PRODUCT DEVELOPMENT OF BROWNIE FORTIFIED WITH PROTEIN

NATTAYA SIRIPHETAMORN

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Thesis

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PRODUCT DEVELOPMENT OF BROWNIE FORTIFIED WITH PROTEIN

BY

NATTAYA SIRIPHETAMORN STUDENT ID. 55080013

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Special Problem Advisor

หัวข้อปัญหาพิเศษ	การพัฒนาผลิตภัณฑ์บราวนี่เสริมโปรตีน
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บทคัดย่อ

ผลิตภัณฑ์โปรตีนบาร์เป็นขนมเพื่อสุขภาพที่กำลังเป็นที่นิยมของผู้บริโภคโดยเฉพาะนักกีฬาและกลุ่มนักเพาะกาย บราวนี่เสริมโปรตีนเป็นทางเลือกใหม่สำหรับขนมที่มีโปรตีนสูง ในการวิจัยครั้งนี้ใช้โปรตีน 3 ชนิด ได้แก่ เวย์โปรตีน โปรตีนไข่ และโปรตีนถั่วเหลือง เพื่อเสริมปริมาณโปรตีนในผลิตภัณฑ์บราวนี่ สารไฮโดรคอลลอยด์ที่ใช้เพื่อปรับปรุงคุณเนื้อสัมผัส คือ เลซิทินจากถั่วเหลืองและ Poly-dextrose ส่วนผสมของชอคโกแลตที่ใช้ในการเคลือบมีการเปลี่ยนแปลงสัดส่วนของกลูโคสไซรัปและนม ใช้การทดลองแบบผสมในการหาสัดส่วนที่เหมาะสมของส่วนผสมโปรตีน สารไฮโดรคอลลอยด์และส่วนผสมชอคโกแลตที่ใช้ในการเคลือบ การเก็บรักษาผลิตภัณฑ์ที่อุณหภูมิแช่เย็นโดยใช้บรรจุภัณฑ์ 3 ชนิด จากผลการทดลองพบว่า คะแนนความพอดีด้านกลิ่นและรสชาติมีคะแนนสูงสุดเมื่อใช้ 16.6% เวย์โปรตีน, 4.2% โปรตีนไข่, 4.2% โปรตีนถั่วเหลือง การเพิ่ม 0.5% เลซิทินถั่วเหลืองต่อ 1.5% Poly-dextrose ทำให้เนื้อสัมผัสของบราวนี่เสริมโปรตีนดีขึ้น และเป็นที่ยอมรับทางด้านประสาทสัมผัสมากที่สุด ส่วนของชอคโกแลตที่ใช้ในการเคลือบบราวนี่เสริมโปรตีนโดยใช้กลูโคสไซรัป 15% และนม 15% ให้การยอมรับทางประสาทสัมผัสโดยรวมมากที่สุด บรรจุภัณฑ์ที่ใช้ในการเก็บรักษาผลิตภัณฑ์บราวนี่เสริมโปรตีน ได้แก่ กล่องพลาสติก ถุงซิปล็อคพร้อมสารดูดซับออกซิเจน และถุงพลาสติกปิดภายใต้สุญญากาศ สามารถเก็บรักษาได้ถึง 7 วัน

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Student name	Nattaya Siriphetamorn Student ID 55080013
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ABSTRACT

Protein bar is recently a very popular healthy snack especially for athletes and weight trainers. Brownie fortified with protein is another choice of high protein snack. In this study, three types of protein were used which were whey protein concentrate (WPC), whole egg protein (WEP), and soy protein isolate (SPI) in order to increase protein contents in brownie. Mixture of soy lecithin and poly-dextrose was used as hydrocolloids for texture improvement. Mixture of glucose syrup and milk was combined with chocolate to cover the brownie. Appropriate proportion of protein mixture, hydrocolloids and covered ingredients were formulated by the mixture design. Shelf-life of brownie fortified with protein was determined in 3 package conditions at chilling temperature. The results shown that high in "Just Right" acceptance for odor and flavor was obtained in fortification of 16.6% WPC, 4.2% WEP and 4.2% SPI in brownie formulation. Addition of 0.5% soy lecithin and 1.5% poly-dextrose was appropriated to improve texture of the high protein brownie texture. The highest in sensory acceptance test was presented in the high protein brownie covered with 15% glucose syrup and 15% milk. Shelf-life of the high protein brownie packed plastic box, zipper bag with oxygen absorber and plastic bag packed under vacuum was approved for 7 days at chilling temperature.

Keywords: Protein bar, High protein Brownie, Protein fortification

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Nattaya Siriphetamorn

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TABLE OF CONTENTS

Page

TABLE OF CONTENTS

LIST OF TABLES

Tables Page

LIST OF ILLUSTRATIONS

Figures Page

Chapter 1

Introduction

1.1. Background and Signification of the Special Problem

Nowadays, people attempt to maintain balanced weight and more concern in their health. Even though, physical activities are necessary in daily life, consumption of healthy diets is also an overwhelming factor for excellent health. Over consumption of carbohydrate and fat could lead to functional disorder symptoms such as obesity and heart attack. However, limitation of some nutrients such as protein is unnecessary for some consumers. In athletes especially weight trainers and body builder, protein intakes have to be in high enough for their requirements.

Protein bar is a snack which is convenience for consumers to carry-on and to eat everywhere and every time as they need. Their ingredients mostly are wheat flour, grain, nut, cereal, flavors and protein sources. The protein sources of protein bar generally are whey protein, milk protein, soy protein and egg. Nutrient compositions of protein bar are mostly high amount in protein and fiber and less amounts in carbohydrate and fat. This snack is packed in many kinds of plastic bags and shelf stable in room temperature. However, most of protein bars is imported from abroad. Prices of this snack is too high for Thai consumers.

According to this imported snack is recently the one of popular snack in Thailand, development and formulation of protein bar product is interesting. However, characteristic of protein bar developed in Thailand should be different from the imported product. Thus, the objective of this research was to develop a homemade brownie fortified with protein as a commercial product in Thailand. It is better to produce a protein bar that suit for everyone, not just for weight trainers or athletes. Additionally, to improve and develop the taste, texture and odor of a protein bar product that suit to Thai consumer's preferences. However, this product can be made from local material, so this would cost the lower price. Therefore, the consumer can make an easier decision.

1.2 Objective

Main objective

To develop a homemade brownie fortified with protein.

Specific objectives

1) To increase protein contents in brownie
2) To study effect of emulsifier types on qualities of brownie fortified with protein
3) To study effect of glucose syrup and milk for chocolate-covered on qualities of brownie fortified with protein
4) To study on effect of packaged conditions during storage

1.3 Expected Benefits

1) To understand the process of baking brownie fortified with protein
2) To understand the effect of protein types on the quality of product
3) To know the acceptance recipe of brownie fortified with protein
4) To understand effect of emulsifier types on the quality of product
5) To understand how to choose the packaging that suit to product
6) To understand the method of product development

Chapter 2

Review Literature

2.1 What is protein bar?

Protein bar is a snack bar with high protein content. It usually is suitable for consumers who concern about their body or who would like to build a body muscle. Currently, protein bar product has a variety choice. Each of them contains different amount of nutrients and nutritional values but it mainly contain high protein. By the way, Thailand has only few brands of imported protein bar such as Promax, MusclePharm and Quest Bar.

