} \text{Gel/coahulum formation}$$

The heat will make the native protein to denaturate, and during the denaturation disulfide bonds will be formed and hydrophobic amino acid residues are exposed (Shimada & Matsushita, 1980). After denaturation and further heating, the proteins will aggregate and interact with other proteins and form either a gel or a coagulum. Which type that is formed depends on conditions like molecular weight, heating time and protein concentration (Raikos *et al.*, 2007; Shimada & Matsushita, 1980). The gel structure is a more structured network compared to the coagulum that is a disorganized aggregation (Raikos *et al.*, 2007).

2.4 Antioxidant activity of proteins

Proteins can also act as free radical scavengers, chelating agents for transition metals, quenchers of singlet oxygen molecule and decreases the radical damage in biological systems (Zieliński & Kozłowska, 2000). The antioxidant activity of proteins depends on the amino acid constitute, for example amino acids that are aromatic donate protons to free radicals, few examples of such amino acids are tyrosine, phenylalanine, and tryptophan as well as sulfur containing amino acids like cysteine. Glutamate, and aspartate are acidic amino acids, and lysine, arginine are basic amino acids. These amino acids scavenge free radicals by metal chelating ions (Zou *et al.*, 2016). Histidine amino acid has imidazole ring, and because of this ring, histidine amino acid has metal chelating and free radical scavenging activity. The positioning of amino acids in the protein has an important role in the protein antioxidant activity. Each peptide has different antioxidant activity depending on the proline at the N-terminus as well as C- terminus. Proline at the N-terminus is better in preventing oxidation of linoleic acid compared to the proline at the C-terminus. Peptides that have histidine at the N-terminus are better metal chelating compared to peptides having histidine at the C-terminus. Hydrophobicity has been reported

to have a close relationship with antioxidant activity of peptides as well as proteins, this has been related to the ability to bind and hide unsaturated lipids. Cationic properties of proteins also is related to its antioxidant activity, since lipid oxidation is inhibited by electrostatic repulsion of transition metals away from lipid droplets by the positively charged groups. Other than antioxidant activity, proteins have other functions such as emulsifying activity, for example protein isolate and whey protein are known to have emulsifying activity. Lactoferrin and phosvitin are examples of protein that possess both antioxidant activity as well as anti-microbial activity. Gel formation, flavour binding as well as increase of viscosity are additional functions of proteins. It has been shown that functional and nutritional properties of proteins isolate as well as protein concentrate has been improved by enzymatic hydrolysis (Griffiths, 2000).

2.5 Noodle (Fu, 2008)

Noodles were spread across Asian to Korea, Japan, Philippines, Thailand, Malaysia and Indonesia by Chinese traders, seafarers and migrants. With the introduction of noodles to other Asian countries, different types of products developed due to varying regional preferences.

Noodles are a traditional food widely consumed throughout Asia. In the past, they were made within the home for consumption by the household. Today they remain a staple of Asian diets and, with improved food technology, offer variety, versatility and high nutritional quality. Noodles are readily available and can be purchased in a variety of forms including fresh, cooked or processed for longer shelf life. Due to their ease of preparation, noodles are now considered as a convenient fast food.

Depending on the processing methods adopted, noodles can be categorized as fresh, dried, boiled, steamed and instant types. For all of these noodles, the primary steps are similar, involving mixing of ingredients (wheat flour, salt and water), resting, sheeting and cutting. Noodles that are sold directly after being processed in this way are known as fresh noodles. These are usually dusted with starch or fine flour immediately after the cutting process to prevent the strands from sticking to each other during handling and transportation. Noodles can also be processed further by drying, boiling, steaming and frying.

Dried noodles are produced from raw wet noodles that have undergone a controlled drying process, whereas boiled noodles are precooked in boiling water. Steamed noodles are produced by treating fresh noodles with steam. “Instant” type noodles are prepared first by steaming the noodles and thereafter dehydrated and this typically is achieved by a deep frying process. The processes involved in manufacture of different types of noodles are shown in Figure 5.

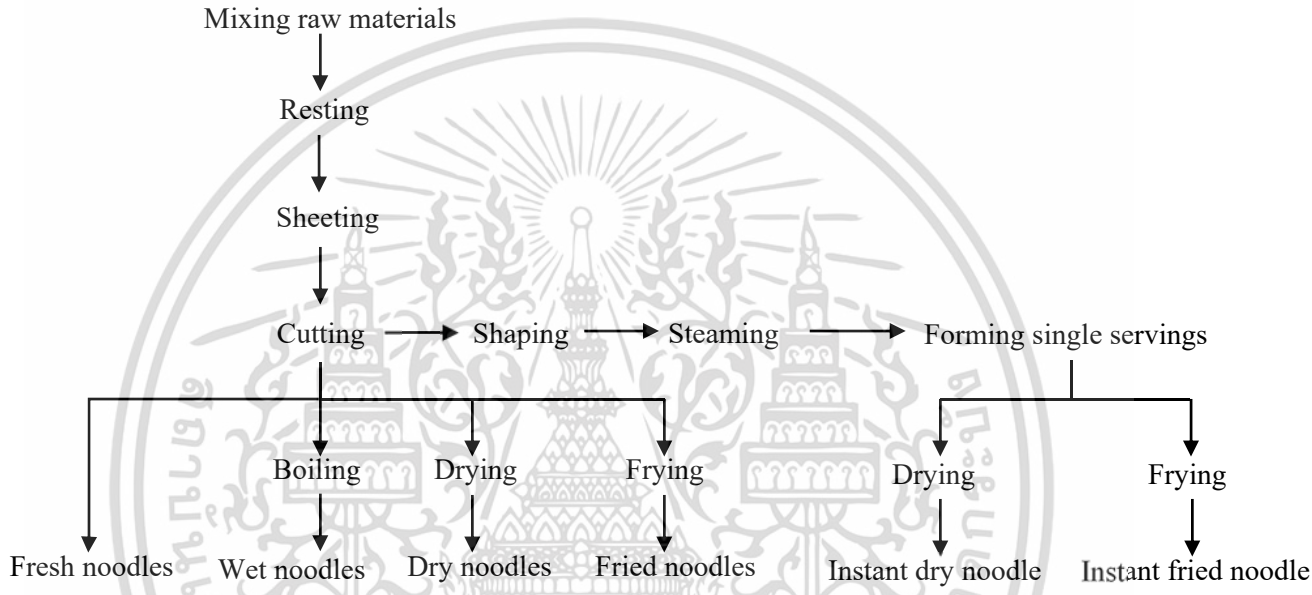


Figure 5. Processing steps used in noodles making

Alternatively, noodles can be classified on the basis of the raw materials used and two types have been widely recognized in the literature: WSN and YAN. The main difference between these is the use or presence of alkaline salts in the formulation. In the case of alkaline noodles, solutions of sodium carbonate, potassium carbonate, sodium bicarbonate or sodium hydroxide, commonly known as lye water or “kan sui” are added. Therefore, depending on the presence or absence of alkaline salts, noodles can be classified as non-alkaline (WSN or commonly known as “udon” in Japan) or alkaline. Asian noodles are not made exclusively from wheat, with many being made from rice, buckwheat and starches derived from mung bean and potato. However, Asian noodles are made primarily from bread wheat flour.

2.5.1 Noodle formulation

Tremendous varieties of Asian noodles exist around the world and within a country (Table 6). These varieties are the result of differences in culture, climate, region and a host of other factors. Table 7 shows the formulation of seven major types of noodles. Both Chinese raw noodles and Japanese udon noodles have the most simplified formulas, containing only flour, water and salt. However, Chinese raw noodles are made from hard wheat and medium to high protein flour, and Japanese udon noodles are produced from soft wheat flour of medium protein content. Chinese raw noodles have been shown to be very useful in screening noodle color due to their simple formulation (Hou *et al.*, 1998).

Chinese wet noodles and chuka-men (alkaline noodle) are characterized by the presence of kan sui (alkali salt), while Malaysian hokkien noodles are characterized by the presence of sodium hydroxide, giving the noodles their characteristic yellowness, alkaline flavor, high pH and improved texture. Both Chinese wet and hokkien noodles are parboiled types, while chuka-men can be either uncooked or cooked. Instant fried noodles usually contain guar gum or other hydrocolloids, making the noodles firmer and easier to rehydrate upon cooking or soaking; polyphosphates allow more water retention on the noodle surface, thus, giving them better mouth-feel. Native or modified potato starch or other equivalent starches are often added in premium instant fried noodles, providing springy texture and improved steaming and cooking quality due to reduced gelatinization temperature. Thailand bamee noodles are characterized by having 10% eggs in the formula. Therefore, egg source and quality are additional variables in bamee noodle quality (Hou *et al.*, 1998).

Table 6. Types of Asian noodles consumed

Region	Type
China/Hong Kong	Instant fried, Chinese raw, dried, hand-made
Indonesia	Instant fried, Chinese wet
Japan	Chuka-men (Chinese style yellow alkaline noodle), Japanese types (include hiramen, udon, hiya-mughi, so-men), soba
Korea	Instant fried, dried, udon, soba
Malaysia	Hokkien, instant fried, Cantonese (alkaline raw), dried
Philippines	Instant fried, dried, Chinese wet, udon
Singapore	Hokkien, Cantonese, instant fried
Taiwan	Chinese wet, Chinese raw, instant fried, dried
Thailand	Bamee, dried, instant fried
Europe, Africa	Instant fried
Latin/South America	Instant fried or dried
North America	Instant fried or dried, Chinese raw, udon, soba

Source: (Hou *et al.*, 1998)

Table 7. The formulation of seven major types of noodles

Ingredient (g)	Noodle Type						
	Chinese Raw	**Chinese Wet**	**Chukamen**	**Japanese (Udon)**	**Malaysian (Hokkien)**	**Thailand (Bamee)**	**Instant Fried**
Flour	100	100	100	100	100	100	100
Water	28	32	32	34	30-33	28	34-37
Salt	1.2	2	1	2	2	3	1.6
Edible Oil	-	-	-	-	-	-	1-3
Potato Starch	-	-	-	-	-	-	0-12
Sodium Hydroxide	-	-	-	-	0.5	-	-
Sodium Carbonate	-	0.45	0.4	-	-	1.5	0.1
Potassium Carbonate	-	0.45	0.6	-	-	-	0.1
Eggs	-	-	-	-	-	10	-
Guar Gum	-	-	-	-	-	-	0-0.2
Polyphosphates	-	-	-	-	-	-	0-0.1

Source: (Hou *et al.*, 1998)

2.5.2 Functionality of main ingredients (Gulia *et al*., 2014)

2.5.2.1 Wheat Flour

The preferences for color, texture, and eating quality of noodles vary widely in different countries and thus wheat flour specifications also vary. Adequate dough strength and extensibility is crucial for noodle flour to withstand sheeting, resist tearing, breakage, and shrinking of dough sheet. Both protein quality and quantity influence characteristics of instant noodles, including fat absorption, color, and textural quality, as well as dough properties like water absorption and color. Flour protein content has a positive correlation with cooked noodle firmness and a negative correlation with noodle brightness. The viscoelasticity of heat-treated gluten, isolated with 2% NaCl solution

significantly correlated with gluten strength and Chinese white salted noodle (WSN) texture and can be used for predicting WSN quality.

Starch properties are also important in determining quality of noodles as their texture depends largely on gelatinized starch. Variations in starch properties have been found to play a major role in noodle softness and viscoelastic properties. Pasting characteristics, peak viscosity, flour swelling volume, amylose: amylopectin ratio, and damaged starch determine the noodle properties. Flours with low gelatinization temperatures are preferred for rapid hydration during cooking. Higher starch damage is associated with poor noodle color, undesirable high cooking loss, and excessive surface swelling. The fine particle flour with lower starch damage, therefore, results in good cooking quality of noodles. The rheological properties of raw WSN have been found to be mainly influenced by the size of starch granules, where the small starch granules exhibited high amounts of water absorption during dough preparation and a dense packing of starch granules inside a thin gluten-strand network. However, the rheological properties of cooked WSN were mainly dominated by the amylose content and fine structure of the amylopectin, which resulted in the differences in water absorption and cooking time required for cooked WSN.

2.5.2.2 Water

Water is another essential ingredient, which is necessary for gluten formation, which provides viscoelastic properties to dough required for noodle processing. The amount of water needed for noodle processing is optimized to hydrate the flour and develop a uniform dough sheet. The optimum water absorption for noodle is affected by protein content, protein quality, damaged starch, and other physical properties of flour. The water absorption level recommended for noodle processing is about 30–38% based on flour weight. Water absorption level has a major impact on the amount of work required in processing as well as color. There was a significant decline in textural characteristics with increasing water absorption. Sheeting, cutting, and drying of noodles become difficult when water absorption deviates more than 2–3% from the optimum level. Insufficient water results in noncohesive stiff dough and less extensible noodle sheet while too much water results in dough stickiness handling problems during processing.

2.5.2.3 Salt and Alkaline Reagents

The amount of salt added in noodles is usually 1–3% of flour weight. Salt has strengthening and tightening effect on the gluten, which may be due to its inhibitory effect on proteolytic enzymes, or by direct interaction of the salt with flour proteins. Thus, it significantly improves sheeting properties of dough, especially at high water absorption levels. Incorporation of salt reduces cooking time, enhances flavor, provides softer but more elastic texture, and inhibits enzyme activities and the growth of microorganisms (Fu, 2008).

Alkaline salt can be used alone or in combination with different salts, depending on local preference. The most commonly used alkaline salts are sodium and potassium carbonates. Other alkaline reagents, such as sodium hydroxide and bicarbonates are also used in some countries. The type of alkaline salt used also affect the quality of noodles. Addition rates of alkaline salts are 0.5–1.5% for noodles with strong alkaline flavor and 0.1–0.3% as a quality improver for instant noodles. The unique yellow color associated with addition of alkaline salts in common wheat used for noodle preparation is due to endogenous flavonoids undergoing a chromophoric shift, i.e., turning yellow, in the presence of alkali. The changes in dough characteristics associated with alkaline pH fundamentally influence the behavior of the gluten proteins resulting in tougher, tighter, and less extensible dough. The toughening of dough with alkali addition has a very significant impact on the processing properties and the texture of the final products. The addition of alkali increases water absorption potential of noodle dough, gives noodles a firmer texture, increases both the breaking and cutting forces of noodles.

2.5.2.4 Hydrocolloids

Hydrocolloids such as guar gum, locust bean gum, alginates, and carboxymethyl cellulose (CMC) are widely used in instant noodle processing. The addition of gums (0.1–0.5%) improves rehydration characteristics of noodles during cooking, modifies the texture and overall “mouthfeel” of finished product, and decreases the fat uptake during frying of instant noodles as they are hydrophilic and have high water binding capacity. Gum and starch improved binding and mechanical network in the dough. Insufficient water in the dough apparently reduced cohesion in the dough, whereas excess

water reduced the functionality of gum and starch. (Choy *et al.*, 2012) also reported that the combined use of acetylated potato starch (APS) and CMC primarily affects textural attributes of hardness and adhesiveness, rather than other quality parameters of instant noodles. However, addition of CMC alone had a negative impact on the cohesiveness values of cooked instant noodles with minimal effects on stickiness and fat uptake, whereas APS enhanced noodle hardness without significantly affecting the cohesiveness values. It was concluded that APS can be used as an ingredient for enhancing noodle eating quality in case of lower protein wheat flours. Lee *et al.* (2008) studied the effects of addition of alginate on physicochemical, rheological, and noodle-making properties of wheat flour. With the addition of alginate, noodles exhibited an increase in cooked weight, cutting and tensile forces, and yellowness while there was a decrease in cooking loss, lightness, and redness.

2.6 Related research

Yi *et al.* (2013) studied the protein extracted from five insect *T. molitor, Z.morio, A.diaperinus, A. domesticus and B. dubia* by distilled water mixed with ascorbic acid. They reported that supernatant containing 17–23% of total protein. Furthermore, Ndiritu *et al.* (2017) studied *Acheta domesticus* proteins extraction using distilled water mixed with ascorbic acid. They reported that yield with protein extracted was 32.72%.

Azagoh *et al.* (2016) studied the extraction of proteins from larvae and larvae meal of *Tenebrio molitor* using solubilisation into water at an alkaline pH. They reported that the protein yield of the larvae and larvae meal were 59.9% and 26.4%, respectively.

Clarkson *et al.* (2018) studied extracted *Locusta Migratoria* protein fractions using an adapted alkali isoelectric precipitation method. The protein content of insoluble fraction, soluble fraction (solids precipitated out at pH 4) and supernatant fraction from locust meal were 81%, 74%, and 69%, respectively. Moreover, protein yield of three fraction was 38%, 10% and 57%, respectively.

