ไฮโดรไลเสตจากถั่วนิ้วนางแดง *Vigna umbellata* ระยะงอก และผลของการทำแห้งแบบโฟม-แมตต่อฤทธิ์ต้านออกซิเดชัน

นายบูรฉัตร ศรีทองแท้

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR)
are the thesis authors' files submitted through the University Graduate School.

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

GERMINATED RICE BEAN *Vigna umbellata* HYDROLYSATE AND EFFECT OF FOAM-MAT DRYING ON ITS ANTIOXIDANT ACTIVITY

Mr. Burachat Sritongtae

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2015
Copyright of Chulalongkorn University

Thesis Title	GERMINATED RICE BEAN *Vigna umbellata* HYDROLYSATE AND EFFECT OF FOAM-MAT DRYING ON ITS ANTIOXIDANT ACTIVITY
By	Mr. Burachat Sritongtae
Field of Study	Food Technology
Thesis Advisor	Assistant ProfessorKiattisak Duangmal, Ph.D.
Thesis Co-Advisor	ProfessorMichael Robert Anthony Morgan, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Science
(Associate ProfessorPolkit Sangvanich, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate ProfessorKanitha Tananuwong, Ph.D.)
.....Thesis Advisor
(Assistant ProfessorKiattisak Duangmal, Ph.D.)
.....Thesis Co-Advisor
(ProfessorMichael Robert Anthony Morgan, Ph.D.)
.....Examiner
(Assistant ProfessorChaleeda Borompichaichartkul, Ph.D.)
.....Examiner
(Assistant ProfessorThanachan Mahawanich, Ph.D.)
.....External Examiner
(ProfessorSoottawat Benjakul, Ph.D.)