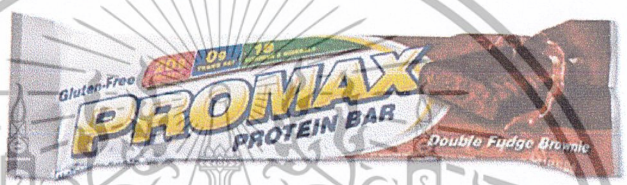

Figure 2.1 Promax Nutriton Corporation's protein bar

Reference: (http://shop.promaxnutrition.com/Double-Fudge-Brownie)

Figure 2.2 MusclePharm's Protein bar

Reference: (http://limitlesssupplements.co.nz/musclepharm-combat-protein-bars)

ภาพที่ 3 Quest Nutrition's Protein bar

Reference: (http://desonaquest.weebly.com/quest-bar-reviews.html)

2.2 Main Ingredient of Protein Bar

2.2.1 Whey protein is a milk protein that separated as a by-product of cheese production. Whey protein is high in nutritional value and essential amino acid. Moreover, it would be immediately absorbed in small intestine, especially Beta-Lacto globulin which is a composition of whey protein. It is not same as the other protein that should be digested by enzyme from pancreas. Furthermore, Whey protein contains high cysteine which is a substrate of glutathione synthesis. There are three types of whey protein which are whey protein concentrate, whey protein isolate and whey protein hydrolyzed.

2.2.1.1 Whey Protein Concentrate contains 30-89% of whey protein by weight. It depends on filtration and other process to separate an unwanted component such as lactose and fat.

2.2.1.2 Whey Protein Isolate is derived from a cross-flow microfiltration of Whey Protein Concentrate powder in order to separate all of lactose and fat. It contains greater than 90% of whey protein by weight.

2.2.1.3 Whey Protein Hydrolyzed is a hydrolyzation of Whey Protein Concentrate or Whey Protein Isolate to reduce a molecule size of whey protein. Peptide is a short chain of amino acid in protein and can be easily absorbed.

2.2.2 Milk protein isolate is manufactured by protein precipitation in low-fat milk. It contains about 80% of casein and 20% of whey protein. It also contains high levels of milk calcium, phosphorous, potassium, and magnesium.

2.2.3 Calcium caseinateis derived from casein in milk. It is produced from neutralization of casein by added sodium hydroxide. Caseinate is role as an emulsifier and bulking agent. It is widely used in food industry such as production of cheese, non-dairy creamer, cream soup and protein supplement.

2.2.4 Soy protein isolate is extracted from soy protein. It contains protein more than 90%. In food industry, it acts as an emulsifier and stabilizing agent. Also, it can be carbohydrate replacer in bakery product.

2.2.5 Natural pre-biotic fiber syrup is a sweetener and generally used in healthy diets. It provides good digestive health because it can activate the stimulation and the growth of pro-biotic microbiology.

2.3 Food additives

2.3.1 Soy Lecithin

Soy lecithin is derived from the soybean oil. Phospholipids is the main component of lecithin. Its molecular structure could be interacted with water and oil molecule. A hydrophilic part attract to water molecules, while a hydrophobic part attract to lipids. Due to its properties, it could acts as an emulsifier or stabilizer in various foods.

2.3.2 Poly-dextrose

Poly-dextros is a polymer of glucose. It is a white amorphous powder, and soluble in water. It probably is a bulking agent and could replace part of sugars and fats in food. Additionally, it could be used as a humectants, thickener, stabilizer, cryoprotectant and emulsifier (Helen, 1996).

2.4 Packaging

2.4.1 Glass container

Glass is made from sand, soda ash and limestone. In the process, wastes of glass are added to reduce a melting point and save the energy during the manufacture.

Glass is hard and resist of compression. Apart from being transparent, it is inexpensive to make, easy to shape when it is molten, reasonably resistant to heat when it is set, chemically, and it can be recycled any number of times. Furthermore, it can against permeation of vapor, gas and water.

2.4.2 Paper

Paper and paperboard are widely used in food packaging. Paper is made from fiber of plant. Since it can resist the compression pressure, it is commonly used for aggregate a pack of product. It is also inexpensive and able to be recycled.

Figure 2.4 Paper packaging
Reference: (http://www.packagingoftheworld.com/2013/10/alliance-bakery-student-project.html)

2.4.3 Metal

There is two main types of metal that are commonly used for food packaging are iron and aluminum. It is a heat stable and high pressure resistance. On the other side, it tends to rust when in a high moisture content. For the aluminum foil, it is usually used with pharmaceutical product. It can be fused with other material such as plastics, in order to improve vapor and gas barrier properties.

2.4.4 Plastics

Plastics or Polymers are formed by heat and pressure. In the manufacture, there are many kinds of material such as Low Density Polyethylene, High Density Polyethylene, Polypropylene, Polystyrene, Polyvinyl Chloride, Nylon and Polyester.

2.4.4.1 Low Density Polyethylene (LDPE)

Properties of LDPE are transparent, flexibility, sealable, and easy to form but low in gas barrier. It can be formed with many process e.g. blow film formation, cast film, extrusion coating, injection molding and blow molding. It is usually used for food packaging.

2.4.4.2 High Density Polyethylene (HDPE)

HDPE is stronger than standard polyethylene, acts as an effective barrier against moisture and remains solid at room temperature. It is typically found in milk jugs, plastic bags and refillable plastic bottles.

2.4.4.3 Polypropylene (PP)

Polypropylene is transparent, heat stable and it has high melting point. It can be found in food storage container, plastic bottle and jugs. Moreover, its moisture barrier properties are better than polyethylene.

2.4.4.4 Polystyrene (PS)

Polystyrene is transparent as glass. It has no flavor and odor. It is not able to use with high temperature and not good in gas and moisture barrier properties.

2.4.4.5 Polyvinyl Chloride (PVC)

The essential materials for polyvinyl chloride are derived from oil and salt. Polyvinyl Chloride is tough and transparent. It is sealable and can be made as refillable plastic bottles, thermoformed tray, and flexible films. However, it is better to use PET instead because PVC can cause a toxin when it is combusted.

2.4.4.6 Nylon

Nylon is a commercial name of Polyamide. It is used in the production of film and fiber, but is also available as a molding compound. The majority of nylons tend to be semi-crystalline and very tough materials with good thermal and chemical resistance. Nylons can be used in high temperature

environments. Heat stabilized systems allow sustained performance at temperatures up to 185°C.

2.4.4.7 Polyester

Polyester is derived from Polyethylene Terephthalate (PET). It combines excellent mechanical, electrical and thermal properties with very good chemical resistance and dimensional stability. Polyesters also offer low moisture absorption and have good flow properties.