Hall *et al.* (2017) studied whole crickets (*Gryllodes sigillatus*) hydrolyzed with alcalase at 0.5, 1.5, and 3.0% (w/w) for 30, 60, and 90 min. Solubility, emulsion and foaming properties were evaluated. They reported that protein solubility of hydrolysates

exhibited over 30% soluble protein at acid pH and 50–90% at alkaline pH. Emulsion activity index ranged from 7 to 32 m^2/g, while foam ability ranged from 100 to 155% for all hydrolysates.

Torruco-Uco *et al.* (2018) studied the functional properties of the grasshopper (*Sphenarium purpurascens*). They reported that foaming capacity, foam stability and gelification capacity of grasshopper meal was 6.17%, 7.13 min and 14%, respectively.

Zielińska *et al.* (2018) studied the functional properties of three species of edible insects: *Gryllodes sigillatus, Schistocerca gregaria*, and *Tenebrio molitor*. The water and oil holding capacity, solubility, and foaming and emulsion properties were evaluated. They reported that the protein solubility showed minimum values at pH 5. The water and oil holding capacity for *T. molitor* protein and *G. sigilltus* protein was 3.95 g/g and 3.33 g/g, respectively. The *G. sigillatus* protein showed the highest foaming capacity, foam stability, and emulsion activity (99.0%, 92.0%, and 72.62%, respectively), while the *S. gregaria* protein exhibited the highest emulsion stability (51.31%).

de Oliveira *et al.* (2017) studied bread quality with adding cinereous cockroach (*Nauphoeta cinerea*) flour in amounts of 5, 10, and 15% (based on wheat flour). They reported that bread enriched with 10% roasted flour presented the best nutritional characteristics, differing little from the white and whole wheat bread.

Osimani *et al.* (2018) studied effects of cricket (*Acheta domesticus*) powder (10 or 30%) on the nutritional value of bread loaves. They reported that the addition of 10% cricket powder produced doughs apparently suitable for bread-making.

Choi *et al.* (2017) studied effects of replacing pork meat with yellow mealworms (5-25%) on the physicochemical properties and sensory characteristics of frankfurters. They reported that the content of protein and ash, pH, and yellowness of frankfurters with yellow mealworm were higher than those of the control and the fat content of treatments decreased with increasing yellow mealworm concentrations. Moreover, color, flavor, off-flavor, and juiciness scores decreased with increasing yellow mealworm concentrations. The addition, replacing lean pork meat with up to 10% yellow mealworm successfully

maintained the quality of frankfurters at a level similar to that of the regular control frankfurters.

Kim *et al.* (2017) studied effects of house cricket (*Acheta domesticus*) flour at 2 different levels (5% and 10%) addition on physicochemical and textural properties of meat emulsion under various formulations. They reported that the replacement of lean meat/fat portion with house cricket flour within 10% level could fortify protein and some micronutrients in meat emulsion, without negative impacts on cooking yield and textural properties (Hardness, Springiness, Cohesiveness, Gumminess and Chewiness).

Park *et al.* (2017) studied physicochemical properties of meat batters prepared with fresh pork meat, back fat, water, and salt and formulated with three different amounts (5%, 10%, and 15%) of silkworm pupae (*Bombyx mori*) powder. They reported that meat batters formulated with silkworm pupae powder showed significantly higher contents of protein and ash than control batter. Moreover, its lower cooking loss than the control. In addition, pH, viscosity, hardness, gumminess, and chewiness were improved after the addition of silkworm pupae. they conclude that silkworm pupae can be added to meat batter to improve its physicochemical properties.

CHAPTER 3

CHARCTERIZATION, FUNCTIONALITY AND ANTIOXIDANT ACTIVITY OF WATER-SOLUBLE PROTEINS EXTRACTED FROM *Bombyx mori* Linn.

3.1 Abstract

The yield of water-soluble protein (WSPB) extracted from silkworm pupae was 3.96% by wet weight basis. The major amino acids were found as glutamic acid, which was the most abundant, followed by histidine, phenylalanine and glycine in that order. The electrophoretic study revealed that proteins with MW of 37, 64 and 75 kDa were the major protein components in WSPB. Based on FTIR analysis, WSPB remained its structural integrity. The surface hydrophobicity, free and total sulfhydryl group contents were 3.52, 22.17 μmol/g and 23.08 μmol/g, respectively. WSPB was highly solubilized in the pH range of 5-11. WSPB exhibited poor emulsifying properties and foaming capacity but the foam stability was comparable to bovine serum albumin (BSA). WSPB had high antioxidant potential based on $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP assay. Therefore, protein from silkworm pupae is a potential source of antioxidant and can be served as an ingredient in processed foods to enhance its desired functionality and nutritional value.

3.2 Introduction

Silkworm pupae (*Bombyx mori* Linn.) are considered a good food source for humans because of their high nutritional value (Wu *et al.*, 2011). They have been consumed in many Asia countries including Thailand, China, Korea, Japan and India. Silkworm pupae are the main by-product of the silk industry and constitute 60% of dry cocoon weight after extracting threads (Hu *et al.*, 2017). The nutritional value of silkworm pupae is rich in lipids (20.1 g/100g) and protein (12 g/100g), exhibiting the high levels of essential amino acids such as valine, methionine and phenylalanine (Mishra *et al.*, 2003). Several studies have shown that silkworm pupae contain 45 to 55% protein (18 amino acids, including 8 essential amino acids) on a dry matter basis. Four kinds of protein components have been

identified in silkworm pupae protein which albumin was found to be the highest at 27.24%, followed by glutelin at 23.72%, prolamine at 11.82% and globulin at 4.21%, respectively (Wang *et al.*, 2011). The hydrolysate of albumin was determined with the highest angiotensin-converting enzyme (ACE) inhibiting effect, followed by globulin. Inhibiting ACE could be decreased hypertension, which recognized as a serious risk factor for cardiovascular diseases (Wang *et al.*, 2011). This means that it is a good-quality protein source and a good source of bioactive peptides (Wang *et al.*, 2011; Yang *et al.*, 2009).

Proteins can be added to foods to improve their functional properties such as solubility, emulsifying and foaming properties (Zielińska *et al.*, 2018). Park *et al.* (2017) also determined the effects of adding flour made from silkworm pupae to meat batter. They found that combining the flour with transglutaminase improved the physicochemical properties of meat batter. Kim *et al.* (2016) found that adding *B. mori* pupae flour to emulsion sausages increased their cooking yield and hardness. *B. mori* pupae have many biomedical advantageous for humans including controlling blood glucose level, improving male sexual function and enhancing memory (Kim *et al.*, 2007; Oh *et al.*, 2012). Wu *et al.* (2011) postulated that animal proteins may have other health benefits, in addition to energy and nutritional functions, including angiotensin I-converting enzyme (ACE) antioxidant activity and free radical-scavenging. Generally, *B. mori* are well known, easily obtained and a favorite in Thailand and are reported to be high in minerals, fatty acids and protein (Hanboonsong *et al.*, 2013; Yhoung-Aree *et al.*, 1997). The aim of these investigations was therefore to study on the characteristics, functional properties and antioxidant activity of water-soluble proteins from silkworm pupae (*B. mori*), in order to determine whether they are a good source of protein that could be extracted and used as a food ingredient.

3.3 Materials and Methods

3.3.1 Materials

Frozen silkworm pupae (*B. mori*) was obtained from a commercial supplier (Mr. BUC FOOD, Phra Nakhon Si Ayutthaya, Thailand). This specie was selected, because they are well known, cheaper, and readily available in Thailand and are reported to have high protein content (Hanboonsong *et al.*, 2013).

3.3.2 Protein extraction

Frozen silkworm pupae was blended with cold water (4°C) at a ratio of 1:4 w/v for 15 min using blender (MMB54G5S, BOSCH, Germany) and stirred overnight at 4°C, to ensure that the proteins were dissolved. The suspension was centrifuged at 12,500 g for 30 min at 4°C. After centrifugation, the sample was separated into 3 layers. The top layer was fat layer. The middle layer contained water soluble protein fraction. The bottom layer was undissolved components. The upper layer, containing the lipid fraction, and undissolved debris layer were removed. The supernatant or middle layer, containing water soluble protein fraction was collected, freeze-dried and referred to as "water-soluble protein from *B. mori*: WSPB". Extraction was performed in triplicate and the protein content of the extract was determined by Kjeldahl method (AOAC, 2000).

3.3.3 Yield and efficiency of extraction

The yield of WSPB was calculated as a percentage of the weight of WSPB powder in comparison with the weight of *B. mori* before extraction and was calculated as follows:

Yield (%) = (weight of WSPB (g) / weight of sample (g)) × 100.

Extraction efficiency was calculated as a percentage of the total protein extracted from *B. mori* of WSPB in comparison with the content of total protein content in *B. mori* in which determined by Kjeldahl method (AOAC, 2000). The extraction efficiency of WSPB was calculated as follows:

Extraction efficiency (%) = (total extracted protein of WSPB (g) / total protein content of *B. mori* (g)) × 100.

3.3.4 Characterization of WSPB

3.3.4.1 Amino acid analysis

The amino acid composition of WSPB was determined by The Central Instrument Facility at Mahidol University, Bangkok, Thailand. The analysis was performed using HPLC (Waters Alliance 2695 with heater, Jasco FP2020 fluorescence detector (EX: 250 and EM: 395 nm)) with a Hypersil gold column C18 (4.6×150 mm, 3µm) at 35 °C. Amino acid standards (Sigma-Aldrich, USA) were used for calibration.

3.3.4.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (1970) with slight modifications. The samples (3 g) were mixed with 27 mL of 5% SDS, heated at 85°C for 1 h, then centrifuged at 8,500 g for 5 min using a centrifuge (5804 R Eppendorf, Germany) to remove undissolved debris. The supernatant was collected and mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% (w/v) SDS, 20% (v/v) glycerol and 0.3% (w/v) bromophenol blue) for non-reducing condition and in the presence of 10% (v/v) β-ME for reducing condition. Samples (15 µg protein, determined by Biuret method) were loaded onto a polyacrylamide gel made of 10% separating gel and 4% stacking gel and subjected to electrophoresis at a constant current of 20 mA/gel, using an electrophoresis apparatus (AE-6440, Atto Co., Tokyo, Japan). After electrophoresis, gels were fixed with a mixture of 50% (v/v) methanol and 10% (v/v) acetic acid for 45 min, followed by staining with 0.05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid for overnight with constant shaking. Finally, gels were destained with the mixture of 30% (v/v) methanol and 10% (v/v) acetic acid until clear background was obtained. The molecular weight protein standard markers, using Precision Plus Protein^{TM} Unstained Standard (10-250 kDa) (Bio-Rad, CA, USA) were run in the same manner used to estimate the molecular weight of proteins. Gels were imaged using a scanner (MFC-L2700DW, Brother, UK) and band intensities were quantified with the public domain digital analysis software, ImageJ (ImageJ 1.51t, National Institutes of Health, Bethesda, USA).

3.3.4.3 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of WSPB was determined by Scientific Instrument Centre at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The analysis was performed using a Nicolet Model 6700 FT-IR Spectrometer (Thermo Scientific, Germany). Spectrum was acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–400 cm^{-1} at room temperature. Automatic signals were collected in 32 scans and evaluated against a background spectrum recorded from the clean, empty cell at 25 °C.

3.3.4.4 Determination of surface hydrophobicity

Surface hydrophobicity of the WSPB samples was determined using a fluorescence probe 1-anilino-8-naphathalenesulfonate (ANS) following the method described by Malik *et al.* (2017) with some modifications. WSPB was prepared at concentrations in the range of 0.05-0.5 mg/mL with a phosphate buffer (0.1 M, pH 7). 20 µL of ANS (8.0 mM in phosphate buffer 0.01 M, pH 7) was added to 4 mL of WSPB solution, vortexed and kept in the dark for 15 min. Relative fluorescence intensity (RFI) of both the buffer (blank) and each protein solution (from the lowest to the highest concentration) was measured using a fluorescence spectrometer (F-2700, Hitachi, Japan) at 390 nm (excitation wavelength) and 480 nm (emission wavelength), with a scanning speed of 5 nm s^{-1}. RFI of each dilution bank was subtracted from corresponding protein solution with the fluorescence probe ANS to obtain the net RFI. The initial slope of the plot of standardized net RFI values versus % protein concentration was expressed as surface hydrophobicity (H_o).

3.3.4.5 Determination of free and total sulfhydryl group content

The method used for determination of the sulfhydryl group content of WSPB was adopted from Malik *et al.* (2017) with some modifications. The protein solution (0.5% w/v) was prepared using a standard buffer pH 8.0 (0.086 M Tris, 0.09 M glycine and 4 mM Na_2EDTA) for free sulfhydryl group determination and a denaturing buffer (standard buffer plus 8 M urea and 0.5% w/v sodium dodecyl sulfate) for total sulfhydryl group determination. The samples were then incubated at room temperature for 30 min and the mixture was centrifuged (12,500 g for 20 min) prior to collecting the supernatant for determination. To each 4 mL aliquot of supernatant, 0.1 mL Ellman's reagent solution (5,5-dithiobis (2-nitrobenzoic acid): DTNB) (4 mg DTNB/mL buffer) was added, rapidly mixed and allowed to stand for 15 min. The solution was then read at 412 nm in an UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) against a blank. The blank was prepared by mixing 4 mL of the respective buffer with 0.1 mL of Ellman's reagent. In order to calculate micromoles of SH/g of protein, a molar extinction coefficient of 1.36×10^4 M^{-1} cm^{-1} was used.

3.3.4.6 Color measurement

The color of WSPB powder was determined using the Colour Quest XE colorimeter (Hunter Lab., Hunter Assoc. Laboratory, USA) The setting for the illuminant was D_{65} source and the observer was standard 10°. Calibration of the instrument was conducted with black and white calibration tiles. WSPB powder was filled in a cuvette quart path length 25 mm, and three observations were measured and expressed as CIE L^* (lightness), a^* (redness), and b^* (yellowness) with 5 readings/samples.

3.3.5 pH measurement

WSPB (1 g) was mixed with 9 mL of distilled water and stirred at 100 rpm for 10 min. The pH value of the mixture was measured at room temperature in triplicate using an electronic pH meter (FE-20, Mettler-Toledo Instruments Co., Ltd., Switzerland).

3.3.6 Determination of protein functional properties

3.3.6.1 Protein solubility

Protein solubility was measured according to the method of Nalinanon *et al.* (2011) with slight modifications. The solubility of WSPB was determined at pH values from 1 to 11. Briefly, 50 mg of WSPB was dispersed in 8 mL of distilled water and the pH of the mixture was adjusted to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 using 1 M HCl or 1 M NaOH. The dispersion was stirred for 30 min at room temperature and then the volume was adjusted to 10 mL and centrifuged at 6,000 g for 10 min. The supernatant was collected and subjected to protein determination using Biuret method. Bovine serum albumin was used as a protein standard. Total protein content in the sample was determined from the soluble portion of the sample in 0.5 M NaOH and relative solubility of protein sample was calculated as follows:

Relative solubility (%) = (protein content in supernatant/ total protein content in sample) ×100

3.3.6.2 Emulsion activity index (EAI) and emulsion stability index (ESI)

The EAI and the ESI were determined according to the method of Pearce and Kinsella (1978), with slight modifications. 2 mL of soybean oil and 6 mL of protein solution (5 mg/mL) were homogenized at 20,000 rpm for 1 min. An aliquot of the

emulsion (50μL) was pipetted from the bottom portion of the container at 0 and 10 min after homogenization and subsequently diluted 100-fold using 0.1% sodium dodecyl sulfate (SDS) solution. Each sample was mixed thoroughly for 10 s using a vortex mixer. A_{500} of the resulting dispersion was measured using a spectrophotometer (UV-1800, Shimadzu, Japan). EAI and ESI were calculated as follows:

$$EAI\ (m^2/g) = (2\times 2.303\times A\times DF)/l\Phi C$$

where $A = A_{500}$, DF = dilution factor (100), l = path length of cuvette (m), Φ = oil volume fraction and C = protein concentration in aqueous phase (g/m^3)

$$ESI\ (min) = (A_0\times t/\Delta A)$$

where $\Delta A = A_0 - A_{10}$ and t = 10 min.