บูรฉัตร ศรีทองแท้ : ไฮโดรไลเสตจากถั่วนิ้วนางแดง *Vigna umbellata* ระยะงอก และผลของการทำแห้งแบบโฟม-แมตต่อฤทธิ์ต้านออกซิเดชัน (GERMINATED RICE BEAN *Vigna umbellata* HYDROLYSATE AND EFFECT OF FOAM-MAT DRYING ON ITS ANTIOXIDANT ACTIVITY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.เกียรติศักดิ์ ดวงมาลย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.โรเบิร์ต แอนโธนี มอร์แกน, 113 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการงอก การย่อยด้วยเอนไซม์ และอุณหภูมิการทำแห้งต่อสมบัติทางเคมีกายภาพ และฤทธิ์ต้านออกซิเดชันของโปรตีนไฮโดรไลเสตจากถั่วนิ้วนางแดงที่ผ่านการทำแห้งด้วยวิธีโฟม-แมต ขั้นตอนแรกศึกษาผลของระยะเวลาการเพาะ และการเตรียมถั่วด้วยกรดซิตริกเข้มข้น 1 % (w v^{-1}) ต่อองค์ประกอบทางเคมี และฤทธิ์ต้านออกซิเดชัน เมื่อนำถั่วนิ้วนางแดงมาแช่น้ำและสารละลายกรดซิตริกเข้มข้น 1 % (w v^{-1}) นาน 6 ชั่วโมง มาเพาะเป็นเวลา 0-24 ชั่วโมง พบว่าเมื่อระยะเวลาการเพาะเพิ่มขึ้น ปริมาณความชื้น ปริมาณสารประกอบฟีนอลิกทั้งหมด และปริมาณน้ำตาลรีดิวซ์ของถั่วทั้งสองชุดการทดลองมีค่าสูงขึ้นอย่างมีนัยสำคัญ ($p\leq0.05$) การเตรียมถั่วก่อนการเพาะส่งผลชัดเจนต่อการเพิ่มของปริมาณสารประกอบฟีนอลิกทั้งหมด ปริมาณโปรตีนหยาบของถั่วทั้งสองชุดการทดลองมีค่าสูงสุดที่ระยะเวลาการเพาะที่ 18 ชั่วโมง ร้อยละกำจัดอนุมูลอิสระ 1,1-diphenyl-2-picrylhydrazyl (DPPH) และค่า ferric reducing antioxidant power (FRAP) ของสารสกัดที่ได้จากการสกัดถั่วเพาะงอกทั้งสองชุดการทดลองด้วยน้ำและเอทานอล มีค่าสูงขึ้นอย่างมีนัยสำคัญ เมื่อระยะเวลาการเพาะเพิ่มขึ้น ($p\leq0.05$) ขั้นตอนที่สอง นำถั่วที่ผ่านการเพาะเป็นเวลา 18 ชั่วโมง (GRB) และถั่วที่ไม่ผ่านการเพาะ (NGRB) มาย่อยด้วย ด้วยเอนไซม์ Flavourzyme® ความเข้มข้น 5% (โดยน้ำหนักแห้งของถั่ว) โดยควบคุมปฏิกิริยาที่อุณหภูมิ 50 ºC เป็นเวลา 24 ชั่วโมง พบว่าปริมาณหมู่อะมิโนอิสระ ระดับการย่อย ปริมาณสารประกอบฟีนอลิกทั้งหมด และฤทธิ์ต้านออกซิเดชัน (กำจัดอนุมูลอิสระ DPPH และค่า FRAP) ของโปรตีนไฮโดรไลเสตที่เตรียมจาก GRB มีค่าสูงอย่างมีนัยสำคัญ เมื่อระยะเวลาการย่อยเพิ่มขึ้น ($p\leq0.05$) โดยค่าที่ได้จากโปรตีนไฮโดรไลเสตที่เตรียมจาก GRB มีค่าสูงกว่าที่เตรียมจาก NGRB ตลอดระยะเวลาการย่อย 24 ชั่วโมง ในการทดลองนี้เลือกระยะเวลาการย่อยที่ 21 ชั่วโมง เป็นวัตถุดิบในการผลิตโปรตีนไฮโดรไลเสต ขั้นตอนที่ 3 ศึกษาจลนพลศาสาตร์การอบแห้ง และแบบจำลองทางคณิตศาสตร์ของการทำแห้งโปรตีนไฮโดรไลเสตด้วยวิธีโฟม-แมต ทำแห้งโฟมโปรตีนไฮโดรไลเสตด้วยอุณหภูมิที่แตกต่างกันสองอุณหภูมิ (60 และ 70 ºC) พบว่าอัตราการอบแห้งของโปรตีนไฮโดรไลเสตที่เตรียมจาก GRB มีค่าต่ำกว่าที่เตรียมจาก NGRB เล็กน้อย แบบจำลอง Weibull distribution และ Midilli มีความเหมาะสมในการทำนายลักษณะการอบแห้งด้วยวิธีโฟม-แมตของโปรตีนไฮโดรไลเสตจากถั่วนิ้วนางแดงทั้งสองชุดการทดลอง อบแห้งตัวอย่างจนมีความชื้นสุดท้ายประมาณ 8.5% (โดยน้ำหนักแห้ง) ในการทดลองนี้ได้มีการศึกษาสมบัติทางเคมีกายภาพของโปรตีนไฮโดรไลเสตที่ผ่านการทำแห้งด้วยวิธีโฟม-แมต พบว่าการอบแห้งที่ 60 ºC ปริมาณสารประกอบฟีนอลิกทั้งหมด และปริมาณฟลาโวนอยด์ทั้งหมดของตัวอย่างแห้งที่เตรียมจากทั้ง GRB และ NGRB มีค่าสูงกว่าการอบแห้งที่ 70 ºC อย่างมีนัยสำคัญ ($p\leq0.05$) องค์ประกอบฟีนอลิกหลักที่พบในตัวอย่างที่ผ่านการทำแห้งด้วยวิธีโฟม-แมต คือ กาลิก แคทีคอล และเอพิแคเทชิน การอบแห้งที่อุณหภูมิสูงขึ้นมีผลให้ค่าร้อยละการกำจัดอนุมูลอิสระ DPPH และค่า FRAP มีค่าลดลงอย่างมีนัยสำคัญ ($p\leq0.05$) จากการทดลองพบว่าที่ระดับความเข้มข้นของโปรตีนไฮโดรไลเสตเดียวกัน ค่าร้อยละการกำจัดอนุมูลอิสระ DPPH ที่วัดด้วยวิธี electron spin resonance (ESR) มีค่าสูงกว่าการวัดด้วยวิธีการเปรียบเทียบความเข้มของสี 1.20-1.32 เท่า โปรตีนไฮโดรไลเสตที่ผ่านการทำแห้งด้วยวิธีโฟม-แมตที่เตรียมจาก GRB มีปริมาณสารประกอบฟีนอลิกทั้งหมด ปริมาณฟลาโวนอยด์ทั้งหมด และฤทธิ์ต้านออกซิเดชัน มีค่าสูงกว่าที่เตรียมจาก NGRB เพปไทด์ที่มีน้ำหนักโมเลกุลเล็ก (น้อยกว่า 3 kDa) มีฤทธิ์ต้านออกซิเดชันสูงกว่าเพปไทด์ที่มีน้ำหนักโมเลกุลใหญ่ (มากกว่า 3 kDa) องค์ประกอบหลักที่มีผลต่อฤทธิ์ต้านออกซิเดชันของโปรตีนไฮโดรไลเสตจากถั่วนิ้วนางแดงที่ผ่านการทำแห้งด้วยวิธีโฟม-แมตคือสารประกอบฟีนอลิก

ภาควิชา เทคโนโลยีทางอาหาร ลายมือชื่อนิสิต .....