2.4.5 Retort pouch packaging

A retort pouch is a heat resistant bag made of laminated plastic films or foil. It generally consists of an outer layer of polyester or nylon for printability and toughness, a middle aluminum foil layer that functions as the principal oxygen and water vapor barrier and an inner heat sealed polypropylene (Varalakshmi and Prince Devadason et al, 2014). There are many types of retort pouch such as four side sealed pouch, three side sealed pouch and stand up pouch. The advantages are reduced shipping costs and storage space for the empty containers. The pouch also has good shelf appeal and a growing acceptance by consumers

2.5 Packaging Technology

2.5.4 Retort Pouch Processing

Food is firstly prepared and sealed into the retort pouch. The pouch is then heated to 240-250°F for several minutes under high pressure inside a retort or autoclave machine. This process reliably kills all commonly occurring microorganisms and preventing from spoiling.

2.5.5 Aseptic packaging

Packaging materials are sterilized by various methods to kill microorganisms contained in the packages during forming and transport through the machine prior to filling under aseptic system (Ansari and Datta, 2003).

2.5.6 Vacuum Packing

Vacuum packing is a method that removes oxygen out of the package before sealing. Product will be under anaerobic condition. It is normally used for extend shelf-life of product.

2.5.7 Modified Atmosphere Packaging (MAP)

Modified Atmosphere Packaging is a sealed package which contains a mixture of natural gases. Oxygen, Carbon dioxide and Nitrogen are commonly used. This kind of package has ability to extend shelf-life of food product (Nick, 1994). By the way, low amount of oxygen inhibits the growth of aerobic microorganism and could slow down the rate of oxidation reaction.

Carbon dioxide also inhibits the growth of mold and aerobic microorganism. However, the efficiency of microorganism growth inhibition depends on component and ingredients in good. It will be absorbed in food with high moisture content. Moreover, the high concentration of carbon dioxide might cause spoilage in fruit and vegetables.

Nitrogen is often used for replace the oxygen in packaging. It extends shelf-life of food and prevents shrinkage of packaging.

2.5.8 Active Packaging

Active packaging is known as an oxygen scavenger or oxygen absorber. It can be defined as a mode of packaging that can prolong the shelf life and maintain the quality of food (Anita and Priyanka, 2014). Oxygen absorber effects in lowering oxidation rates due to a lower oxygen concentration (Berenzon and Sam, 1997).

Chapter 3

Materials and Methods

3.1 Ingredients and chemicals

3.1.1 Ingredients

Whey protein concentrate	Vicchi Enterprise Co.,Ltd
Soy protein isolate	Thai Food and Chemical Co.,Ltd
Whole egg powder	Thai Food and Chemical Co.,Ltd
All purpose flour (Royal fan)	United Flour Mill Public Co., Ltd
Sugar	Mitr Phol Sugar Co., Ltd.
Lin Sugar half-calorie	Baanrai Sugar Industry Co., Ltd.
Semi-sweet chocolate chips	Hershey (Thailand) Co.,Ltd
Dutch Cocoa powder	Berli Jucker Co., Ltd.
Allowrie Pure Creamery Salted Butter	KCG Corporation Co., Ltd
Vanilla extract	Jr F&b Co., Ltd
Dark chocolate compound	PT. Freyabadi Indotama
Glucose syrup	Nakornluang Glucose Co., Ltd
Milk	Meiji Holdings Co., Ltd
Poly-dextrose	Tate and Lyle
Soy lecithin	Bronson and Jacobs International

3.1.2 Chemicals

Sulfuric acid	RLC Labscan Limited, Thailand
Boric acid 2%	Carlo ERBA Reagent SpA., Rodona
Hydrochloric solution 37%	RLC Labscan Limited, Thailand
Sodium hydroxide 40%	Carlo ERBA Reagent SpA., Rodona
Catalyst: copper sulphate and potassium sulphate	Carlo ERBA Reagent SpA., Rodona
Methyl red	Carlo ERBA Reagent SpA., Rodona
Methylene blue	Qualigens Fine Chemicals Pvt. Ltd., India

3.2 Equipment

Analytical balance	Bangkok Advanced Technology Co., Ltd
Food mixer	Nesco Group Co.,Ltd

Oven	SEVERIN Elektrogeräte GmbH
Texture analyzer TA-XT2i	Charpa Techcenter Co., Ltd
Water activity meter	AquaLab Series 3TE
Kjeldahl apparatus	Kjeldahl Model 530GAK, Scientific

3.3 Methods

3.3.1 The basic recipe and cooking directions

Brownie was made in a homemade cooking style. Recipe of homemade brownie was basically composed of all-purpose flour, sugar, cocoa powder, chocolate chip, butter and egg. The brownie recipe was modified from Pailin, 2016. Ratio of ingredients is presented in **Table 3.1**. Amount of nutrients calculated by using food composition data base is presented in **Table 3.2**. Brownie cooking was mainly divided into 2 steps that were batter preparation and baking. Detailed instructions of each step are presented in **Figure 3.1**.

Table 3.1 Recipe of homemade brownie

Ingredients	Percent by weight
All purpose flour	13
Sugar	11
Cocoa powder	10
Chocolate chip	10
Butter	33
Egg	23

Table 3.2 Nutrients of basic brownie

Nutrients	Ingredients	Weight (g)	Percent
Carbohydrate	All purpose flour	46.4	32
	Sugar	50	
	Cocoa powder	22.5	
	Semisweet chocolate chip	26.7	
Fat	Butter	160	44
	Semisweet chocolate chip	10.7	
	Cocoa powder	8.4	
	Eggs fat	21.6	

Protein	Eggs protein	35.6	9.1
	All purpose flour	5.9	
	Cocoa powder	11.3	
Other	Water	67.9	14.9