3.3.6.3 Foaming capacity (FC) and foam stability (FS)

FC and FS of WSPB solution was determined as described by Nalinanon *et al.* (2011) with slight modification. Sample (35 mL), with 5 mg/mL protein concentration, was transferred into a 100-mL cylinder. The solutions were homogenized at 16,000 rpm for 1 min at room temperature (about 25ºC) and the samples were allowed to stand for 0 and 60 min. FE and FS were then calculated using the following equations:

$$FC\ (\%) = V_T/V_0\times 100$$

$$FS\ (\%) = V_{60}/V_0\times 100$$

where V_T is total volume after whipping, V_0 is the original volume before whipping, and V_{60} is total volume after leaving at room temperature for 60 min.

3.3.7 Determination of antioxidant activity

3.3.7.1 DPPH radical scavenging activity

DPPH radical scavenging activity was measured following the method of Murakami *et al.* (2004) with a slightly modification. Briefly, the reaction mixture contained 5.4 mL of WSPB at different concentration and 0.6 mL of 0.8 mM DPPH in 95 % ethanol. The mixture was incubated at room temperature for 30 min in dark, and then the absorbance at 517 nm was measured using a spectrophotometer (UV-1800,

Shimadzu, Japan). The control was prepared in the same manner except that distilled water was used instead of the sample. The percentage of DPPH· scavenging activity of the sample was calculated as:

$$\text{Scavenging activity (\%)} = (1-A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is the absorbance of the assay without sample and A_{sample} is the absorbance in the presence of the WSPB.

The result was expressed as the IC_{50} value. The IC_{50} (concentration providing 50% inhibition) value was calculated from the plotted graph of scavenging activity against the concentrations of the sample.

3.3.7.2 ABTS radical scavenging activity

ABTS radical scavenging activity was measured following the method of Rice-Evans *et al.* (1997) with a slightly modification. The ABTS radical ($ABTS^{\bullet+}$) was produced by reacting 7.4 mM ABTS stock solution with 2.45 mM potassium persulphate at a ratio of 1:1 (v/v). The mixture was allowed to react for 12-16 h at room temperature in the dark. This working solution of $ABTS^{\bullet+}$ solution was diluted with 95% ethanol, in order to obtain an absorbance of 0.700 ± 0.020 at 734 nm. The reaction mixture contained 0.15 mL of WSPB at different concentrations and 2.85 mL of $ABTS^{\cdot+}$ solution. The mixture was incubated at room temperature for 6 min in dark. Then, the absorbance at 734 nm was measured using a spectrophotometer (UV-1800, Shimadzu, Japan). The control was prepared in the same manner except that distilled water was used instead of the sample. The percentage of $ABTS^{\cdot+}$ scavenging activity and IC_{50} of the sample was calculated in the same manner as described in section 3.3.7.1.

3.3.7.3 Ferric reducing antioxidant power (FRAP)

FRAP was determined by the method described by Benzie and Strain (1996). Briefly, the FRAP reagent was freshly prepared by mixing of 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ solution and 300 mM acetate buffer, pH 3.6 (1:1:10 *v*/*v*/*v*). A sample (0.1 mL) was mixed with 3mL of FRAP reagent and the mixture was left at room temperature for 8 min in the dark. The absorbance was measured at 593 nm using a spectrophotometer (UV-1800, Shimadzu,

Japan). The blank was prepared in the same manner, except that distilled water was used instead of the sample. A standard curve was prepared using Trolox in the range of 20-120 μg. The activity was expressed as μg Trolox equivalent (TE)/g protein.

3.3.8 Statistical analysis

All results were performed in triplicate. Data were presented as means ± standard deviation and a probability value of <0.05 was considered significant. For pair comparison, T-test was used. SPSS statistic program (SPSS 11.0 for Windows, SPSS Inc., Chicago, USA) was used for data analysis.

3.4 Results and Discussion

3.4.1 Yield and characteristics of WSPB

The yield and some physicochemical characteristics of water-soluble protein from *B. mori* (WSPB) are shown in Table 8. The silkworm pupae were simply extracted with distilled water. The yield of the resultant freeze-dried counterpart or WSPB was 3.62% based on wet weight basis. Kim *et al.* (2016) reported that the yield of defatted silkworm pupae flour was about 35.84% (dry weight), which composed of ground whole insect without fat. The difference is probably due to different preparation and extraction procedures. According to the total protein content of *B. mori* (6.04%) determined by Kjeldahl method, the extraction efficiency of WSPB was calculated to be 65.62%, indicating high efficacy of protein extraction. The protein extraction method using water in the present work is low cost, environmental friendly and practical of use in large scale of protein extraction.

The pH of WSPB was 6.64, which was slightly higher than that reported for untreated silkworm pupae flour (pH 6.43) (Kim *et al.*, 2016). This might be due to the difference in the source or cultivation of the silkworms. The color of WSPB expressed as L^*, a^* and b^* values was 75.21, 2.11 and 24.67, respectively, presenting a bright-light reddish-yellow color. However, a general observation of the visible color of WSPB powder tended to be bright yellow. The predominant pigment of silkworm pupae is melanin, which can be black, brown or yellow in color (Wittkopp & Beldade, 2009). Kim *et al.* (2016) dried silkworm pupae at high temperature, which might be one cause for the color to be darker (L^* = 42.95) in their experiments compared to this result.

Table 8. Yield and physicochemical characteristics of water-soluble protein from *B. mori* (WSPB)

		WSPB†
Yield (%)		3.96±0.14
Extraction efficiency (%)		65.62±2.29
pH		6.64±0.01
Color	*L**	75.42±0.17
	*a**	2.22±0.11
	*b**	24.70±0.13
Free sulfhydryl content (μmol/g)		22.18±0.05
Total sulfhydryl content (μmol/g)		23.08±0.07
Surface hydrophobicity (H_0)		3.52±0.03

†Mean ± SD from triplicate determinations.

The total sulfhydryl content and free sulfhydryl content of WSPB were 23.08 and 22.18 μmol/g, respectively. It was suggested that WSPB may have a good reducing power as a function of –SH group. The surface hydrophobicity (H_0) of WSPB was 3.52±0.03, which was lower than previously reported by Azagoh *et al.* (2016) who found surface hydrophobicity of the mealworm beetle larvae (*Tenrbrio molitor)* to be 102.5. Higher surface hydrophobicity was also previously found in soy protein isolate (Wagner *et al.*, 2000) and soluble protein from *T. molitor* larvae meal (Azagoh *et al.*, 2016). They explained that aggregating proteins are more hydrophobic and hydrophobic zones are buried inside the structure of proteins. Elias *et al.* (2005) proposed that amino acids in protein including those with sulfhydryl groups (methionine and cysteine) or aromatic ring (tryptophan, tyrosine, and phenylalanine) contain a hydrogen atom that can interact with free radicals. With high free sulfhydryl content and low surface hydrophobicity, WSPB could be easily extracted into water fraction and might also ready to modify its structure to have better functionality as desired.

3.4.2 Amino acid composition

The amino acid composition of WSPB is presented in Table 9. WSPB composed of 15 amino acids of which 7 are essential amino acids and 8 nonessential amino acids. The predominant essential amino acids were histidine, lysine, threonine and valine and the predominant nonessential amino acids were glutamic acid, proline, glycine and tyrosine. Glutamic acid, histidine, proline and glycine were the major amino acid found in WSPB with descending amount in order.

Table 9. Amino acid composition of water-soluble protein from *B. mori* (WSPB) (residues/1000 residues)

Amino acids	WSPB (residues/1000 residues)
Essential amino acids	
Histidine	129
Isoleucine	29
Leucine	36
Lysine	54
Methionine	ND
Phenylalanine	23
Threonine	53
Tryptophan	ND
Valine	50
Nonessential amino acids	
Alanine	52
Arginine	37
Aspartic/Asparagine	62
Cysteine	ND
Glutamic/Glutamine	210
Glycine	66
Proline	77
Serine	59
Tyrosine	53

ND is not detectable.

Rao (1994) also reported that glutamic acid was the most abundant amino acid in silkworm larvae. These results are similar to those reported by Wu *et al.* (2011) for *B. mori*, although, the level of amino acid content was lower when compared to Rao (1994)

and Longvah *et al.* (2011). These differences might be due to differences in the extraction method and source of insects. Generally, protein functionality and bioactivity govern by its amino acid composition as well as amino acid sequence (Nalinanon *et al.*, 2010). In addition, the total hydrophobic amino acid, including isoleucine, leucine, methionine, phenylalanine, valine, alanine, glycine and proline of WSPB was calculated to be 333 residues/1000 residues, indicating that WSPB had slightly low molecular hydrophobicity.

3.4.3 SDS-PAGE

Protein pattern and molecular weight distribution of WSPB was analyzed by SDS-PAGE using 10% separating gel under reducing and non-reducing conditions as shown in Figure 6. There was a wide range of molecular weights in the WSPB ranging from lower 20 kDa to 250 kDa. Four major groups of protein bands under reducing condition were found to be 50-75 kDa, 37-50 kDa, 25-37 kDa and less than 25 kDa with the observed protein bands at 37 kDa, 45 kDa, 64 kDa, 75 kDa and 80 kDa being abundant. Four major groups of protein bands under non-reducing condition were found to be over 150 kDa, 50-100 kDa, 37-50 kDa and less than 25 kDa with the observed protein band intensity 37 kDa, 64 kDa and 75 kDa being abundant. The protein patterns of WSPB under non-reducing condition were difference from reducing condition. The absence of protein bands with molecular weight over 250 kDa and lower band intensity of three major bands in WSPB under non-reducing condition and with the presence of protein bands with 80 kDa, 54 kDa and 45 kDa under reducing condition indicated that WSPB contained disulfide bonds. Wang *et al.* (2011) reported four major protein components in silk worm pupae, including albumin (97.4 kDa, 61.4 kDa, 44.4 kDa and 26.7 kDa), glutelin (200 kDa and 15 to 60 kDa), globulin (130.0 kDa and 26.8 kDa) and prolamin (15.3 to 46 kDa).

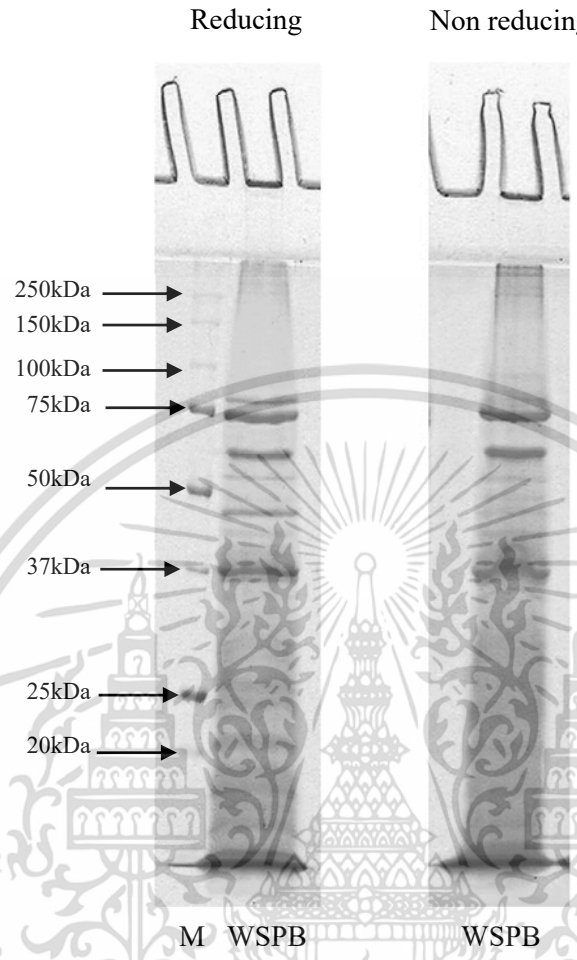


Figure 6. SDS-PAGE patterns of water-soluble protein from *B. mori* (WSPB) under reducing and non-reducing conditions. M denotes standard molecular weight protein markers.

3.4.4 Fourier-transform infrared (FTIR) spectrum of WSPB

The FTIR spectrum of WSPB is depicted in Figure 7. The result showed that WSPB had three characteristic amide bands representing amide *B* (2900–3200 cm^{-1}), amide *I* (1600–1700 cm^{-1}) and amide *III* (1200–1400 cm^{-1}). This result was in accordance with those previously reported in silkworm pupae protein modified by ultrasound or micronization techniques (Zhou *et al.*, 2017). The major peaks were found at wavenumbers of 2924.47, 1609.58, and 1398.30 $cm^{-1}$for amide *B*, amide *I* and amide *III*, respectively. Amide *B* corresponded to asymmetric stretch vibration of =C–H as well as $–NH_3^+$ and

amide *I* bands originated from C=O stretching vibrations coupled to N–H bending vibrations, CN stretch and CCN deformation (Je *et al.*, 2009). Amide *III* represented the combination peaks between N–H deformation and C–N stretching vibrations and was involved with the triple helical structure of protein (Muyonga *et al.*, 2004). As a result, WSPB remained its structural integrity after extraction.

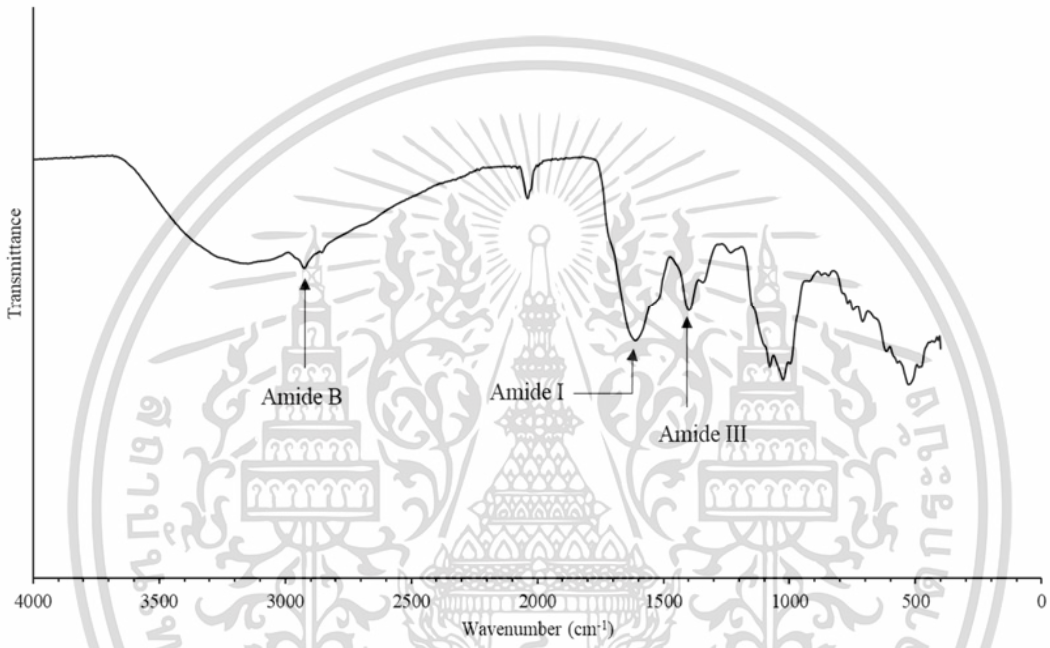


Figure 7. Fourier transform infrared spectrum of water-soluble protein from *B. mori* (WSPB)

3.4.5 Functional properties of WSPB

The solubility of WSPB in the pH range of 1 -11 was depicted in Figure 8. The result showed that WSPB was highly solubilized more than 80% in the pH range of 5-11, indicating that the protein in silkworm pupae can be solubilized at neutral to alkaline pH. At acidic pH, the solubility of WSPB was generally low (< 40%). The lowest solubility was found in the pH range of 3-4, indicating isoelectric pH (*pI*) of WSPB. Wang *et al.* (2011) reported that the *pI* of albumin, globulin, glutelin and prolamin of silk worm protein was 2.5, 2.7, 4.0 and 4.5, respectively. This was due to a reduction in electrostatic repulsive forces between the proteins, leading to protein aggregation (Azagoh *et al.*, 2016). This

effect is similar to those reported for several legumes, animal protein and protein isolates (Azagoh *et al.*, 2016; Bußler *et al.*, 2016; Horax *et al.*, 2017)

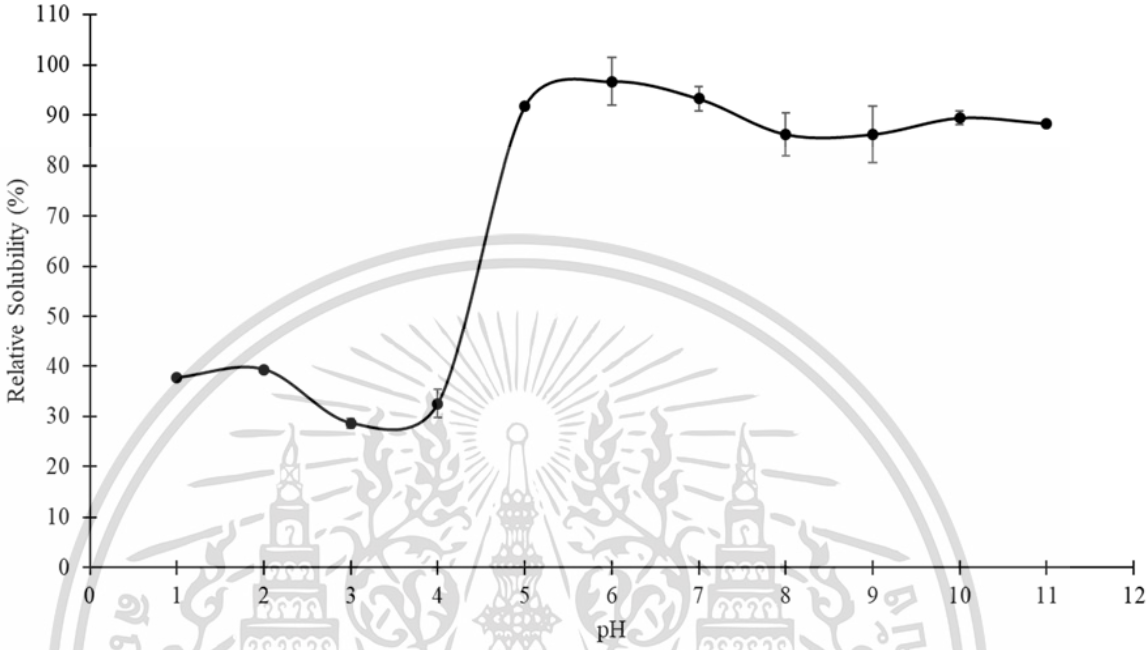


Figure 8. Relative solubility (%) of water-soluble protein from *B. mori* (WSPB) as affected by different pHs.