สาขาวิชา เทคโนโลยีทางอาหาร ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5373811323 : MAJOR FOOD TECHNOLOGY
KEYWORDS: GERMINATED RICE BEAN / HYDROLYSATE / FOAM-MAT DRYING / ANTIOXIDANT

BURACHAT SRITONGTAE: GERMINATED RICE BEAN *Vigna umbellata* HYDROLYSATE AND EFFECT OF FOAM-MAT DRYING ON ITS ANTIOXIDANT ACTIVITY. ADVISOR: ASST. PROF.KIATTISAK DUANGMAL, Ph.D., CO-ADVISOR: PROF.MICHAEL ROBERT ANTHONY MORGAN, Ph.D., 113 pp.

The aims of this research were to study the effect of germination, enzymatic hydrolysis, and foam-mat drying temperature on physico-chemical properties and antioxidant activities of foam-mat dried rice bean protein hydrolysate. Firstly, the effects of germination period and pretreatment with 1% (w v^{-1}) citric acid on chemical composition and antioxidant activities were studied. Rice beans were soaked in water and 1% (w v^{-1}) citric acid solution for 6 h before germination for 0-24 h. Moisture content, total phenolic content (TPC) and the reducing sugar content of beans from both treatments increased with increasing germination time ($p \leq 0.05$). Pretreatment showed strong impact on an increase in TPC. The 18 hour-germinated rice beans (GRB) of both treatments showed the highest crude protein content. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) of the water and ethanolic extracts obtained from both treatments increased as germination period increased ($p \leq 0.05$). Secondly, the GRB and non-germinated rice bean (NGRB) were then hydrolysed using 5% (by dry weight of rice bean) Flavourzyme®. The reaction was conducted at 50°C for 24 h. The results showed that free amino group, degree of hydrolysis, TPC, and antioxidant activities (DPPH radical scavenging activity and FRAP) of hydrolysate of GRB significantly increased with increasing hydrolysis period ($p \leq 0.05$). All parameters tested from hydrolysate of GRB were higher than those of NGRB over 0-24 h of hydrolysis. The hydrolysis period for 21 h was chosen for preparation of protein hydrolysates in this study. Thirdly, drying kinetic and mathematical model of foam-mat dried protein hydrolysate were studied. Foamed protein hydrolysate was dried at two different drying temperatures (60 and 70 °C). Drying rate of protein hydrolysate of GRB was slightly lower than that of NGRB protein hydrolysate at both drying temperatures. Weibull distribution and Midilli model could suitably describe the drying characteristic of both foam-mat dried rice bean protein hydrolysate. The samples were dried until reaching a moisture content of about 8.5% (dry basis). Physico-chemical properties of foam-mat dried protein hydrolysate were investigated. TPC and total flavonoid content of both GRB and NGRB foam-mat dried at 60 °C were higher than those of 70 °C ($p \leq 0.05$). Gallic acid, catechol and epicatechin were major phenolic compounds in both foam-mat dried samples. DPPH radical scavenging activity and FRAP significantly decreased with increasing drying temperatures ($p \leq 0.05$). It was found that %DPPH radical scavenging activity measured using electron spin resonance (ESR) was 1.20-1.32 times higher than using colorimetric method, at the same concentration of protein hydrolysate. The higher TPC, total flavonoid content, and antioxidant activities of foam-mat dried protein hydrolysate of GRB compared to NGRB were observed. The low molecular weight peptide (lower than 3 kDa) showed the higher antioxidant activities than high molecular weight peptide (higher than 3 kDa). The main components responsible for the antioxidant activity of foam-mat dried rice bean protein hydrolysates was phenolics.

Department:	Food Technology	Student's Signature	.....
Field of Study:	Food Technology	Advisor's Signature	.....
Academic Year:	2015	Co-Advisor's Signature	.....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Assistant Professor Dr. Kiattisak Duangmal and my co-advisor Professor Dr. Michael Robert Anthony Morgan for their valuable suggestion and great kindness throughout my doctoral degree pursuit.