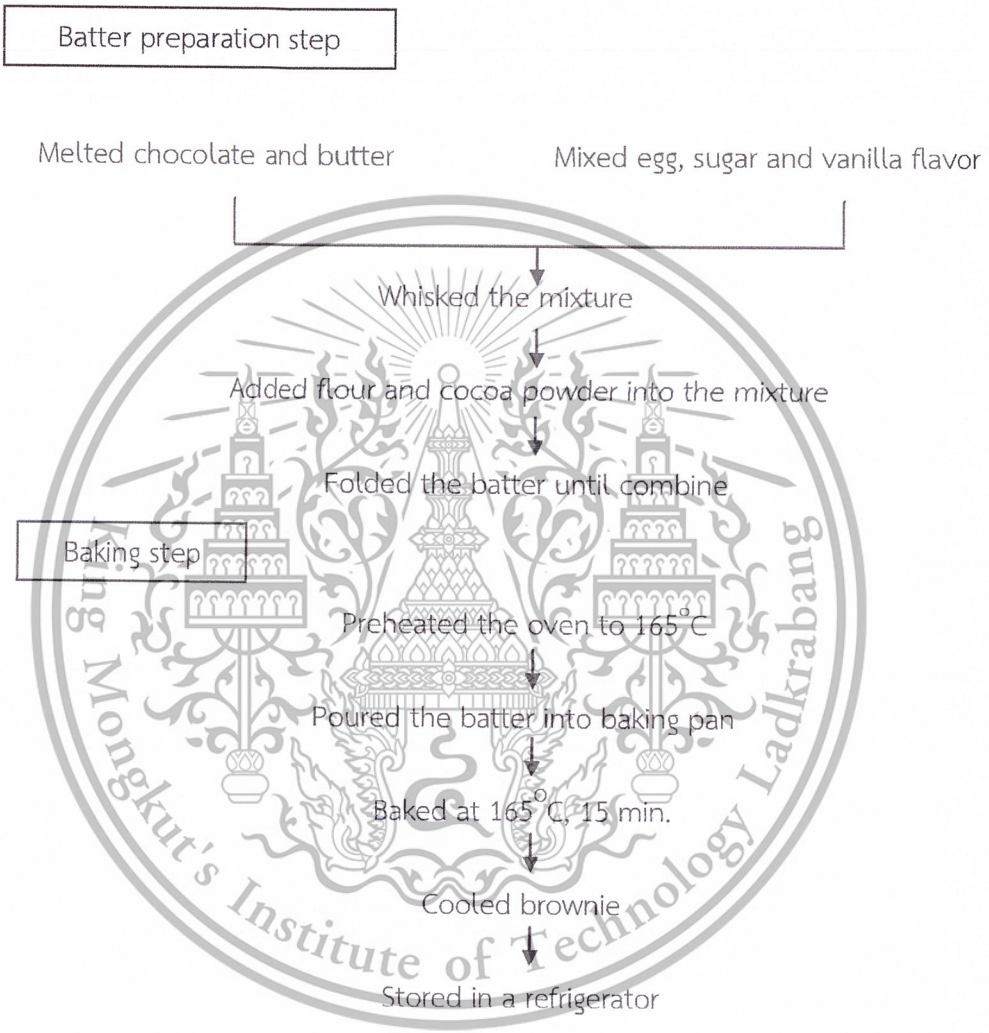

Figure 3.1 Instruction of homemade brownie cooking
(Ref: Modified from Pailin, 2016)

3.3.2 Increase of protein contents in low carbohydrate brownie

In order to increase protein content in brownie, ratio of carbohydrate calculated by summary of carbohydrate contents in all ingredients was partially replaced by protein. Whey protein, whole egg powder and soy protein isolate were used for protein sources. Proportion of whey protein, whole egg powder and soy protein were formulated by mixture design (Program Design Expert version 7.0) corresponding to approximately 25% protein in the final product. All protein formulations are presented in **Appendix A.** The selected protein formulations are presented in **Table 3.3.**

Table 3.3 Formulation of whey protein, whole egg powder and soy protein

Formulation	Percent by weight		
	Whey Protein Concentrate	Whole Egg Protein Powder	Soy Protein Isolate
1	16.6	4.2	4.2
2	4.2	16.6	4.2
3	4.2	4.2	16.6

A homemade brownie fortified with protein was prepared as in **Figure 1** with some modifications. Proteins were fortified in the batter preparation step. Baking of the brownie fortified with protein is presented in **Figure 2.**

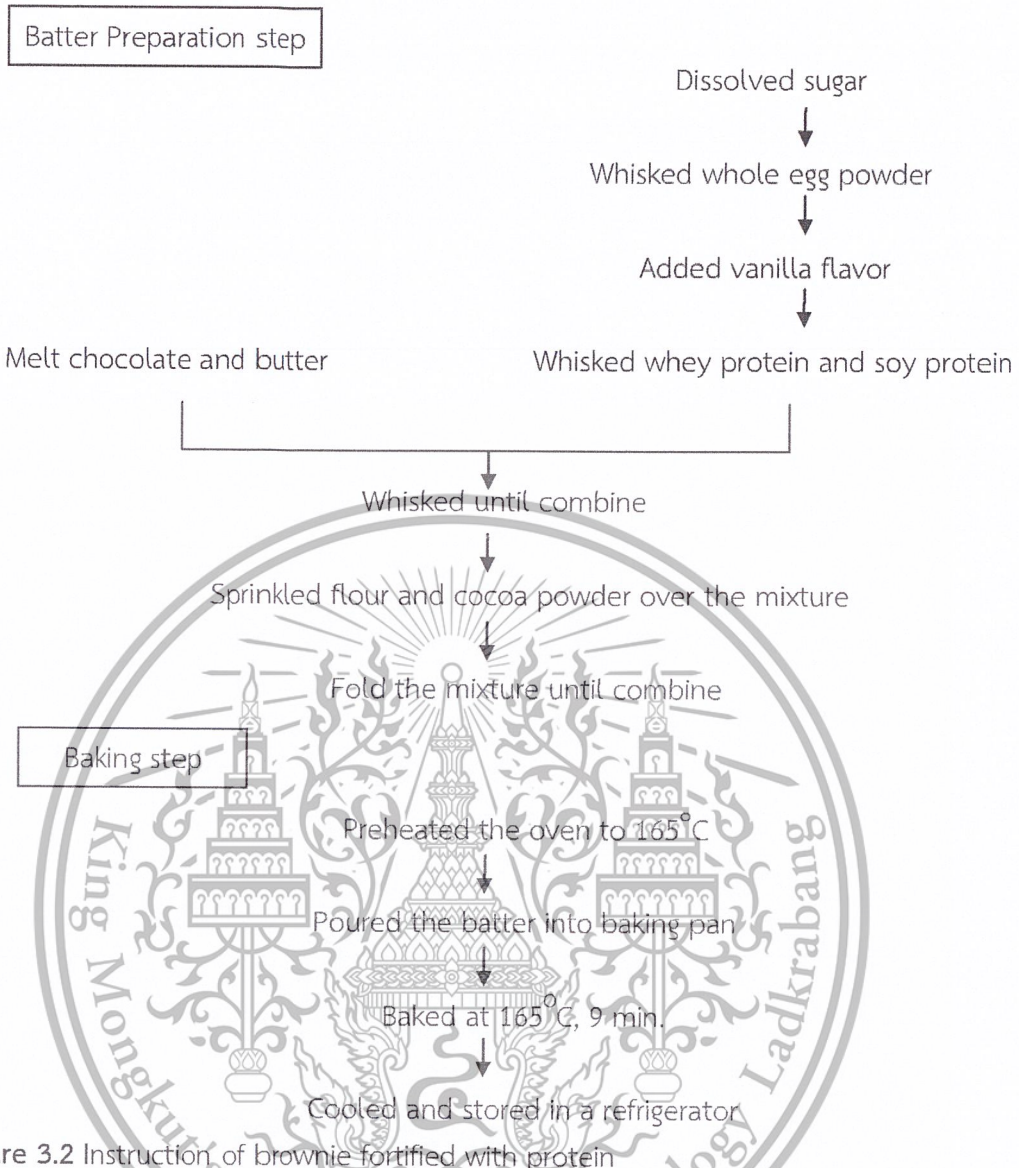

Figure 3.2 Instruction of brownie fortified with protein

Sensory acceptance of brownie fortified with protein was evaluated by using Just About Right test (JAR) in texture, odor and flavor attributes in 10 untrained panelists. The JAR test was analyzed by firstly input data into table and compute as a percentage of each attribute. Set the criteria of just about right in 70%. If the just right score of each attribute is greater than or equal to 70%, it means should not improve that attribute. The JAR test sample is presented in **Appendix B**