Emulsifying and foaming properties of WSPB are shown in Table 10. The emulsion activity index (EAI) and emulsion stability index (ESI) of WSPB were 25.09 m^2/g and 21.15 min, respectively. The EAI and ESI of WSPB were significantly lower than bovine serum albumin (BSA) ($p<0.05$). The differences between the emulsion activity and emulsion stability are related to the amphiphilicity of the protein surface, protein contents (soluble and insoluble) and other components (Zielińska *et al.*, 2018).

Table 10. Emulsifying and foaming properties of water-soluble protein from *B. mori* (WSPB) and bovine serum albumin (BSA) †

Functional properties	WSPB	BSA
Emulsion activity index (EAI) (m^2/g)	25.09±1.34^{b*}	295.24±2.30^{a}
Emulsion stability index (ESI) (min)	21.15±0.22^{b}	38.55±0.77^{a}
Foam capacity (FC) (%)	9.29±1.01^{b}	81.23±7.27^{a}
Foam stability (FS) (%)	93.46±0.06^{a}	67.41±2.95^{b}

† Mean ± SD from triplicate determinations.

* Different superscript letters in the same row indicate significant differences ($p<0.05$).

WSPB exhibited 9.29% and 93.46% for foam capacity (FC) and foam stability (FS), respectively. The FC of WSPB was low when compared with BSA (81.23%) ($p<0.05$). This might be due to the variation of molecular weights of protein component in WSPB interrupted the formation of protein film at the lamellae of air bubble. The result was similar to those reported by Adebowale *et al.* (2005) for whole giant cricket (*Gryllidae* sp.) powder that had a FC of 6%, and Omotoso (2006) who reported the FC from *Cirina forda* as 7.1%. However, FS of WSPB was significantly higher than that of BSA (67.41%) ($p<0.05$). Johnson and Zabik (1981) explained that intermolecular protein-protein interaction enhances the cohesive nature of the film, therefore imparting stability and elasticity to the membrane. This interaction appears to be dependent on the presence of a high ratio of nonpolar/polar side chains in the protein which was found in WSPB, according to its amino acid component. Zielińska *et al.* (2018) reported the FC of *Gryllidae sigillatus* flour as 41% and FS was 34.67%. The differences between FC and FS of proteins may be due to their different compositions in different species and their different conformational characteristics (Zielińska *et al.*, 2018). The differences in FS is also probably due to components such as carbohydrates, which reduces protein-protein interactions and leads to

formation of weak interfacial membranes that are unable to stabilize the foams (Zielińska *et al.*, 2015).

3.4.6 Antioxidant activities

Antioxidant activities as determined by ABTS, DPPH and FRAP assays of WSPB are shown in Table 11. WSPB exhibited strong scavenging activity on DPPH and ABTS radicals with IC_{50} of 43.11 and 16.57 µg/mL, respectively.

Table 11. Antioxidant activity of water-soluble protein from *B. mori* (WSPB)

Antioxidant assays	WSPB[†]
DPPH (IC_{50} µg/mL)	43.11±0.11
ABTS (IC_{50} µg/mL)	16.57±0.04
FRAP (µg TE/g protein)	54.20±0.13

[†] Mean ± SD from triplicate determinations

Wu *et al.* (2011) reported that protein hydrolysates from larval instars of silkworm, obtained after digestion with gastrointestinal proteases, had $DPPH^{\bullet}$ scavenging capacity (IC_{50}) of 57.91 µg/mL. Pachiappan *et al.* (2016) reported that silkworm pupae powder had $DPPH^{\bullet}$ scavenging capacity (IC_{50}) of 60.58 µg/mL. These reports found less effective on $DPPH^{\bullet}$ scavenging activity when compared with the present result (IC_{50} of 43.11 µg/mL). In contrast, the results had low potential on DPPH inhibition when compared with methanolic silkworm pupae extract from muga silkworm (*Antheraea* assamensis) (IC_{50} of 25.83 µg/mL as reported by Deori *et al.* (2014). Additionally, Zielińska *et al.* (2015) reported that the antiradical activity against $DPPH^{\bullet}$ for the hydrolysates obtained after digestion of five edible insects ranged from 19.1 to 76.3 µg/mL. The antiradical activity against $ABTS^{\bullet+}$ (IC_{50}) ranged from 4.6 to 25.9 µg/mL (Zielińska *et al.*, 2015). The more stable products could be formed and the radical chain reaction terminated from these peptides since they are electron donors that could react with free radicals (Jemil *et al.*, 2014). These results indicate that insect protein could be a good source of antioxidant peptides.

As per FRAP assay, WSPB also presented reducing power on Fe^{3+} of 54.20 µg TE/g protein. Bousopha *et al.* (2016) reported that collagen hydrolysate from pharaoh cuttlefish skin with 10-30% DH had ferric reducing power values of 23.50 to 26.50 µmolTE/g protein. The increase or decrease in ferric reducing power for protein hydrolysates may be related to the exposure of electron-dense amino acid side chain groups, such as polar or charged moieties during hydrolysis (Zambrowicz *et al.*, 2012). Chalamaiah *et al.* (2015) reported that the reducing power of three carp roe protein hydrolysates increased with increasing concentrations. Compounds with higher reducing power were shown to have a better ability to donate electrons or hydrogen and serve as a significant indicator of their potential for use as an antioxidant (Je *et al.*, 2009). The antioxidant activities of $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and FRAP scavenging ability, appear to be dependent on the molecular weight of the peptide (Chalamaiah *et al.*, 2015). In addition, those with a low-molecular weight had lower antioxidant activity. This suggests that the hydrolysates obtained from some edible insect protein can be used as compounds that are able to donate electrons and thus show antioxidant activity.

3.5 Conclusion

B. mori could be a good source of protein. Its water-soluble protein fraction exhibited beneficial physicochemical and functional properties as well as high antioxidant activity. This edible insect protein can be used as an alternative food ingredient in many food applications.

CHAPTER 4

CHARCTERISTICS, FUNCTIONALPROPERTIES AND ANTIOXIDANT ACTIVITIES OF WATER SOLUBLE PROTEINS EXTRACTED FROM GRASSHOPPERS, *Patanga succincta* AND *Chondracris roseapbrunner*

4.1 Abstract

Water soluble proteins extracted from two species of grasshoppers, *Patanga succincta* (WSPP) and *Chondracris roseapbrunner* (WSPC) were characterized as well as their functional properties and antioxidant activities were investigated. The extraction yield, on a wet weight basis, was 7.35% and 7.46% for WSPP and WSPC, respectively. The most abundant amino acid in both proteins were glutamic acid, followed by aspartic, alanine and leucine, in that order. The electrophoretic study revealed that proteins with MW of 29, 42, 50, 69 and 146 kDa were the major protein components in WSPP and WSPC. FTIR analysis showed that those proteins remain their structural integrity. The surface hydrophobicity at pH 7 of WSPC was higher than WSPP, but the sulfhydryl group content did non-significant between the proteins from two species. Both grasshopper proteins were mostly soluble in strong acidic and alkaline aqueous solutions with a minimum value at pH 4. Those proteins exhibited poor emulsifying properties and foaming capacity but they had greater foaming stability compared to bovine serum albumin (BSA) ($p<0.05$). WSPC showed greater $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities and ferric reducing antioxidant power (FRAP) than did WSPP ($p<0.05$). Therefore, based on characteristics and functional properties, water soluble proteins from both edible grasshoppers can be used as an ingredient in food applications.

4.2 Introduction

In 2050, the world population is estimated at more than 9 billion people, resulting in an additional need for food and feed outputs (FAO, 2009). Conventional sources of

protein will not be sufficient for the global human population, and alternatives sources such as insects will be required (Zielińska *et al.*, 2015). Approximately 1,900 species of edible insects are traditionally consumed in many parts of the world, for example in Africa, Asia and Latin America and are considered as having potential to contribute to the world's food security (van Huis, 2013). In countryside of Northern and Northeastern Thailand, people consume several species of insects including grasshoppers, crickets, beetles, silkworm pupae and bamboo worm.

Edible insects offer an important source of minerals, lipids and above all proteins. Edible insects have higher crude protein content and have been reported to be a good source of essential amino acids (Belluco *et al.*, 2013). Currently, most insect consumption is as a component ingredient of processed foods, and their successful utilization depends on fulfilling one or more functional requirements of good solubility, emulsion/foam capacity and stabilization, and gel formation (Chalamaiah *et al.*, 2012; Garcés-Rimón *et al.*, 2016). (Omotoso, 2006) evaluated the functional properties of the larva of Pallid Emperor Moth (*Cirina forda*) and found that they had good solubility and emulsion properties. Yi *et al.* (2013) reported poor foam and gelling properties of five different acid extracted insect proteins, including those from a cricket (*Acheta domesticus*). Kim *et al.* (2016) determined the effects of adding flour made from defatted mealworm larvae (*Tenebrio molitor*) and defatted silkworm pupae (*Bombyx mori*) and found that the added insect flours increased their cooking yield and hardness on emulsion sausages. Park *et al.* (2017) also found that adding transglutaminase to silkworm pupae flour resulted in improved physicochemical properties of meat batter.

From previous reports, 32 insect species have been evaluated for their nutrition value in Thailand (Yhoung-Aree *et al.*, 1997). Their protein content ranged from 6.12 to 25.88 g/100g wet weight and their fiber levels ranged from 1.00 to 12.42 g/100g wet weight (Klinhom *et al.*, 1984 ; Lewvanich *et al.*, 1999). Yang *et al.* (2006) reported polyunsaturated fatty acid content of 6 species of edible insects from Thailand to range from 726 to 2883 mg/100g, and monounsaturated fatty acid content to range from 714 to 5889 mg/100g. This lends to the fact that edible insects are a potential source of fat and protein, but there is limited information on the characteristics and functional properties of

extracted edible protein from specific insects. The aim of this investigation was, therefore, to characterize and compare the functional properties as well as antioxidant activities of water soluble proteins from two species of grasshoppers (*P. succincta* and *C. roseapbrunner*), commonly found in Thailand, for their potential use as an alternative source of protein in food ingredients.

4.3 Materials and Methods

4.3.1 Materials

Frozen Bombay Locust (*P. succincta*) and Spur-throated grasshopper (*C. roseapbrunner*) were obtained from a commercial supplier (Mr. BUC FOOD, Phra Nakhon Si Ayutthaya, Thailand). These two species were selected, because they are well known, cheaper, and easily available in Thailand and are reported to have high protein content (Hanboonsong *et al.*, 2013; Yhoung-Aree *et al.*, 1997).

4.3.2 Samples preparation

The grasshopper samples were thawed, removed hindleg/long jumping legs and washed with running portable tap water, rinsed with distilled water and drained using a plastic sieve for 1 h. The prepared samples were then stored in plastic containers at -20°C until further experiments.

4.3.3 Protein extraction

This method was described as same as previous method in 3.3.2. The supernatant or middle layer was collected, freeze-dried and referred to as “WSPP” and “WSPC” for the protein from *P. succincta* and *C. roseapbrunner*, respectively.

4.3.4 Calculation of extraction yield and efficiency

The yields and efficiency of WSPP and WSPC were described as same as previous method in 3.3.3.

4.3.5 Physicochemical characterization

4.3.5.1 Amino acid composition

The amino acid compositions of WSPP and WSPC were described as same as previous method in 3.3.4.1.

4.3.5.2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

This method was described as same as previous method in 3.3.4.2.

4.3.5.3 Fourier transform infrared spectroscopy (FTIR)

This method was described as same as previous method in 3.3.4.3.

4.3.5.4 Determination of surface hydrophobicity (H_0)

The surface hydrophobicity of WSPP and WSPC were described as same as previous method in 3.3.4.4.

4.3.5.5 Determination of free and total sulfhydryl group content

The free and total sulfhydryl group content of WSPP and WSPC were described as same as previous method in 3.3.4.5.

4.3.5.6 Color measurement

The colors of WSPP and WSPC were described as same as previous method in 3.3.4.5.

4.3.5.7 pH measurement

The pH of WSPP and WSPC were described as same as previous method in 3.3.5.

4.3.6 Evaluation of functional properties

4.3.6.1 Protein solubility

The protein solubility of WSPP and WSPC were described as same as previous method in 3.3.6.1.

4.3.6.2 Emulsifying properties

The emulsion activity index (EAI) and emulsion stability index (ESI) of WSPP and WSPC were described as same as previous method in 3.3.6.2.

4.3.6.3 Foaming properties

The foaming capacity (FC) and foam stability (FS) of WSPP and WSPC were described as same as previous method in 3.3.6.3.

4.3.7 Determination of antioxidant activities

4.3.7.1 DPPH radical scavenging activity

DPPH radical scavenging activity of WSPP and WSPC were described as same as previous method in 3.3.7.1.

4.3.7.2 ABTS radical scavenging activity

ABTS radical scavenging activity of WSPP and WSPC were described as same as previous method in 3.3.7.2.

4.3.7.3 Ferric reducing antioxidant power (FRAP)

This method was described as same as previous method in 3.3.7.3.

4.3.8 Statistical analysis

The statistical analysis of WSPP and WSPC were described as same as previous method in 3.3.8.

4.4 Results and discussion

4.4.1 Yield and extraction efficiency

The yield and extraction efficiency of water soluble protein from grasshoppers, WSPP and WSPC are shown in Table 12. The proteins from both grasshoppers were simply extracted with distilled water. The yields of the resultant freeze-dried counterparts called WSPP and WSPC were 7.35±0.19 and 7.49±0.19% (wet weight basis), respectively. Clarkson *et al.* (2018) found that protein yield in soluble locust fraction from *L. migratoria* was 9.83%. The difference in extraction yield was probably due to different preparations and extraction procedures. The crude protein contents determined by Kjeldahl method of *P. succincta* and *C. roseapbrunner* were 15.07 and 17.40% (wet weight basis), respectively (data not shown), which can expressed in dry weight to be 65.55 and 71.75%, respectively. The protein content of those grasshoppers was lower than that of previously reported in whole freeze-dried locust and alkali extracted defatted locusts (65.87 and 82.26% dry weight, respectively) (Purschke *et al.*, 2018). Clarkson *et al.* (2018) reported that crude protein content of *L. migratoria* was 50.79% (dry weight). The extraction efficiency of WSPP and WSPC were calculated to be 48.77 and 42.86%, indicating high efficacy of protein extraction. Therefore, water soluble protein might be

one of major protein component in grasshoppers and was suitable for protein extraction by water, an environment friendly method.

Table 12. Yield, extraction efficiency and physicochemical characteristics of water soluble protein extracted from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC)[†].