I am deeply grateful to Associate Professor Dr. Kanitha Tananuwong, Assistant Professor Dr. Chaleeda Borompichaichartkul, Assistant Professor Dr. Thanachan Mahawanich and Professor Dr. Soottawat Benjakul for constructive comments as thesis committees.

Sincerest appreciation also extends to Thailand Research Fund (Royal Golden Jubilee Ph.D. Program, Grant No. PHD/0154/2553) and the Newton Fund for financial support of this research.

A special thanks to my family (father, mother, uncle and my sister). They are supporting me and encouraging me with their best wishes. Finally, I am deeply grateful to Seksan Wongsiri, Thasanporn Sangsukiam and all friends at Chulalongkorn University and University of Leeds for their help, encouragement and gave me useful suggestions.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CONTENTS

Page

LIST OF TABLES

Page

Page

Page

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF FIGURES

Page

Page

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER I

INTRODUCTION

Rice bean (*Vigna umbellata*) is one of the economically important agricultural products of Thailand, and has been used for making pastry filling, rice bean sprouts and traditional Thai desserts. Rice bean seeds are a good source of protein (14-26% on dry basis). It also contains phenolic compounds which exhibit antioxidant activity. Legume seed germination results in biochemical changes leading to increased levels of, for example, amino acids, peptides and structural proteins and breakdown of seed storage compounds. These processes affect nutritional value of legume seeds. Moreover, germination causes an increase in beneficial bioactive compounds such as phenolics and vitamins C and E.

Modification of proteins by enzymatic hydrolysis is an effective way to improve the functional properties and leads to alternative uses of protein. To produce protein hydrolysate, enzymatic hydrolysis is performed via utilising proteolytic enzyme to hydrolyse peptide bond. This process modifies its physico-chemical, functional and sensorial properties of the native protein. Some hydrolysed proteins have excellent potential activity as antioxidant in food. Antioxidant activity of protein hydrolysate mainly depends on amino acid residues within peptides.

The hydrolysate may be dried and stored in a dry form. Foam-mat drying is a process in which a liquid or semisolid material is transformed into a stable foam by incorporation of foaming agent into liquid foods with subsequent whipping to stiff foam. The foam is then spread as a thin sheet and dried in a hot air oven. This drying method can increase the drying rate by making more porous of material, thus allowing rapid moisture movement within the material. The products can be dried rapidly and without losing much of its nutritional and antioxidant activity.

The bean hydrolysate has been reported to show antioxidant activities. However, there has been no reported study on foam-mat drying on antioxidant activity of germinated bean hydrolysate. Therefore, the objectives of this thesis were as follows:

1) To study the effect of acid pretreatment and germination period on chemical composition and antioxidant activity of rice bean during germination.

2) To study the effect of hydrolysis period on degree of hydrolysis and antioxidant activity of rice bean hydrolysate.

3) To study the influence of foam-mat drying temperature on phenolic composition and antioxidant activity of foam-mat dried rice bean hydrolysate.

The knowledge on physico-chemical properties and antioxidant activities of foam-mat dried rice bean hydrolysate obtained from this research would provide meaningful information for a new opportunity to use foam-mat dried rice bean hydrolysate as functional ingredients which improve the quality and health benefits of food products.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER II
LITERATURE REVIEW

2.1 Rice bean

Rice bean (*Vigna umbellata*) is in the same genus as mung bean (*Vigna radiata*), azuki bean (*Vigna angularis*) and black bean (*Vigna mungo*). It is a small edible beans, 6-8 mm seed with a concave hilum, and varies in color from greenish-yellow to black. However, the dominant color is red and yellow. It has been reported that rice bean is resistant to pests and diseases during storage and shows higher percentage of seed viability than mung bean and black bean (Mukherjee and Sarkar, 1991).