3.3.3 Using emulsifiers to improve qualities of brownie fortified protein

Poly-dextrose and soy lecithin were added as emulsifiers into brownie fortified with protein selected from the previous experimentation (3.3.2). Proportion of poly-dextrose and soy lecithin was formulated by using mixture design in order to equivalent 2% by weight of brownie. All emulsifier formulations are presented in

Appendix C. The selected emulsifier formulations are shown in **Table 3.4.** Poly-dextrose was dissolved in water before adding sugar. Lecithin was added before sprinkle the mixture of cocoa powder and flour.

Table 3.4 Formulation of Soy lecithin and Poly-dextrose

Formulation	Percent by weight	
	Soy Lecithin	Poly-dextrose
1	0.25	1.75
2	0.50	1.50
3	1.000	1.000
4	1.25	0.75
5	1.50	0.50
6	1.75	0.25

A two-level factorial design was used for water activity and texture analysis measurements. The experiments were determined in triplicate. Sensory acceptances of the brownie were evaluated by using Just about right test in texture, odor and flavor attributes in 10 untrained panelists. Data analysis was similar to 3.3.2.

3.3.4 Using glucose syrup and milk for chocolate-covered in brownie fortified with protein

A brownie fortified with protein recipe received from the experiment in 3.3.3 was dipped by mixture of chocolate, milk and glucose syrup. Amount of chocolate was fixed at 70% by weight of covered materials. Proportion of milk and glucose syrup was formulated by mixture design in order to equivalent in 30% by weight of covered mixture. All milk and glucose formulations are presented in **Appendix D.** The selected milk and glucose formulations are shown in **Table 3.5**.

Table 3.5 Formulations of Glucose syrup and Milk

Formulation	Percent by weight	
	Glucose syrup	Milk
1	7.5	22.5
2	10.0	20
3	15	15

In order to prepare the chocolate-covered, milk and glucose syrup were firstly heated. After that, mixture of milk and glucose syrup was poured into a chocolate bowl and stirred immediately until it completely combined. Brownie fortified with protein was dipped into the mixture and then cooled on the cooling stack at room temperature. The covered brownie was stored in a refrigerator before qualities determinations.

Texture and water activity of chocolate-covered brownie were determined in triplicate. The experiment was performed in a two-level factorial design. Sensory acceptance of chocolate-covered brownie was evaluated by 7-point hedonic scale in 5 attributes (appearance, flavor, texture, odor, and overall appearance) in 30 untrained panelists. The hedonic scale test is presented in **Appendix E**.

Protein determination of the final product was analyzed by Kjeldahl method to confirm that brownie could be claimed as a fortified product. Kjeldahl method is shown in **Appendix F**.

3.3.5 Study on effect of packaged conditions during storage

Brownie fortified with protein prepared as a selected method from 3.3.4 was stored in 3 types of packaged condition. The packages were plastic box, laminate zipper bag included with oxygen absorber and plastic bag packed in vacuum condition. Samples were stored in a refrigerator (4°C). Texture and water activity of the brownie were determined at day 0, 3, 5 and 7 of storage. Yeast and mold of the brownie were checked at day 7 of storage. The experiments were performed in a two-level factorial design in duplicate.

Chapter 4

Results and Discussion

4.1 Effect of protein types on sensory acceptance of brownie

In order to increase protein content in brownie from 6% to 25%, whey protein concentrate (WPC), whole egg powder (WEP) and soy protein isolate (SPI) were carried out for brownie production. Proportions of each protein sources were formulated and selected by mixture design (**Table 3.3**). Sensory acceptances of the brownie were evaluated by using Just About Right Test (JAR). The results shown that protein types resulted in sensory acceptance scores (**Table 4.1**).

As explain in the materials and method section, the criteria of just about right was set at 70%. Score of using high amount of WEP and SPI was less than 70% in all sensory attributes suggesting significant affect of WEP and SPI on sensory qualities of the brownie. High amounts of WEP lead to too much scores in odor attribute, while high amounts of SPI caused of too much scores in odor, flavor and texture of brownie. Those sensory acceptances may because specific smell of soy bean and egg which are unlike in some people. In addition, hard texture of samples was obtained in high amount of SPI. On the other hand, whey is normally separated from milk, its smell is similar to original milk recognized by general consumer. Therefore, using high amount of WPC provided greater than 70% of scores in the just right level in odor and flavor attributes. However, scores in the texture attribute was less than 70% in the just right level and high score in the too little level. Too much amount of WPC caused in sandy and loosely texture. Therefore, addition of 16.6% WPC, 4.2% WEP and 4.2% SPI was selected for further experiments to improve the texture characteristics of brownie.

Table 4.1 Just About Right Score of brownie fortified with protein

WPC:WEP:SPI	Sensory score by percent								
	Odor			Flavor			Texture		
	Too little	Just right	Too much	Too little	Just right	Too much	Too little	Just right	Too much
16.6: 4.2: 4.2	0	80	20	10	80	10	80	20	0
4.2: 16.6: 4.2	0	20	80	50	10	40	60	30	10
4.2: 4.2: 16.6	0	10	90	20	10	70	0	20	80

WPC=Whey Protein Concentrate, WEP=Whole Egg Powder, SPI=Soy Protein Isolate; n = 10

4.2 Effect of emulsifier types on qualities of brownie fortified with protein

According to the previous experiments, the texture of brownie fortified with protein was less accepted by sensory acceptance tests. Therefore, emulsifiers (2% by weight) included soy lecithin and poly-dextrose were used to improve texture characteristics of brownie fortified with protein. The results shown that texture characteristics of samples were improved by using of soy lecithin and poly-dextrose (**Table 4.2**). The decrease of hardness and springiness were caused by increase of soy lecithin level and decrease of poly-dextrose level. Soy lecithin is an emulsifier which is an ampiphilic structure. It could be bound with water and lipid molecules in brownie to form spring and soft texture. For poly-dextrose, it is a bulking agent characterized as a polysaccharide. It could absorb free water in the product to from a stiff gel after heating. Therefore, the hardness of brownie was increased when increase of poly-dextrose amounts. On the other hand, level of emulsifier types does not affect the water activity (Aw).