		WSPP	WSPC
Yield (%)		7.35±0.19[ns]	7.46±0.19[ns]
Extraction efficiency (%)		48.77±0.37[a‡]	42.86±0.09[b]
pH		6.08±0.01[ns]	6.07±0.00[ns]
Color	*L**	47.26±0.17[b]	55.80±0.13[a]
	*a**	6.04±0.10[a]	5.48±0.09[b]
	*b**	16.80±0.09[b]	18.20±0.08[a]
Free sulfhydryl content (μmol/g)		0.48±0.03[ns]	0.48±0.01[ns]
Total sulfhydryl content (μmol/g)		1.06±0.04[ns]	1.14±0.04[ns]
Surface hydrophobicity (H_0)		15.92±0.21[b]	22.59±0.22[a]

[†]Mean ± SD from triplicate determinations.

[‡] Different superscript letters in the same row indicate significant differences ($p<0.05$).

ns = no significant difference.

4.4.2 Physicochemical characteristics of grasshopper proteins

4.4.2.1 pH and color

The color and pH of WSPP and WSPC are shown in Table 12. WSPP and WSPC exhibited a faintly acidic pH with a value of 6.08 and 6.07, respectively. The color of WSPP and WSPC expressed as *L**, *a** and *b** values was 47.26, 6.04 and 16.80, respectively for WSPP and 55.80, 5.48 and 18.20, respectively for WSPC. The WSPC had a higher value than WSPP for *L** and *b**, but lower values for *a**, indicating more light green color in WSPC than WSPP. The green coloration could be due to a common green

pigment identified in insects call insectoverdin (Clarkson *et al.*, 2018). Goodwin (1952) postulated that a mixture of two chromoproteins, one yellow component from carotenoids and the other a bile component (blue) creating the green color in locust. However, a general observation of the visible color of both WSPP and WSPC tended to be brown, which could be attributed to melanin (Wittkopp & Beldade, 2009).

4.4.2.2 Surface hydrophobicity and sulfhydryl content

The surface hydrophobicity of WSPC and WSPP was found to be 22.59±0.22 and 15.92±0.21, respectively Table 12. From the result, surface hydrophobicity of both insect proteins was lower than the previous report of Azagoh *et al.* (2016). They reported that surface hydrophobicity of *T. molitor* larvae meal was 102.5. Aggregating proteins are more hydrophobic and hydrophobic zones are buried inside the structure of proteins (Wagner *et al.*, 2000). The surface hydrophobicity of protein extracted from edible insects has been shown to vary depending on the extraction protocol (Gould & Wolf, 2018). Surface hydrophobicity might also be dependent on the size, conformation, amino acid composition and sequence of a protein.

The total sulfhydryl content and free sulfhydryl content of WSPC (1.06 and 0.48 μmol/g) was comparable to WSPP (1.14 and 0.48 μmol/g) Table 12. This was expected from the amount of sulphur-containing amino acids in found in both proteins. Differences in sulfhydryl content found in different proteins have been reported. Van der Plancken *et al.* (2005) reported total sulfhydryl content of untreated egg white solution was 58.5 μM/g protein. Malik *et al.* (2017) found that the free and total sulfhydryl content of sunflower protein isolate was about 7.7 and 80.1 μmol/g. Thus, the quantity of free and total sulfhydryl content might be depended on source of protein, part of animal and species of samples.

4.4.2.3 Protein patterns

Protein patterns and molecular weight distribution of WSPP and WSPC analyzed by SDS-PAGE using 10% separating gel under reducing and non-reducing conditions are shown in Figure 9. There was a wide range of molecular weights in the WSPP and WSPC ranging from lower 20 kDa to 250 kDa. Six major groups of protein bands under reducing condition were found to be more than 250 kDa,75-150 kDa, 50-75

kDa, 37-50 kDa, 25-37 kDa and less than 25 kDa with the observed WSPP protein bands at 24 kDa, 29 kDa, 45 kDa, 52 kDa, 61 kDa, 69 kDa, 124 kDa and 146 kDa being abundant. WSPC protein bands were abundant at 22 kDa, 29 kDa, 35 kDa, 37 kDa, 42 kDa, 47 kDa, 50 kDa, 61 kDa, 69 kDa and 146 kDa. Under reducing condition, WSPC found more protein bands than WSPP, and 50-75 kDa and 37-50 kDa were generous protein bands.

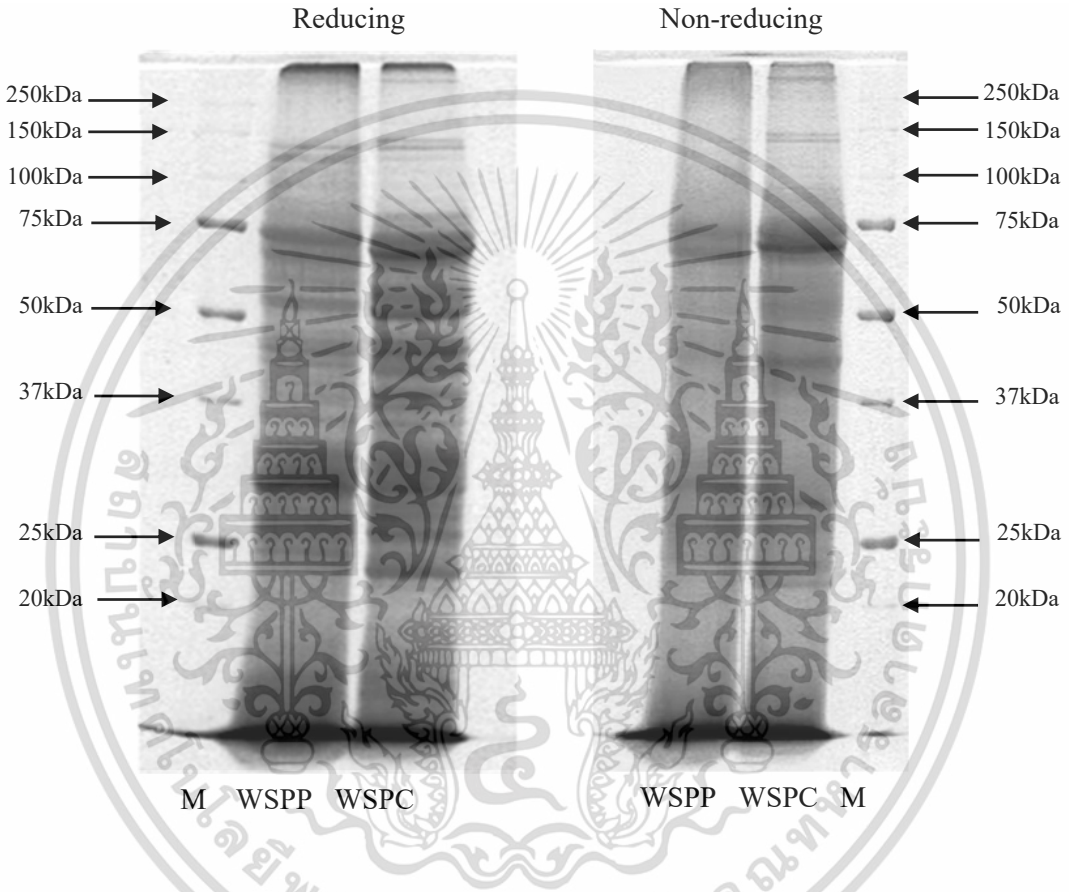


Figure 9. SDS-PAGE patterns of water soluble protein from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) under reducing and non-reducing conditions.

Five major groups of protein bands under non-reducing condition were found to be 100-150 kDa, 50-75 kDa, 37-50 kDa, 25-37 kDa and less than 25 kDa with the observed WSPP protein band intensity 24 kDa, 29 kDa, 42 kDa and 61 kDa being abundant. WSPC protein bands were abundant at 22-23 kDa, 27 kDa, 42 kDa, 61 kDa and 146 kDa. Purschke *et al.* (2018) reported that protein concentrate from *Locusta migratoria*

included proteins in the range of 6–100 kDa. The characteristic bands (40, 50 and 100 kDa) of *L. migratoria* protein contained muscle protein tropomyosin and high amounts of tubulin, which are responsible for the formation of the microtue bules (Purschke *et al.*, 2018). Zielińska *et al.* (2017) reported that raw locust *S. gregaria* had protein band with molecular weight range between 29.0-44.3 kDa and 97.2 kDa, while boiled locust found low intensity of protein band at range 29.0-44.3 kDa and baked locust disappeared protein band. The differences in protein patterns under reducing and non-reducing conditions might be depended on species of insects, extraction method, insects processing. Furthermore, protein functionalities and antioxidant activities are also governed by molecular weight distribution, amino acid composition and amino acid sequence of the protein itself (Nalinanon *et al.*, 2011).

4.4.2.4 Amino acid composition

The amino acid compositions of WSPP and WSPC are presented in Table 13. Generally, both grasshopper proteins had similar amino acid profile. WSPP and WSPC composed of 15 amino acids of which 7 essential amino acids and 8 nonessential amino acids. Methionine, tryptophan and cysteine were not detectable, possibly due to they had very low ratio or were not present in the proteins studied. The predominant essential amino acids were leucine and lysine which are similar to those reported for *Locusta migratoria* (Purschke *et al.*, 2018), *Tenebrio molitor* larvae (Azagoh *et al.*, 2016) and silkworm larvae protein isolates (Wu *et al.*, 2011). The amount of essential amino acids in WSPP and WSPC were sufficient to meet the adult nutritional requirements based on the FAO/WHO guidelines (FAO, 2009). The predominant nonessential amino acids were glutamic acid, aspartic acid, alanine and arginine. Glutamic acid, aspartic acid, leucine and alanine were the major amino acid found in both WSPP and WSPC with descending amount in order. Glutamic acid was the most abundant amino acid in locust and grasshopper (Makkar *et al.*, 2014; Ruiz *et al.*, 2015). These results are similar to those reported by Makkar *et al.* (2014) for grasshoppers (glutamic acid 4.5-15.2 g/16 g nitrogen). These differences might be due to the differences in the extraction method, species and source of insects. In general, protein functionality and bioactivity govern by its amino acid composition as well as amino acid sequence (Nalinanon *et al.*, 2010). Both WSPP and WSPC contained higher amount of hydrophobic amino acids than hydrophilic amino acids.

It has been shown that hydrophobic amino acids and one or more residues of histidine, proline, methionine, cysteine, tyrosine, tryptophan and phenylalanine can enhance the activities of the antioxidant peptides (Chalamaiah *et al.*, 2012). However, although both WSPP and WSPC had similar amino acid profile, they might have same or different molecular properties, functionalities and bioactive potentials which also depending on their amino acid sequence.

Table 13. Amino acid composition of water soluble protein extracted from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) (residues/1000 residues).

Amino acids	**WSPP**	**WSPC**
Essential amino acids		
Histidine	22	23
Isoleucine	48	44
Leucine	86	81
Lysine	76	74
Methionine	ND	ND
Phenylalanine	44	43
Threonine	49	49
Tryptophan	ND	ND
Valine	59	60
Nonessential amino acids		
Alanine	85	88
Arginine	83	79
Aspartic/Asparagine	98	98
Cysteine	ND	ND
Glutamic/Glutamine	156	151
Glycine	62	63
Proline	55	57
Serine	52	50
Tyrosine	27	39
Hydrophobic amino acids*	439	436
Hydrophillic amino acids**	382	387

ND is not detectable.

* Hydrophobic amino acids include isoleucine, leucine, methionine, phenylalanine, valine, alanine, glycine and proline.

**Hydrophillic amino acids include serine, threonine, cysteine, aspartic/asparagine, glutamic/glutamine and tyrosine.

4.4.2.5 Fourier transform infrared spectra

The FTIR spectra of WSPP and WSPC are depicted in Figure 10. The result showed that WSPP and WSPC had five characteristic amide bands representing amide *A* (3200–3300 cm^{-1}), amide *B* (2900–3200 cm^{-1}), amide *I* (1600–1700 cm^{-1}), amide *II* (1500–1600 cm^{-1}) and amide *III* (1200–1400 cm^{-1}), which confirms those previously reported in collagen from skins of young and adult Nile perch (*Lates niloticus*) by Muyonga *et al.* (2004). The major peaks of WSPC were found at wavenumbers of 3268.80, 2924.28, 1623.80, 1515.21, and 1398.25 cm^{-1} for amide *A*, amide *B*, amide *I*, amide *II* and amide *III*, respectively, whereas WSPP were found at wavenumbers of 3259.16, 2921.09, 1621.87, 1514.87 and 1400 cm^{-1}, respectively. Ramappa *et al.* (2016) reported that the major absorption peaks in FTIR spectra of various silkworm pupae powder were also ranged in amide regions of 1630-1680 (N-H bending), 1600-1650 (C=O stretching) and 1500-1570 (N-H bending). Amide *A* corresponds to the stretching vibrations of N–H group, amide *B* corresponds to asymmetric stretch vibration of =C–H as well as $–NH_3^+$ and amide *I* bands originated from C=O stretching vibrations coupled to N–H bending vibrations, CN stretch and CCN deformation (Bandekar, 1992). Amide *II* representing N–H bending vibrations coupled to C–N stretching vibrations. Amide *III* represented the combination peaks between N–H deformation and C–N stretching vibrations and was involved with the triple helical structure of protein (Muyonga *et al.*, 2004). Those typical amide bands of the protein corresponded to particular stretching and bending vibrations of the protein backbone (Barth, 2007). Amide *I* arises from α-helix (1650-1658 cm^{-1}) and β-sheet (1638 cm^{-1}, 1687 cm^{-1}) , while N-H bending vibrations coupled to C-N stretching vibrations attributed to amide *II* (Du *et al.*, 2018). For amide *III*, it was obtained from a complex mix of α-helix (1290-1340 cm^{-1}) and β-sheet (1181-1248 cm^{-1}) along with random coil (1255-1288 cm^{-1}) (Du *et al.*, 2018). As a result, both WSPP and WSPC remained their structural integrity after extraction. However, the FTIR spectra of WSPP and WSPC were generally similar but the differences in a few characteristic peaks were detected (Figure 10), indicating slight differences in the structure, amino acids and functional groups of proteins (Glassford *et al.*, 2013).

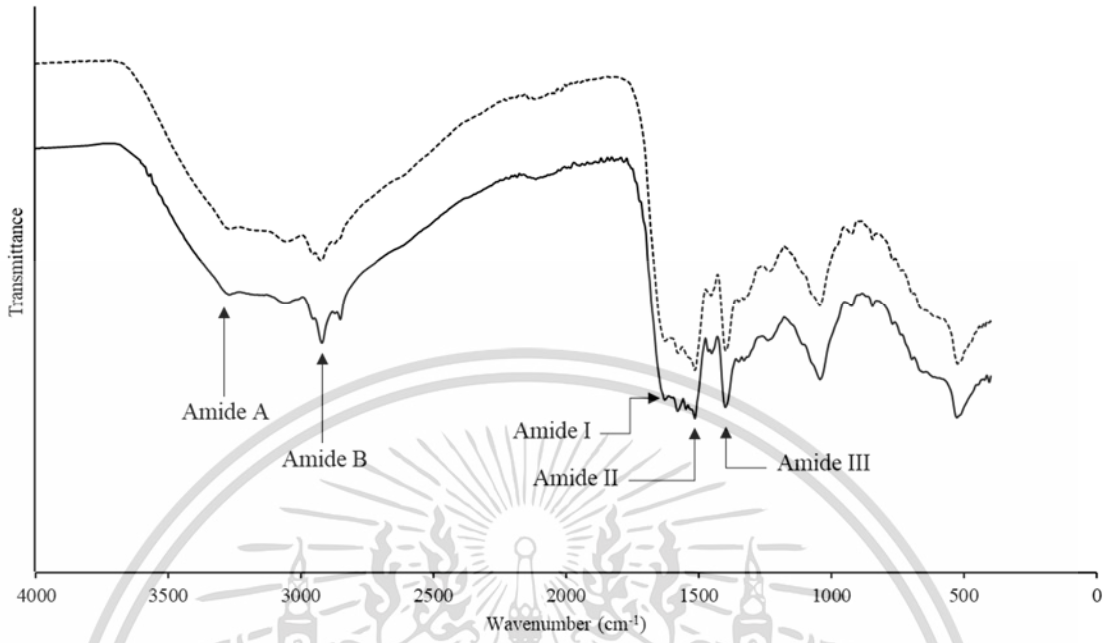


Figure 10. Fourier-transform infrared spectra of water soluble protein from *P. succincta* (WSPP) (—) and *C. roseapbrunner* (WSPC) (----).