Table 4.2 Texture characteristics and Aw of brownie fortified with protein

Soy lecithin : Poly-dextrose (%)	Texture characteristics		Aw^{ns}
	Hardness	Springiness	
0.00:0.00 (control)	44.61^{c}	33.63±3.43^{b}	0.887±0.00
0.25:1.75	21.07^{ab}	27.96±4.62^{ab}	0.883±0.01
0.5:1.50	26.82^{ab}	33.82±5.76^{b}	0.859±0.04
1.0:1.0	32.23^{bc}	30.18±2.25^{b}	0.856±0.02
1.25:0.75	16.09^{a}	23.01±1.36^{a}	0.868±0.02
1.50:0.5	14.20^{a}	22.38±0.70^{a}	0.864±0.00
1.75:0.25	14.27^{a}	23.13±2.94^{a}	0.847±0.04

Means having different letters in a column are significantly different ($P \leq 0.05$).

ns not significantly different ($P > 0.05$). Mean±SD; n =3

For sensory evaluation, emulsifiers could improve the texture of brownie fortified with protein. It was found that amounts of soy lecithin and poly-dextrose resulted in sensory acceptance (**Table 4.3**). Texture scores of Just About Right test after addition of emulsifies were higher than the previous experimentation. The

criteria of just about right was set at 70%. The highest score of just right in odor (70%), flavor (90%) and texture (80%) was using soy lecithin and poly-dextrose at 0.5% and 1.5% respectively. However, the other formulation was less than 70% of just right score. In additon, added high amount of soy-lecithin affected the soy odor and sticky texture. More hardness was caused by an increase amount of poly-dextrose. Therefore, addition of 0.5% of Soy lecithin and 1.5% of poly-dextrose was chosen as an appropriate formulation for further study.

Table 4.3 Effect of soy lecithin and poly-dextrose on sensory qualities of brownie fortified with protein

Soy Lecithin : Poly-dextrose	Sensory score by percent								
	Odor			Flavor			Texture		
	Too little	Just right	Too much	Too little	Just right	Too much	Too little	Just right	Too much
0.25:1.75	30	40	30	30	50	20	10	70	20
0.5:1.50	10	70	20	0	90	0	10	80	10
1.0:1.0	50	50	0	50	40	10	40	40	20
1.25:0.75	30	60	10	40	60	0	40	30	30
1.50:0.5	30	40	30	20	50	30	50	20	30
1.75:0.25	20	30	50	30	40	30	0	60	40

Just about right scale; n= 10

4.3 Effect of covered ingredients on qualities of brownie fortified with protein

In order to bake a brownie fortified with protein and covered with chocolate mixture, the selected formulation of brownie fortified with protein and added emulsifier was used. The mixture of chocolate-covered was composed of 70% of chocolate and varied glucose syrup and milk within 30% by weight. The result show that varying of glucose syrup and milk affect the hardness of chocolate-covered (**Table 4.4**). As glucose syrup level increased and milk level decreased, the hardness significantly decreased. On the other side, the proportion of glucose syrup and milk did not affect the water activity (Aw) of chocolate-covered. Sensory acceptances of brownie fortified with protein and covered with chocolate were evaluated by using 7-hedonic scale sensory test. It was shown that glucose syrup and milk affect the sensory acceptance scores (**Table 4.5**). The highest score in

flavor and overall acceptance was obtained in using 15% of glucose syrup and 15% of milk. Odor, glossiness and appearance were not significantly different. Thus, 15% of glucose syrup and 15% of milk were selected as a final product.

Table 4.4 Effect of glucose syrup and milk on hardness and water activity (Aw) of chocolate-covered brownie fortified with protein

Glucose syrup : Milk	Hardness(N)	Aw^{ns}
0	3.53±0.2^{b}	0.872±0.03
7.5:22.5	2.89±0.5^{ab}	0.859±0.01
10:20	1.93±0.9^{a}	0.857±0.01
15:15	1.90±0.7^{a}	0.855±0.01

ns is not significantly different (P > 0.05). Mean±SD; n =3

Table 4.5 Effect of glucose syrup and milk on sensory acceptances of chocolate-covered brownie fortified with protein

Glucose syrup : Milk	Sensory Score				
	Odor^{ns}	Flavor	Glossiness^{ns}	Appearance^{ns}	Overall acceptance
7.5:22.5	5.03±0.95	4.52±0.97^{a}	5.51±1.03	5.24±1.15	4.88±0.89^{a}
10:20	5.33±0.82	4.97±0.95^{b}	5.30±0.95	5.03±1.05	5.15±0.83^{a}
15:15	5.24±1.06	5.55±1.09^{c}	5.33±0.96	5.30±0.95	5.82±0.95^{b}

7-point hedonic scale (1 = extremely dislike; 7 = extremely like)

Means having different letters in a column are significantly different (P ≤ 0.05).

ns is not significantly different (P > 0.05). Mean±SD

4.4 Effect of packaging conditions during storage

The latest formulation of brownie fortified with protein and covered with chocolate selected from the previous section was used. Results in **Table 4.7**, **Table 4.8** and **Table 4.9** are shown that conditions of packaging affect hardness, springiness and water activity (Aw) during the storage. Hardness of the sample stored in plastic box is harder than the sample vacuumed in plastic bag at day 3, 5 and 7 of storage. Springiness of sample vacuumed in plastic bag was significantly different. Water activity (Aw) of sample stored in plastic box was significantly decreased at day 7 of storage. The results of yeast and mold determination were less than 10cfu/g (Appendix G). Thus, brownie fortified with protein and covered with chocolate could be stored at chilled temperature up to 7 days storage.

Table 4.6 Effect of packaging condition on hardness of chocolate-covered brownie fortified with protein

Day of storage	Packaging conditions		
	Plastic box	Laminate zipper bag including oxygen absorber	Vacuum plastic bag
0	21.45±3.43	21.45±3.43	21.45±3.43
3	28.79±3.59^{b}	21.98±2.21^{a}	20.35±2.79^{a}
5	34.55±2.46^{b}	26.27±2.56^{ab}	24.69±3.88^{a}
7	39.25±4.17^{b}	31.53±6.10^{ab}	24.88±2.11^{a}

Means having different letters in a row are significantly different ($P \leq 0.05$). Mean±SD; n=2

Table 4.7 Effect of packaging condition on springiness of chocolate-covered brownie fortified with protein

Day of storage	Packaging conditions		
	Plastic box	Laminate zipper bag including oxygen absorber	Vacuum plastic bag
0	24.42±6.17	24.42±6.17	24.42±6.17
3^{ns}	17.87±10.30	21.64±11.20	26.53±9.16
5^{ns}	25.11±2.05	25.23±6.08	26.00±2.79
7	29.22±3.00^{b}	31.12±0.68^{b}	26.06±1.21^{a}

Means having different letters in a row are significantly different ($P \leq 0.05$). Mean±SD; n=2

Table 4.8 Effect of packaging condition on water activity (Aw) of chocolate-covered brownie fortified with protein

Day of storage	Packaging conditions		
	Plastic box	Laminate zipper bag including oxygen absorber	Vacuum plastic bag
0	0.863	0.863	0.863
3 ^{ns}	0.871±0.01	0.871±0.01	0.870±0.01
5 ^{ns}	0.851±0.02	0.867±0.00	0.862±0.00
7	0.833±0.01^{b}	0.864±0.00^{a}	0.864±0.01^{a}

Means having different letters in a row are significantly different ($P \leq 0.05$). Mean±SD; n=2

4.5 Characteristics of brownie fortified with protein

4.5.1 Formulation

The main ingredients of brownie are composed of all propose flour, sugar, chocolate and butter. Whey protein concentrate, whole egg protein powder and soy protein isolate were used to fortified brownie. Addition of soy lecithin and poly-dextrose were used for improve the texture attributes. For the chocolate-covered, it is composed of chocolate, milk and glucose syrup. The final formulation of brownie fortified with protein shown in **Table 4.9**

Table 4.9 The final formulation of brownie fortified with protein

Brownie fortified with protein (Inner layer)	
Ingredients	Percent by weight
All propose flour	11
Sugar	9
Cocoa powder	8
Chocolate chip	8
Butter	25
Water	17
Whey protein concentrate	13
Whole egg protein powder	4
Soy protein isolate	3

Soy lecithin	0.5
Poly-dextrose	1.5
Chocolate-covered section	
Ingredients	Percent by weight
Dark Chocolate compound	70
Glucose syrup	15
Milk	15

4.5.2 Nutrient compositions

Nutrient compositions of brownie fortified with protein are approximately composed of 16% protein analyzed by Kjeldahl method, 32% carbohydrate and 44% fat calculated from the formulation. The protein content is higher than 10% of protein in basic homemade brownie which gives only 6% of protein (United States Department of Agriculture). Therefore, it can be claimed as a brownie fortified with protein if followed by Notification of the Ministry of Public Health (No. 182).

4.5.3 Safety

As describe above, the developed brownie products which is packed in plastic box, laminate zipper bag including oxygen absorber and vacuum plastic bag were safe in chilling condition for 7 days storage.

4.5.3 Cost

According to the final formulation of the brownie, costs of product depended on raw material prices. Cost of product calculated based on 100 grams of product was shown in **Table 4.10**. Originate cost of 100 grams of brownie fortified with protein was about 48 Baht. However, it is reasonable to sell this product at 50 Baht for one serving size (30 grams) (Notification of the Ministry of Public Health, 1998). That price included package cost and others for example labeling and marketing cost.

Table 4.10 Cost of brownie fortified with protein

Inner layer	Price
Ingredients	Baht per 100 grams
All propose flour	0.5
Cocoa powder	5.0
Chocolate chip	7.5
Butter	5.1
Half calorie sugar	0.5
Sugar	0.1
Whey protein concentrate	7.2
Egg protein powder	1.2
Soy protein isolate	0.8
Chocolate-covered	Price
Ingredients	Baht per 100 grams
Chocolate compound	10.4
Chocolate chip	7.5
Milk (ml)	1.0
Glucose syrup	0.8
Total (Baht)	48

CHAPTER 5
CONCLUSION

5.1 Conclusion

The study of product development of brownie fortified with protein by increase protein content, added emulsifiers, improve the chocolate covered qualities and study the types of packaging. It was found that:

5.1.1 The appropriate proportion of protein fortified in brownie is composed of 16.4% WPC, 4.2% WEP and 4.2% SPI.

5.1.2 The optimized formulation of emulsifier is 0.5% soy lecithin and 1.5% poly-dextrose.

5.1.3 The accepted formulation of glucose syrup and milk mixture on quality of the chocolate-covered is 15% glucose syrup and 15% milk.

5.1.4 Brownie fortified with proteins could be stored up to 7 days in packages (plastic box, laminate zipper bag with O_2 absorber, and vacuum plastic bag) at chilled temperature.

5.1.5 Homemade brownie fortified with proteins is feasible to produce as a commercial product.

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APPENDIX

APPENDIX A

The formulation of protein mixture within 25% by weight

Whey Protein Concentrate	Whole Egg Protein	Soy Protein Isolate
12.500	12.500	0.000
12.500	0.000	12.500
0.000	0.000	25.000
16.667	4.167	4.167
25.000	0.000	0.000
8.333	8.333	8.333
4.167	4.167	16.667
0.000	25.000	0.000
0.000	12.500	12.500
12.500	12.500	0.000
25.000	0.000	0.000
0.000	25.000	0.000
0.000	0.000	25.000
4.167	16.667	4.167

APPENDIX B

Sensory Acceptance Test

Just About Right

Product: Brownie fortified with protein Date: .....

Panelist Name : .....

Please assess the codified sample and indicate your opinion, based on the scale below.

Sample 351

Attributes	Too little	Just right	Too much
Odor			
Flavor			
Texture			

Sample 847

Attributes	Too little	Just right	Too much
Odor			
Flavor			
Texture			

Sample 233

Attributes	Too little	Just right	Too much
Odor			
Flavor			
Texture			

Other

.....

.....

.....

Thank you

APPENDIX C

The formation of Lecithin and Poly-dextrose within 2% by weight

Soy Lecithin	Poly-dextrose
1.000	1.000
2.000	0.000
0.000	2.000
1.244	0.756
0.504	1.496
0.252	1.748
1.743	0.257
1.496	0.504

APPENDIX D

The formation of glucose syrup and milk mixture

Glucose syrup	Milk
15	15
7.5	22.5
20	10
30	0
0	30
10	20
22.5	7.5

APPENDIX E

Sensory Acceptance Test

7-Point Hedonic-Scale

Product: Brownie fortified with protein and covered with chocolate

Date: .....

Panelist Name: .....

Please assess the codified sample and indicate, based on the scale below, whether you liked or did not like each attributes.

1 = disliked extremely 2 = disliked moderately 3 = disliked slightly

4 = neither liked nor disliked 5 = liked slightly 6 = liked moderately 7 = liked extremely

Attributes	Samples		
	653	988	131
Odor			
Flavor			
Glossiness			
Appearance			
Overall appearance			

Other

.....

.....

.....

.....

.....

Thank you

APPENDIX F

Kjeldahl Method (AOAC, 2000)

Procedure:

Digestion

1. Weigh approximately 2.5 g ground sample into digestion flask.
2. Add 10 g catalyst. Then add 25 mL sulfuric acid.
3. Place flask on the digestion rack and start digestion at 380°C, 45-1 hr until solution change into blue color.
4. Cooling the solution to room temperature.

Distillation

1. Prepare Erlenmeyer flask by adding 60 mL boric acid. Then drop a drop of methylene blue and methyl red.
2. Place both digestion flask and Erlenmeyer flask into the distillation apparatus.
3. Start digestion and remove the flask after finish.