4.4.3 Functional properties

4.4.3.1 Relative solubility

The relative solubility (%) of WSPP and WSPC in the pH range of 1 to11 was depicted in Figure 11. The result showed that WSPC generally had higher relative solubility than WSPP in all pHs tested. WSPP and WSPC were highly solubilized more than 85% in the pH range of 6-11, indicating that the protein in grasshopper can be solubilized at neutral to alkaline pH. At strong acidic condition (pH 1-2), the solubility of grasshopper proteins was relatively high with relative solubility of 73-78% and 88-90% for WSPP and WSPC, respectively. However, the solubility of those proteins decreased with increasing pH to 3 and 4. The lowest relative solubility was found at the pH 4 for both proteins, indicating their isoelectric pH (p*I*). This was due to a reduction in electrostatic repulsive forces between the proteins, leading to protein aggregation (Azagoh *et al.*, 2016). These results correspond well with the protein from migratory locusts *S. gregaria* (Zielińska *et al.*, 2018). The p*I*s of migratory locust (Purschke *et al.*, 2018), edible meal worm and black soldier fly (Bußler *et al.*, 2016) were also found at pH 4. Additionally, the

solubility of insect proteins was similar to those reported for several legumes, animal proteins and protein isolates (Azagoh *et al.*, 2016; Barth, 2007; Bußler *et al.*, 2016; Horax *et al.*, 2017; Purschke *et al.*, 2018).

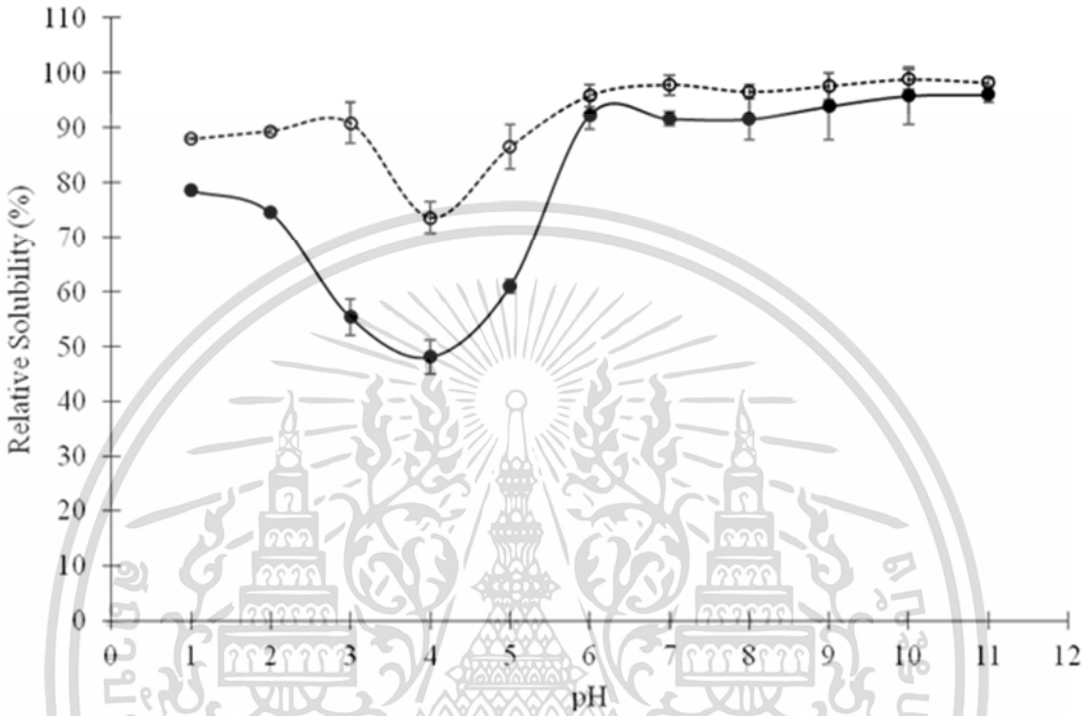


Figure 11. Relative solubility (%) of water soluble protein from *P. succincta* (WSPP) (—) and *C. roseapbrunner* (WSPC) (----) as affected by different pHs.

4.4.3.2 Emulsifying properties

Emulsifying and foaming properties of WSPP and WSPC are shown in Table 14. The emulsion activity index (EAI) and emulsion stability index (ESI) of WSPP and WSPC ranged from 29.23 to 36.69 m^2/g and 15.67 to 33.34 min, respectively. The EAI and ESI of those proteins were significantly lower than bovine serum albumin (BSA) ($p<0.05$). Low emulsifying properties found in both insect proteins might be due to they contained high amount of low molecular weight components. When peptides are shorter and less globular, they will form less stable protein layers around the oil droplets that offer less resistance to coalescence or Ostwald ripening (Guan *et al.*, 2007). Adebowale *et al.* (2005) reported an adequate emulsification but poor stability in whole giant African cricket (*Gryllidae* sp.) powder. In contrast, both high emulsion formation and stability in moth

(*Cirina forda*) larva and silkworm (*Bombyx Mori*) powders have been report by Omotoso (2015). Although WSPC exhibited greater EAI and ESI than WSPP, they had poor emulsifying properties when compared to BSA and protein from locusts *S. gregaria* (Zielińska *et al.*, 2018). Emulsifying properties of other proteins have been determined and reported. Tirgar *et al.* (2017) reported that EAI and ESI of flaxseed protein were 46.5 m^2/g and 12.51 min, respectively. Hall *et al.* (2017) reported EAI of 27 to 32 m^2/g for cricket (*G. sigillatus*) protein hydrolysates. EAI and ESI of WSPP was similar to that found by Nalinanon *et al.* (2011), who reported EAI and ESI for protein hydrolysate at concentration of 0.5% from ornate threadfin bream (*Nemipterus* sp.) muscle and DH10-30% using skipjack tuna (*Katsuwonus* sp.) pepsin digestion were 29.9 to 30.3 m^2/g and 14.1 to 18.6 min, respectively. WSPC exhibited greater emulsifying properties than WSPP. This might be due to a greater surface hydrophobicity of WSPC had a positive effect on emulsification (Table 14). The ability of a protein to rapidly lower free energy of a newly created interface is controlled by (1) how rapidly it can adsorb to the interface and (2) how rapidly and easily it can undergo conformational rearrangement and reorientation at the interface (Panpipat & Chaijan, 2017). The differences between the emulsion activity and emulsion stability are related to the amphiphilicity of the protein surface, protein contents (soluble and insoluble) and other components (Zielińska *et al.*, 2018). The lower ESI of WSPP and WSPC than BSA which could be attributed to partial denaturation of proteins and change in the distribution of molecular charge that exposes hydrophobic amino acid (Zielińska *et al.*, 2018).

Table 14. Emulsifying and foaming properties of water soluble protein extracted from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) and bovine serum albumin (BSA)[†].

Functional properties	**WSPP**	**WSPC**	**BSA**
Emulsion activity index (EAI) (m^2/g)	29.23±0.79^{c‡}	36.96±0.59^{b}	295.24±2.30^{a}
Emulsion stability index (ESI) (min)	15.67±0.18^{c}	33.34±4.37^{b}	38.55±0.77^{a}
Foaming capacity (FC) (%)	8.57±4.04^{c}	25.71±4.04^{b}	81.23±7.27^{a}
Foam stability (FS) (%)	98.72±1.81^{a}	86.41±2.78^{b}	67.41±2.95^{c}

[†]Mean ± SD from triplicate determinations.

[‡] Different superscript letters in the same row indicate significant differences ($p<0.05$).

4.4.3.3 Foaming properties

Foam capacity (FC) and foam stability (FS) of WSPP and WSPC are shown in Table 14. WSPP and WSPC exhibited 8.57, 25.71% and 86.41, 98.72% for FC and FS, respectively. WSPC showed higher FC than WSPP, although the FC of those proteins were low when compared with BSA (81.23%) ($p<0.05$). The result of FC from WSPC was similar to those reported by (Adebowale *et al.*, 2005) for whole giant cricket (*Gryllidae* sp.) powder that had a FC of 6%, and Omotoso (2006) who reported the FC from *Cirina forda* larvae powder as 7.1%. However, WSPC had lower FS than WSPP. The FS of WSPP and WSPC (~80%) was significantly higher than that of BSA (67.41%) ($p<0.05$), indicating the excellent capacity to stablilize foam against collapse. Generally, foam collapse takes place by any of these three mechanisms including (1) indisproportionation of bubbles; (2) coalescence of bubbles due to instability of thin film between them; and (3) drainage of water from the surface of the bubbles down to the liquid layer, thereby leading to the removal of protein from film around the bubble (Panpipat & Chaijan, 2017). Zielińska *et al.* (2018) reported the FC of protein preparations with alkali extraction from locusts (*S. gregaria*) as 32% and FS was 6.17%. The differences between FC and FS of proteins may be due to the factors influencing foam formation include hydrophobic amino acid content, surface hydrophobicity, location of hydrophobic amino acid residues on the protein surface, presence of thiol groups, cations and anions, carbohydrates and lipids (Zielińska *et al.*, 2018). Johnson and Zabik (1981) explained that intermolecular protein-protein interaction enhances the cohesive nature of the film, imparting stability and elasticity to the membrane. This interaction appears to be dependent on the presence of a high ratio of nonpolar/polar side chains in the protein (Johnson & Zabik, 1981). Additionally, Nalinanon *et al.* (2011) suggested that low MW peptide could not maintain well-ordered orientation of the molecule at the interface.

4.4.4 Antioxidant activities

Antioxidant activities as determined by ABTS, DPPH and FRAP assays of WSPP and WSPC are shown in Table 15. WSPP and WSPC exhibited strong scavenging activities with IC_{50} of 204.67 and 176.31 µg/mL for DPPH radical and 81.97 and 69.12 µg/mL for ABTS radical, respectively. Zielińska *et al.* (2017) reported that the antiradical activities against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ for the hydrolysates obtained after digestion of

locusts *L. migratoria* were found to be 67 µg/mL and 25.9 µg/mL of their IC_{50}, respectively. These reports found less effective on $DPPH^{\bullet}$ scavenging activity when compared with the present result (IC_{50} of 176.31 to 204.67 µg/mL). Additionally, Zielińska *et al.* (2017) reported that the protein hydrolysates prepared by alkali extraction from locusts (*S. gregaria*) exhibited IC_{50} for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities were 28.5 and 16.6 µg/mL, respectively. With high ABTS radical-scavenging activity, it was postulated that antioxidative compounds were most likely hydrophilic (Nalinanon *et al.*, 2011). Also, with high DPPH radical-scavenging activity, the results obtained suggest that the grasshopper proteins contained amino acids or peptides that were electron donors and could react with free radicals to convert them to more stable products and terminate the radical chain reaction. The protein from various species of edible insect might give different scavenging activity on $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals. It might be depended on molecular weight of protein or peptide as well as its amino acid composition. Zhang *et al.* (2010) postulated that peptides with large molecular weight have less antioxidant activity. Nalinanon *et al.* (2011) found that peptides in hydrolysates from ornate threadfin bream muscle with various degree of hydrolysis (DH) might differently scavenge two different radicals, ABTS and DPPH radicals. In addition, the presence of hydrophobic sequences in the peptides could interact with lipid molecules and could scavenge by donating protons to lipid derived radicals (Chalamaiah *et al.*, 2012).

Table 15. Antioxidant activity of water soluble protein from *P. succincta* (WSPP) and *C. roseapbrunner* (WSPC) [†].

Antioxidant assays	**WSPP**	**WSPC**
DPPH (IC_{50} µg/mL)	$204.67\pm2.02^{a‡}$	176.31 ± 2.02^{b}
ABTS (IC_{50} µg/mL)	81.97 ± 0.07^{a}	69.12 ± 0.26^{b}
FRAP (µg TE/g protein)	22.26 ± 0.45^{b}	27.59 ± 0.06^{a}

[†]Mean ± SD from triplicate determinations.

[‡]Different superscript letters in the same row indicate significant differences ($p<0.05$).

As per FRAP assay, WSPP and WSPC also presented reducing power on Fe^{3+} of 22.26 and 27.59 µg TE/g protein. Similar results were obtained by Xia *et al.* (2012) who

reported that the antioxidant properties of the barley glutelin hydrolysates had ferric reducing power values of 24.0 μg Fe^{2+}/mg, respectively. Bousopha *et al.* (2016) reported that collagen hydrolysate from pharaoh cuttlefish skin with 10-30% DH had ferric reducing power values of 23.50 to 26.50 μmol TE/g protein. The increase or decrease in ferric reducing power for protein hydrolysates may be related to the exposure of electron-dense amino acid side chain groups, such as polar or charged moieties during hydrolysis (Zambrowicz *et al.*, 2012). Compounds with higher reducing power were shown to have a better ability to donate electrons or hydrogen and serve as a significant indicator of their potential for use as an antioxidant (Je *et al.*, 2009).

4.5 Conclusion

The proteins from edible insects, *P. succincta* and *C. roseapbrunner* could be effectively extracted by water. Based on physicochemical and functional properties as well as antioxidant activities, water soluble proteins from both insect are beneficial for human nutrition and had potential use as food ingredient. This opens up the possibility for these edible insect proteins to be used as in suitable food applications.

CHAPTER 5

QUALITY AND PROPERTIES OF NOODLE PARTIAL REPLACEMENT WITH DEFATTED POWDER OF BOMBAY LOCUST (*Patanga succincta*)

5.1 Abstract

The grasshopper is one of the edible insects recommended as an excellent alternative food, due to its high protein content. The quality properties of noodles partial replacement wheat flour with 5, 10, 15, 20, and 30% defatted bombay locusts powder (DBLP) were characterized as well as their chemical, physical, cooking quality, texture properties and sensory characteristics were evaluated. The results of noodle formula development indicated that as the amount of DBLP increased, the L^* value of the noodles decreased and the appearance became darkness red-brown. The cooking yield and cooking loss were ranged 189.41 to 232.57% and 5.81 to 8.89%, respectively. The tensile strength and elasticity of cooked noodles were ranged 4.67 to 9.31 g.force and 8.11 to 25.60 mm, respectively. The physical, cooking qualities and textural of noodle were decreased, when adding DBLP high level ($p<0.05$). The results of noodle after adding guar gum 0.5% to improved characteristic and properties of insect noodle was showed that the noodle DBLP 5% had cooking qualities and texture properties similar to the control. The results of consumer evaluation showed that the overall liking of cooked noodles DBLP 5% were at the like level. The present study indicated that DBLP is a potential source of protein when substituted for wheat flour in noodle products. Noodles with DBLP 5% were composed of 24.07% moisture content, 4.04% fat, 2.44% ash and 16.65% protein. The incorporation of DBLP in the noodle ingredients significantly increased protein content.

5.2 Introduction

Noodles are a traditional food widely consumed throughout Asia. In the past, they were made within the home for consumption by the household. Noodles are readily available and can be purchased in a variety of forms including fresh, cooked or processed for longer shelf life. Due to their ease of preparation, noodles are now considered as a

convenient fast food (Fu, 2008). Asian noodles made from wheat may be divided into two general classes based on the ingredients used: white salted noodles (WSN) made from flour, sodium chloride and water, and yellow alkaline noodles (YAN) made from flour, alkaline salts (such as sodium and potassium carbonate) and water (Asenstorfer *et al.*, 2006). Traditional noodles are claimed to lack other essential nutritional components such as dietary fiber, vitamins and minerals (Fu, 2008).

The high nutritional value of edible insects has been attracting the attention of researchers and food industry for their potential use in the formulation of foods with enhanced nutritional characteristics. Indeed, insects are very rich in proteins and essential amino acids (Belluco *et al.*, 2013). Edible insects include Lepidoptera, Coleoptera, Orthoptera, Isoptera, and Hymenoptera (Yhoung-Aree *et al.*, 1997), silk worm pupae and grasshopper are typically used as a food source in Thailand. One of the problems related to edible insect consumption as a human food is unfavorable consumer perception (Tan *et al.*, 2015). This problem may be solved by processing edible insects in a less recognizable form and incorporating them in food products (Verkerk *et al.*, 2007). de Oliveira *et al.* (2017) studied bread quality with adding cinereous cockroach (*Nauphoeta cinerea*) flour in amounts of 5, 10, and 15% (based on wheat flour). They reported that bread enriched with 10% roasted flour presented the best nutritional characteristics, differing little from the white and whole wheat bread. Osimani *et al.* (2018) studied effects of cricket (*Acheta domesticus*) powder (10 or 30%) on the nutritional value of bread loaves. They reported that the addition of 10% cricket powder produced doughs apparently suitable for bread-making. The aim of this study was to use of defatted bombay locusts powder (*Patanga succincta*) in noodle with enhanced nutritional value. Experimental noodle was obtained from doughs produced using different blends of wheat flour and defatted bombay locusts powder. The effect of replacement levels on physical, chemical, cooking quality, textural and sensory of the locust noodles were determined.