Titration

1. Erlenmeyer flask was titrated with 0.01N HCl solution to pink endpoint. Record volume and calculate by using the formula below.

$$\text{Nitrogen in sample (\%)} = \frac{(A-B) \times N \times 14 \times 100}{W \times 1000}$$

$$\text{Protein (\%)} = \%\text{Nitrogen in sample} \times 6.25$$

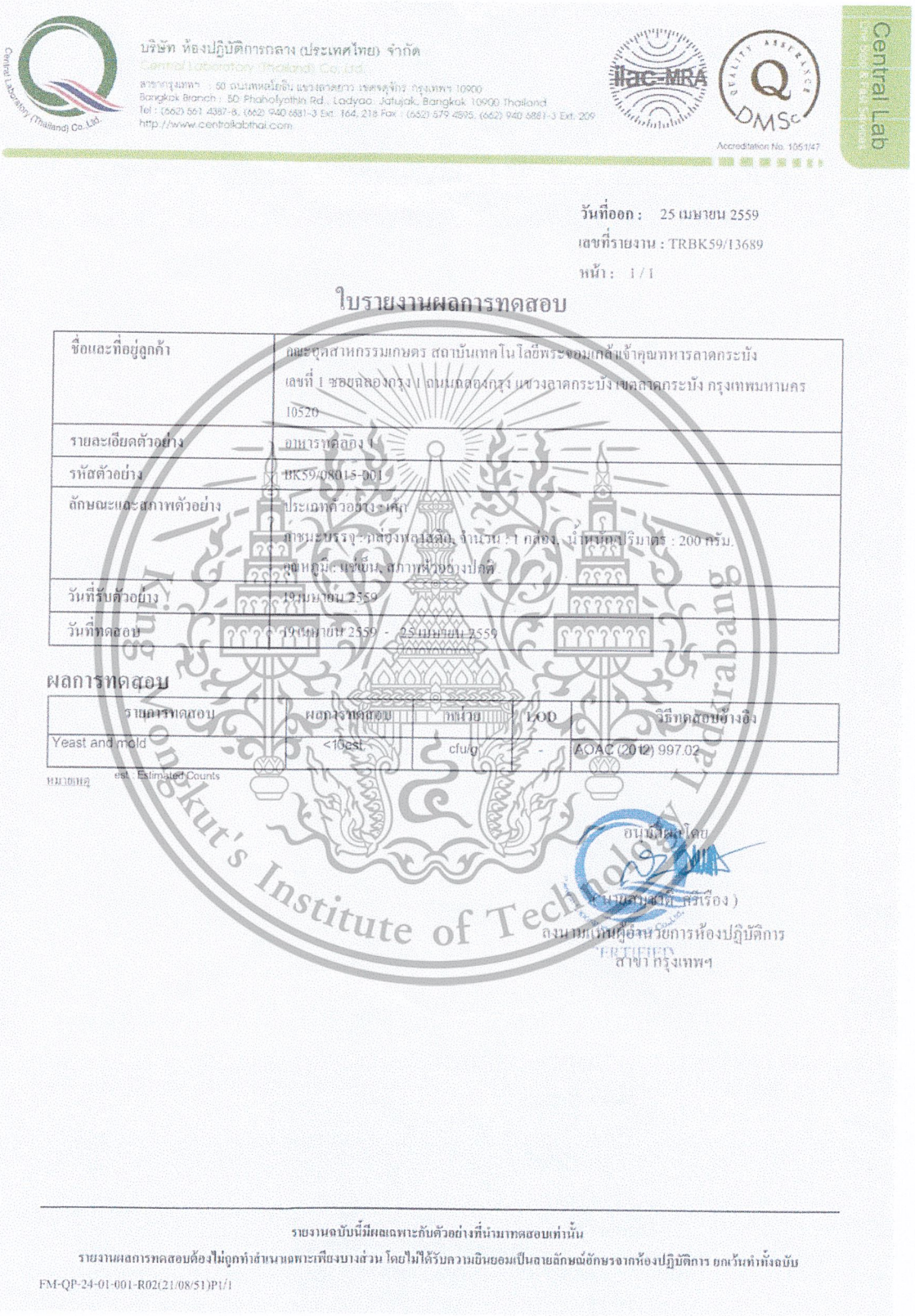
A ; Volume of HCl used in titration (ml)

B ; Volume of HCl used in blank titration (ml)

N ; Normality of standard acid(N)

W ; Weight of sample (g)

APPENDIX G



บริษัท ห้องปฏิบัติการกลาง (ประเทศไทย) จำกัด
Central Laboratory (Thailand) Co., Ltd.
สาขากรุงเทพฯ : 50 ถนนพหลโยธิน แขวงลาดยาว เขตจตุจักร กรุงเทพฯ 10900
Bangkok Branch : 50 Phaholyothin Rd., Ladyao, Jatujak, Bangkok 10900 Thailand
Tel : (662) 561 4387-8, (662) 940 6831-3 Ext. 164, 218 Fax : (662) 579 4995, (662) 940 6861-3 Ext. 209
http://www.centralabthai.com
Accreditation No. 1051/47
Central Lab

วันที่ออก : 25 เมษายน 2559
เลขที่รายงาน : TRBK59/13689
หน้า : 1 / 1

ใบรายงานผลการทดสอบ

ชื่อและที่อยู่ลูกค้า	คณะอุตสาหกรรมเกษตร สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง เลขที่ 1 ซอยฉลองกรุง 1 ถนนฉลองกรุง แขวงลาดกระบัง เขตลาดกระบัง กรุงเทพมหานคร 10520
รายละเอียดตัวอย่าง	อาหารทดลอง 1
รหัสตัวอย่าง	BK59/08015-001
ลักษณะและสภาพตัวอย่าง	ประเภทตัวอย่าง : เค้ก ลักษณะบรรจุ : กล่องพลาสติก, จำนวน : 1 กล่อง, น้ำหนักบริมาตร : 200 กรัม. ลักษณะ, ปริมาณ, สภาพห่อของปิดดี
วันที่รับตัวอย่าง	19 เมษายน 2559
วันที่ทดสอบ	19 เมษายน 2559 - 25 เมษายน 2559

ผลการทดสอบ

รายการทดสอบ	ผลการทดสอบ	หน่วย	LOD	วิธีทดสอบอ้างอิง
Yeast and mold	<10est.	cfu/g	-	AOAC (2012) 997.02

หมายเหตุ est. Estimated Counts

อนันต์ สุดโดย
(นายอนันต์ ศรีรอง)
ผู้จัดการห้องปฏิบัติการ
สาขา กรุงเทพฯ

รายงานฉบับนี้มีผลเฉพาะกับตัวอย่างที่นำมาทดสอบเท่านั้น
รายงานผลการทดสอบต้องไม่ถูกทำสำเนาเฉพาะเพียงบางส่วน โดยไม่ได้รับความยินยอมเป็นลายลักษณ์อักษรจากห้องปฏิบัติการ ยกเว้นทำทั้งฉบับ
FM-QP-24-01-001-R02(21/08/51)P1/1

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Name- Surname		Miss Nattaya Siriphetamorn
Date of Birth		5 October 1993
Education History		
2007	Junior High School	Wells International School
2009	Junior High School	Interkids Bilingual School
2010	Senior High School	Saint Joseph Convent School
2012	Enrollment Year	King Mongut's Institue of Technology Ladkrabang