5.3 Materials and methods

5.3.1 Preparation of defatted bombay locusts powder

Bombay locusts were thawed, removed hindleg/long jumping legs and washed with running portable tap water, rinsed with distilled water and drained using a

plastic sieve for 1 h. Bombay locusts was ground in a blender (MMB54G5S, BOSCH, Germany) with the highest speed for 5 minutes. Bombay locusts paste was defatted according to the procedure reported by Dervas *et al.* (1999) using cold hexane (1:8 w/v). The slurry was kept with periodical stirring for 45 min, at 4 °C and the hexane with fat was decanted. The fat extraction was repeated twice and the defatted bombay locusts was desolventized in a fume hood at room temperature for 2 hours. After that defatted bombay locusts was dried in a tray drier at 70 °C for 5 h. Dried defatted bombay locusts was ground and sieved (250 micron), finally stored in zip bags in desiccator until used.

5.3.2 Noodle preparation

The formula of the control noodle and locust noodle were shown in Table 16. All dried ingredients were combined and mixed to produce homogenize mixture. The mixture was placed in a mixing bowl and hand kneaded with egg and water until the dough formed (~20 min) and smooth. Place it in a mixing bowl, covered with plastic wrap and let rise in a room temperature about 30 min. Punch dough down by hand kneaded for 1 min, divided the dough into approximately 50-g portions and sheeted using pasta machine (ATLAS 150 WELL.AS.P, Italy) by rolling at position one and repeated at position six. Thereafter, the sheet of dough was passed through a hand operated pasta machine. The dough was cut into strips of 25 cm. Each noodle strand was 1.5 mm width and 1.5 mm thickness.

The effect of guar gum on the quality of the noodle were also elucidated. All the experimental formulas were showed in Table 17. The noodles were prepared following the methods described above. All dried ingredients excepted guar gum were combined and mixed to produce homogenize mixture. The mixture was placed in a mixing bowl and mixed with tap guar gum-egg and water until the dough formed (~20 min).

Table 16. The formula of the control and locust noodle.

Ingredients	formulation					
	0% (Control)	**5%**	**10%**	**15%**	**20%**	**30%**
Wheat flour (g)	100	95	90	85	80	70
Locust powder (g)	0	5	10	15	20	30
Water (g)	3	3	3	3	3	3
Whole egg (g)	47	47	47	47	47	47
Sodium carbonate (g)	2	2	2	2	2	2
Salt (g)	1	1	1	1	1	1

Source: modified from Hou *et al.* (1998)

Table 17. The formula of the control and locust noodle with guar gum.

Ingredients	formulation					
	0% (Control)	**5%**	**10%**	**15%**	**20%**	**30%**
Wheat flour (g)	100	95	90	85	80	70
Locust powder (g)	0	5	10	15	20	30
Water (g)	3	3	3	3	3	3
Whole egg (g)	47	47	47	47	47	47
Sodium carbonate (g)	2	2	2	2	2	2
Guar gum (g)	0.5	0.5	0.5	0.5	0.5	0.5
Salt (g)	1	1	1	1	1	1

Source: modified from Hou *et al.* (1998)

5.3.3 Color analysis

The colors of noodle were measured as previous method in 3.3.4.6. Twenty grams of noodle was filled in a cuvette quart path length 25 mm, and three observations were measured and expressed as CIE L^* (lightness), a^* (redness), and b^* (yellowness) with 5 readings/samples.

5.3.4 Noodle cooking quality

Noodle cooking quality (cooking yield and cooking loss) were modified by American Association of Cereal Chemists (AACC, 2000). Ten grams of noodles were cooked in 150 mL of boiling distilled water in a 250-mL beaker for 1.30 min, rinsed in cold water, and drained for 15 min before weighed. Percentage of increased weight was calculated as a cooking yield. Solids content in the cooking water was determined by drying at 105 °C overnight. The cooking loss was determined by the weight of noodles prior to cooking and the weight of the dried residue after the cooking water had been completely dehydrated.

5.3.5 Texture analysis

Noodles were cooked 90 seconds, cooled for 1 minute under running distilled water, drained, stored for exactly 10 minutes at 25 °C as described by Kruger *et al.* (1994) and submitted to tensile testing using the TA-XT2i texture analyzer (Lu *et al.*, 2009). The distance between the parallel friction rollers was 2 cm. Instrument settings were tension mode; pretest speed 5.0 mm/s; post-test speed 10 mm/s; test speed 3.0 mm/s; and distance 60 mm. The data acquisition rate was 200 pps. The trigger force was 1.0 g with a trigger type of auto. From force-distance curves, two texture parameters were obtained: tensile strength (maximum force; g) and elasticity (distance at maximum force; mm). Each sample was measured 10 times.

5.3.6 Sensory evaluation

Noodle samples were prepared for sensory evaluation. The samples were boiled using tap water for 90 seconds. The samples were then stored for not more than 30 minutes in tightly covered plastic food containers before testing. The cooked noodle would be served in ramen soup. The ramen soup was prepared by mixed Tsuyu with water ratio of 1:6 (v/v). The proportion of noodle with soup was 1:3 (w/v). The cooked noodles were evaluated for appearance, flavor, taste, texture and overall liking of the samples by 30 untrained panelists using seven-point hedonic scales, where 7 = like very much and 1 = dislike very much.

5.3.7 Proximate Analysis

Defatted bombay locusts powder and noodle samples were determined for moisture, crude protein, crude fat and ash contents according to the Official methods of analysis of the Association of Official Analytical Chemists (AOAC, 2000). All measurements were performed in triplicate and expressed as on a dry weight basis.

5.3.8 Statistical Analysis

Except for the color *L*, a*, b**, which were repeated five times, the tensile strength and elasticity tests for the cooked noodles, which were repeated ten times, all other experiments were repeated three times. SPSS 11.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Analysis of variance was used to determine significant differences between the results, and Duncan's multiple range test was used for means comparison with the confident level ($p < 0.05$).

5.4 Results and discussion

5.4.1 Color characteristics

The color appearance of defatted bombay locusts powder (DBLP) was dark red-brown color as shown in Figure 12. DBLP had proximate composition; 3.92±0.08% moisture content, 12.36±0.04% fat, 3.05±0.02% ash, and 70.09±0.14% protein. While the protein content of the wheat flour was 12.50% (Ritthiruangdej *et al.*, 2011) The color parameters of noodles partial replacement with DBLP samples are shown in Table 18. Among the noodle partial replacement with DBLP levels, a significant ($p < 0.05$) different color intensity in terms of *L*, a**, and *b** could be observed. Noodle samples partial replacement with all DBLP level had the lower *L**and *b** values than control, while *a** values of all DBLP level had the higher than control and significance (Figure 13a and 12b). The results indicated that as the amount of DBLP increased, the appearance of the noodles partial replacement with DBLP grew darker (Figure 14). The addition of DBLP caused the noodle became darker because DBLP had red-brown color and high protein content. Therefore, the brownish pigments generated by the Maillard reaction between the reducing sugars and free amino acids may happened higher (Mohamed *et al.*, 2010).

Table 18. Color characteristics of noodles partial replacement with defatted bombay locusts powder (DBLP) (%).

Noodle	*L**	*a**	*b**
Control	56.72±0.82[a]	4.54±0.18[e]	28.06±0.74[a]
DBLP 5%	32.39±1.41[b]	9.99±0.30[a]	12.94±1.75[b]
DBLP 10%	31.63±0.67[b]	8.49±0.61[b]	11.62±0.35[b]
DBLP 15%	28.80±0.48[c]	8.12±0.09[b]	8.41±0.19[c]
DBLP 20%	27.74±0.24[d]	6.97±0.45[c]	7.04±0.11[d]
DBLP 30%	26.61±0.33[e]	5.11±0.08[d]	5.83±0.83[e]

Values are shown as mean ± SD; n = 3.

Different superscript letters in a column indicate significant differences ($P \leq 0.05$).

Figure 12. Defatted bombay locusts powder (DBLP)

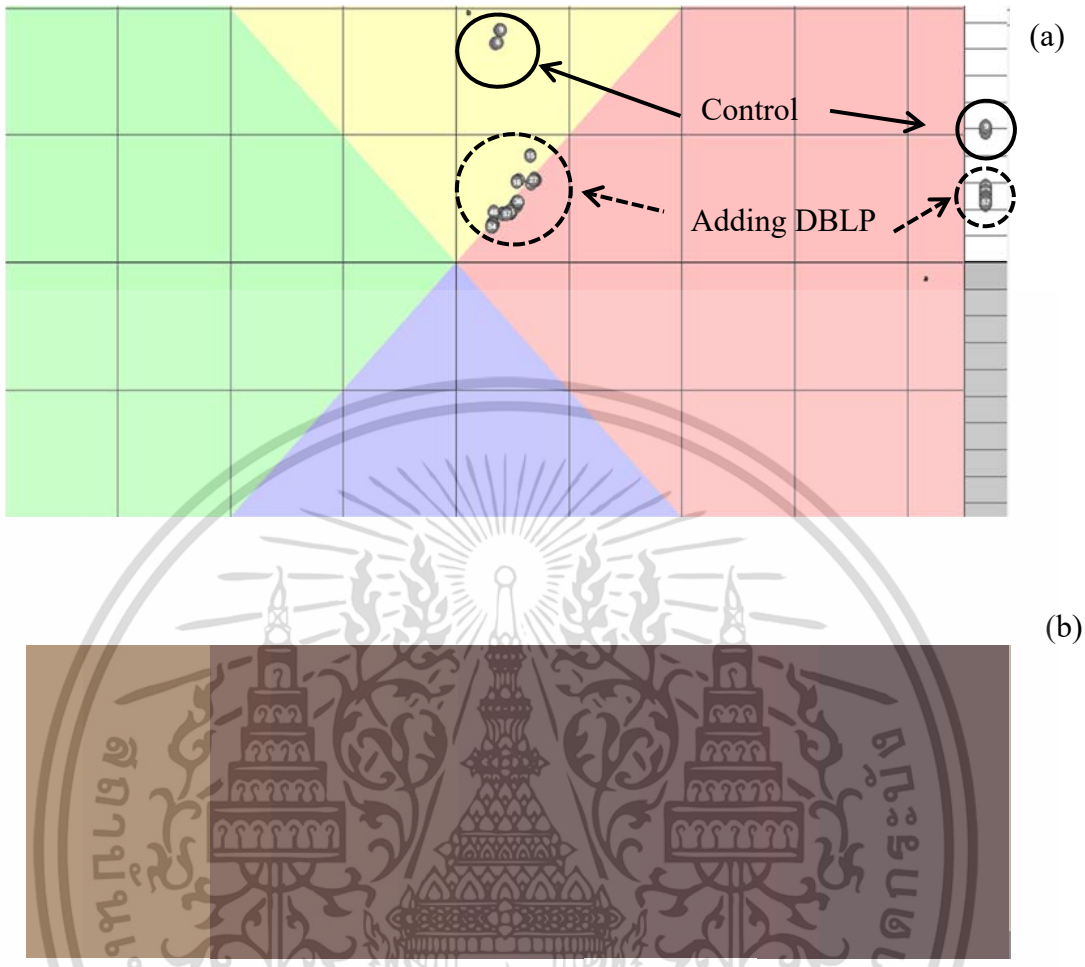


Figure 13. Color plot of cooked noodle partial replacement with defatted bombay locusts powder (DBLP); (a) sample position and (b) color shade of cooked noodle.

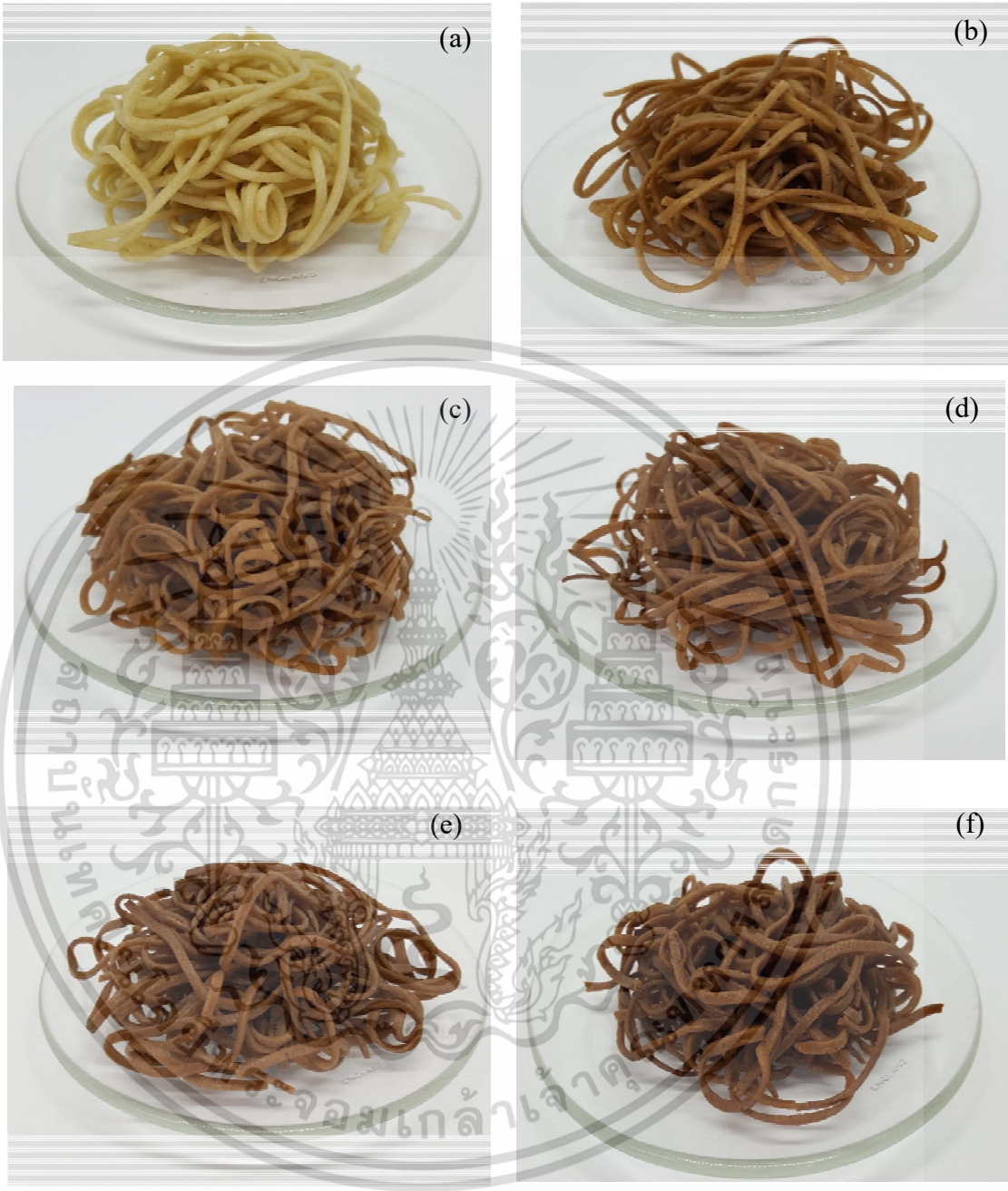


Figure 14. Cooked noodles partial replacement with defatted bombay locusts powder (DBLP); control 0%: (a), DBLP 5%: (b), DBLP 10%: (c), DBLP 15%: (d), DBLP 20%: (e) and DBLP 30%: (f)

5.4.2 Cooking qualities

Cooking qualities (cooking yield and cooking loss) of DBLP noodles are shown in Table 19. Percent cooking yield varied from 189.41 to 234.30% from control to noodles with DBLP 30%. Cooking yields for noodles decreased considerably as levels of DBLP increased. Cooking yield indicated to water absorption of the noodle. Control sample showed more water absorption in noodles than DBLP noodles and significant difference from DBLP level at 10 to 30% (p<0.05). It might suggest that the cooking yield was mostly influenced by adding DBLP. However, control sample was non-significant with DBLP 5%.

Table 19. Cooking yield and cooking loss of noodle partial replacement with defatted bombay locusts powder (DBLP) (%).

	Guar gum (0%)		**Guar gum (0.5%)**	
Noodle	**Cooking yield (%)**	**Cooking loss (%)**	**Cooking yield (%)**	**Cooking loss (%)**
Control	234.30±2.02^{a}	4.14±0.30^{f}	230.51±4.60^{a}	3.19±0.07^{e}
DBLP 5%	232.57±6.73^{a}	5.81±0.05^{e}	229.28±3.20^{ab}	4.03±0.01^{d}
DBLP 10%	217.79±4.87^{b}	6.22±0.12^{d}	220.25±5.33^{b}	5.92±0.33^{c}
DBLP 15%	216.74±9.46^{b}	7.23±0.01^{c}	219.44±2.32^{b}	7.24±0.76^{b}
DBLP 20%	204.79±5.93^{c}	7.96±0.29^{b}	207.48±6.65^{c}	7.59±0.55^{ab}
DBLP 30%	189.41±1.66^{d}	8.89±0.29^{a}	192.98±3.50^{d}	8.19±0.13^{a}

Values are shown as mean ± SD; n = 3.

^{abc} Different superscript letters in a column indicate significant differences ($p \leq 0.05$).

Cooking loss is a critical factor for noodle and pasta quality in terms of the index of resistance to disintegration during cooking (Larrosa *et al.*, 2016). Low amount of cooking loss indicates a high quality of the cooked noodle. Generally, 7.0-8.0% of cooking loss is acceptable (Susanna & Prabhasankar, 2013). In this study, the cooking loss of noodle 5 partial replacement with DBLP 5% to 30% ranged between 5.81 and 8.89%. Replacement with level of DBLP strongly exhibited significant differences in cooking loss (p< 0.05). Thus, all DBLP noodles lack a gluten network, starch polymers were less efficaciously

entrapped in the matrix (Aydin & Gocmen, 2011; Izydorczyk *et al.*, 2005), resulting in a product with high cooking loss when compared with control.

When adding guar gum, the results also showed that the noodles DBLP prepared with guar gum 0.5% had significantly ($p< 0.05$) lower cooking losses than the noodles DBLP without guar gum which was similar to the result of Lü *et al.* (2014) who reported the cooking loss reduced with the addition of guar gum. Silva *et al.* (2013) explained the lower cooking loss of noodles containing hydrocolloids by noting that the hydrated polysaccharide network could encapsulate the starch granules and this created stable-starch granules in the matrix system (Zhou *et al.*, 2013). Menon *et al.* (2015) offered supporting evidence that hydrocolloids created a strong association of soluble starch such as amylose and increased the stability and viscosity which led to lower leaching of amylose into the cooking water.

5.4.3 Texture properties

Noodles control (without DBLP) showed higher tensile strength and elasticity than that of DBLP noodles. Noodles required more tensile strength to fracture. The tensile strength was ranged 4.32 to 9.31 g.force for the sampled with DBLP. The elasticity was ranged 10.52 to 25.60 mm. for the sample with DBLP. On the other hand, the lowest tensile strength and elasticity were the DBLP 30% noodles (Figure 15a and 15b). There was significant difference in tensile strength and elasticity between samples made with 5% to 30%. As levels of DBLP increased, the tensile strength and elasticity of noodles decreased. Textural properties of noodles are mainly affected by the matrix structure of starches, glutens, additional proteins and other ingredients (Ritthiruangdej *et al.*, 2011). Gluten proteins are composed of gliadins and glutenins, which are responsible for gluten or dough extensibility (viscosity) and strength (elasticity), respectively. Therefore, removal of wheat flour would weaken the gluten matrix, hence leading to the weakening of noodle texture.

When adding guar gum 0.5%, DBLP noodles were improved texture (tensile strength and elasticity) better than no added guar gum (Figure 15a and 15b). The tensile strength of DBLP 5% with added guar gum 0.5% was closed to control without guar gum. Guar gum can improved noodle texture because the interaction between the hydrocolloids and wheat protein to improve the gluten network strength and the increase in the wheat starch viscosity during gelatinization (Ribotta *et al.*, 2005). Moreover, hydrocolloid

improved the structure of noodles by ionic charges of the hydrophilic component interacting with proteins (Gull *et al.*, 2016).

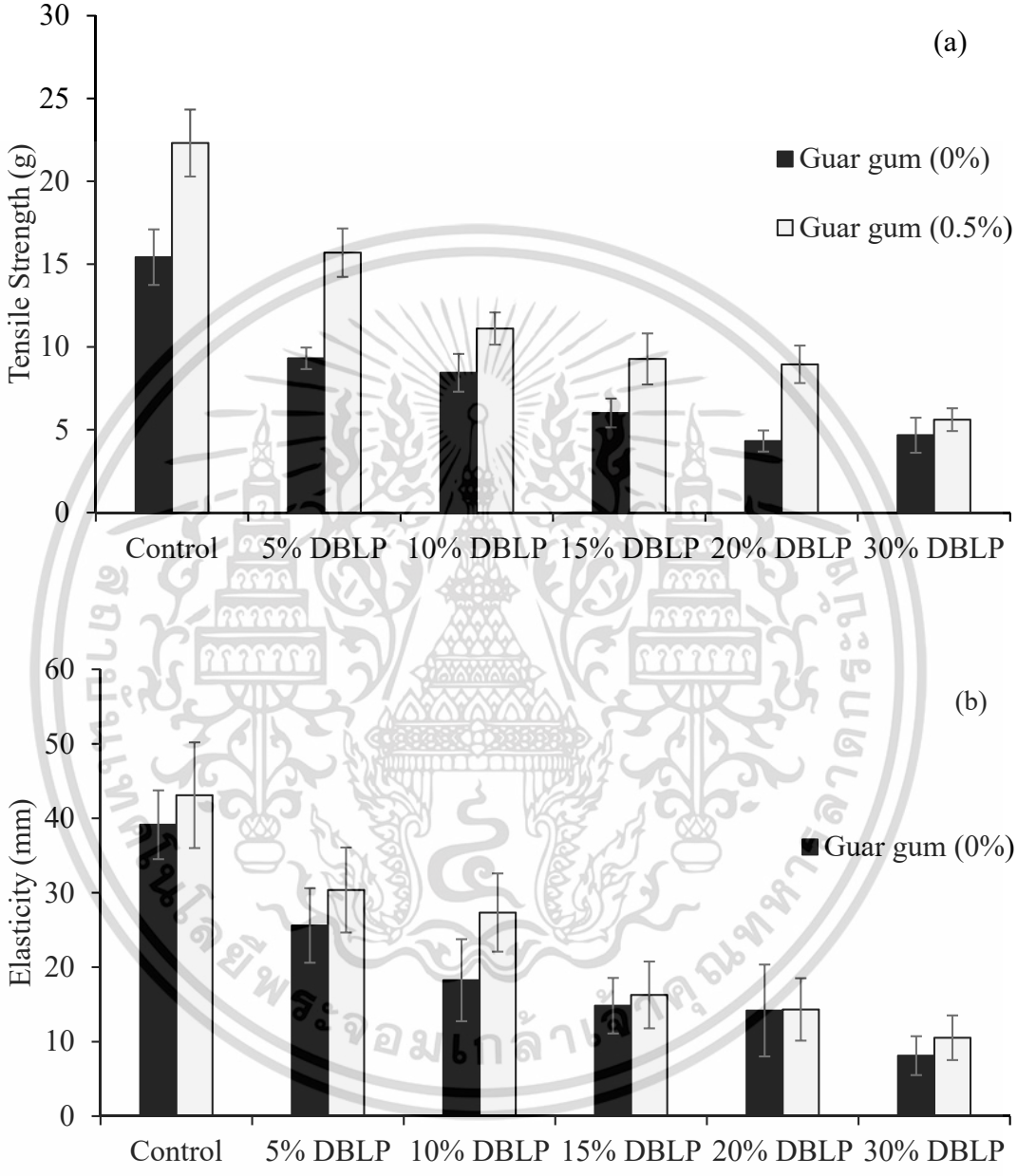


Figure 15. Effect of defatted bombay locusts powder (DBLP) (%) on: tensile strength; and elasticity of cooked noodle partial replacement with defatted bombay locusts powder (DBLP). The vertical bars on each column indicate the standard deviation.

5.4.4 Sensory evaluation

Noodle partial replacement with DBLP 5% was selected to sensory because the cooking qualities and textural was similar to the control. Noodle samples was cooked in boiling water for 1.30 min and served in ramen soup at 75–80°C (Figure 17). The sensory evaluation of control noodle was shown in Figure 16a. Hedonic score for appearance, color, flavor, texture and overall was 6.1, 6.0, 5.3, 6.1 and 6.3, respectively. Moreover, the sensory scores for all the parameters for DBLP 5% noodle was shown in Figure 16b. Hedonic score for appearance, color, flavor, texture and overall was 6.0, 5.7, 5.9, 5.9 and 6.1, respectively. When DBLP 5% noodle compared with control noodle, hedonic score of appearance, texture and overall were in the same range score (6 to 7 score). Although, color of DBLP 5% noodle had lower than control. On the other hand, the flavor score of DBLP 5% had higher than control. From the results, it was found that both noodle had liking score in the range of like to like very much. Thus, it was indicating that the insect noodle was well accepted by the panelists.

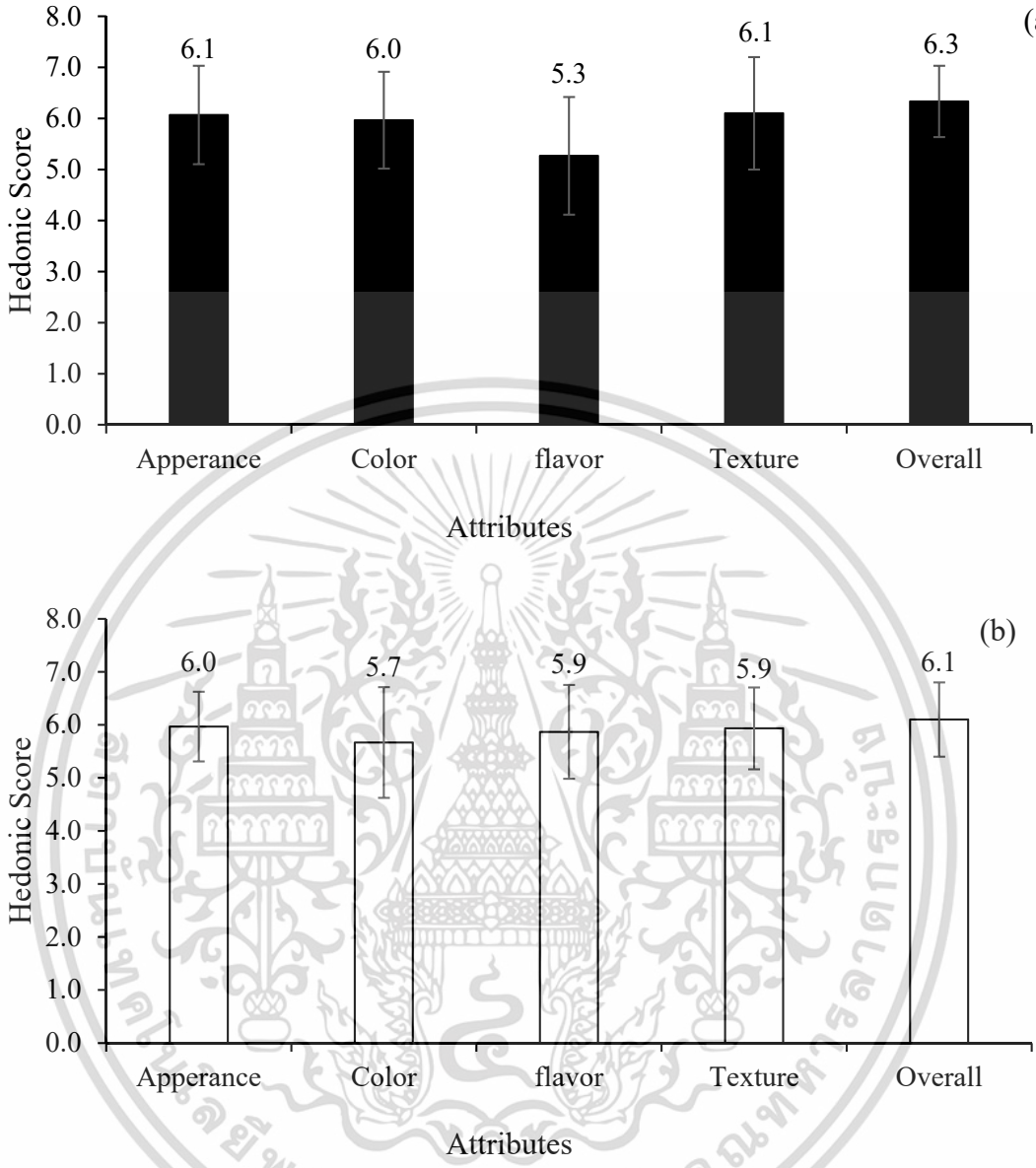


Figure 16. Sensory evaluation of control noodle (a) and noodle partial replacement with defatted bombay locusts powder 5% added guar gum 0.5% (b). The vertical bars on each column indicate the standard deviation.

Figure 17. The noodles partial replacement with defatted bombay locusts powder in ramen soup.

5.4.5 Proximate analysis

The chemical compositions of noodle partial replacement wheat flour with defatted bombay locusts powder 5% and the control noodle was shown in Table 20.

Table 20. Chemical composition of noodles partial replacement with defatted bombay locusts powder 5% added guar gum 0.5%.

Sample	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Carbohydrate* (%)
Control 0%	24.35±0.19	4.52±0.02	2.21±0.02	14.32±0.23	54.6
DBLP 5%	24.07±0.91	4.04±0.05	2.44±0.01	16.65±0.36	52.8

* The resulted from calculation.

It can be observed that the moisture content of noodles partial replacement with defatted bombay locusts powder 5% decreased from control when adding DBLP in the noodles. Ovando-Martinez *et al.* (2009) described that this pattern is related to the decrease in the protein content or protein component (glutenins and gliadins) with the increase in the amount of DBLP in the noodles where the network produced by the gluten is reduced. The fat content slightly changed with the addition of DBLP. Lower fat contents for noodles with added DBLP might be caused by the dilution that is contributed by the DBLP. The ash content increased when DBLP amount in the noodles increased. The ash content depends on the quality of the flour and thus corresponds to the higher mineral content (Kim, 1996). The higher ash in DBLP noodles might be DBLP is rich vitamins and minerals. The protein content increased when DBLP amount in the noodles increased. It depended on the quality of DBLP which is rich protein (70%).

5.5 Conclusion

The noodles partial replacement with defatted bombay locusts powder to provide a higher protein content and supply essential nutrition as well as health benefits were produced. Further addition with guar gum was related to an improvement in the textural characteristics due to the higher tensile strength and elasticity. Moreover, an improvement in the cooking quality of partial replacement with DBLP 5% by adding guar gum gave the product well accepted by panelists.

CHAPTER 6

SUMMARY AND FUTURE WORKS

Three Thai general edible insects; silkworm pupae: *Bombyx mori* Linn., Bombay locust: *Patanga succincta* and Spur-throated grasshopper: *Chondracris roseapbrunner* were extracted with neutral water to obtain water-soluble protein (WSP). These proteins in the native form were characterized as well as their functional properties and antioxidant activities were evaluated.

The visible color of WSPB powder tended to be light yellow-brown. Moreover, the color of WSPP and WSPC was red-brown. The amino acids of three WSPs were found essential and non-essential amino acid, the most abundant was glutamic acid. The proteins with MW of around 40 and 70 kDa were the protein components that found in three WSPs. The FTIR spectra indicated that structure of three WSPs composed of amide A, B, I, II, and III bands. The surface hydrophobicity, free and total sulfhydryl group contents and functional properties depended on edible insect species. Almost edible insect proteins were mostly soluble in strong acidic and alkaline aqueous solutions with a minimum value at pH 4. All WSPs exhibited poor emulsifying properties and foaming capacity but they had greater foam stability with comparable to bovine serum albumin. All WSPs had high antioxidant potential based on DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP assay.

The quality properties of noodle partial replacement wheat flour with defatted Bombay locusts powder (DBLP) were examined. When adding DBLP increased, the L^* value of the noodles decreased and the appearance became darkness red-brown. The cooking and textural qualities of the resulted noodle were lower than control ($p<0.05$). After improving characteristic and properties of insect noodle by added guar gum, the noodle with 5% DBLP had cooking qualities and texture properties like the control. The results of consumer evaluation showed that the overall liking of the cooked insect noodle was well accepted by panelist. Moreover, the protein content of DBLP noodle significantly increased when compared to that of control.

The present study indicated that Thai edible insects are a good source of protein based on their physicochemical and functional properties as well as antioxidant activities.

Their water-soluble protein counterparts and opens up the possibility for proteins extracted from these edible insects to be used as ingredients in processed food.

Future works

1. Modification of functional properties of insect protein should be studied
2. Utilization of insect powder in instant noodle should be investigated.

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