2.1.1 Rice bean in Thailand

Rice bean is grown in various tropical countries such as India, China, the Philippines and Thailand. In Thailand, it is mainly cultivated in Eastern and North-eastern regions. It is also used for mixed-cropping. The growing season in Thailand is during May and August. Rice bean is one of economically important agricultural produces in Thailand. In 2014, the export value for rice bean was 385 million baht for the amount of 2,700-3,200 metric ton (Thailand Foreign Agricultural Trade Statistics, 2014). Rice bean seeds have been used for making pastry filling, and traditional Thai desserts.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

2.1.2 Chemical composition

The dry seed of rice bean is a good source of protein. The amino acid composition of rice bean is well balanced for human consumption. Rice bean is particularly rich in methionine compared to black bean and mung bean. The content of lysine, tyrosine and valine is more or less equal to the content of comparable bean species. Moreover, they are good source of minerals and vitamin B (Andersen, 2007; Yao *et al.*, 2012). Katoch (2013) reported the chemical composition of 16 rice bean genotypes collected from India as follows: crude protein (23.17-25.57%), lipids (1.92-3.42%), dietary fiber (4.11-5.56%), carbohydrate (52.23-55.65%), ascorbic acid (15.14-29.19 mg 100 g^{-1}) and niacin (3.48-4.28 mg 100 g^{-1}). Rice bean also contains phenolic compounds which exhibit an antioxidant activity. Yao *et al.* (2012) reported that different varieties of rice bean from China had significant differences in total

phenolic and flavonoid contents with values ranging from 3.27-6.43 mg gallic acid equivalents g^{-1} and 55.95-320.39 µg catechin equivalents g^{-1}, respectively. Vitexin was the dominant phenolic compound in all studied rice beans.

2.2 Germination

Germination in seed is defined as the sequential series of morphogenetic events that result in the transformation of an embryo into a seedling (Berlyn, 1972).

2.2.1 Physico-chemical changes during germination

The germination of legume seed causes biochemical changes such as synthesis of amino acids, peptides and structural proteins including breakdown of seed-storage compounds. These processes affect nutritional value of legume seed. Ghavidel and Prakash (2007) reported that 24-hour germination caused a significant increase in protein and thiamin levels of all legume samples tested (*Phaseolus aureus*, *Vigna catjang*, *Lens culinaris* and *Cicer arietinum*), while phytic acid and tannin were reduced by 18–21% and 20–38%, respectively. Germination also causes an increase in bioactive compounds such as phenolic compounds, vitamins C and E, considered beneficial as antioxidants. Fernandez-Orozco *et al.* (2008) studied vitamins C and E, and total phenolic content of 7-day germinated mung bean (*Vigna radiata*) and two cultivars of soybean seeds (*Glycine max* cv. jutro and *Glycine max* cv. merit) after germination at 4 and 6 days, respectively. The results showed that vitamin C of mung bean seed increased ($p \leq 0.05$) while no change was observed in sprouts of *Glycine max* cv. jutro. An increase in vitamin C was observed after 5 days of germination in *Glycine max* cv. merit. Phenolic compounds and vitamin E of mung bean and soybean significantly increased after germination. Wongsiri, Ohshima and Duangmal (2015) reported that during 24 hours of germination total phenolic content, crude protein content and amino acid content of germinated mung bean significantly increased with increasing germination period.

In natural environment, seed-sprouting survives during germination by enhancing its defensive response through phenolic biosynthesis and phenolic compounds considering beneficial as antioxidants (Randhir, Lin and Shetty, 2004). Pająk *et al.* (2014) reported that total phenolics and total flavonoids content of 5-day

germinated mung bean were 10.7 and 2.3 times higher than those of non-germinated mung bean seeds.

The process of seed germination is complex and can be affected by internal and environmental factors. These factors are necessary for seed germination in plants. The major internal factors are seed viability, genotype and seed maturation. The most important environmental factors are moisture, gas, temperature and light. Moreover, other factors such as chemicals can affect the germination of seed. Some chemical substances (e.g. potassium nitrite, thiourea, etc.) and some hormones such as gibberellins and cytokinins promote seed germination (Mayer and Poljakoff-Mayber, 1982)

2.2.2 Environmental stress on germination

The germination of seed under stress condition is different from normal condition. Plants show many adaptive strategies in response to different stress such as high osmotic pressure, water deficit, cold, heat and high salt, which ultimately affect plant growth and productivity. The reactions of plants to environmental stresses are complex and involve many kinds of physiological and biochemical responses (Dubey, 1999). Gill *et al.* (2002) studied germination of sorghum seed (*Sorghum bicolor*) on the filter sheets moistened with osmotic solution (0.75 M mannitol solution, -1.86 MPa) for 14 hours. The total sugar and reducing sugar content of germinated sorghum was markedly increased after 14 hours of germination. The increase of soluble sugar seems to play an important role in osmotic regulation of cell during germination of seed. Esen *et al.* (2012) found that 10 days of germination of *Gypsophila oblanceolata* (salt-tolerant plant) in distilled water containing 300 mM NaCl caused a significant increase in activity of antioxidant enzymes (superoxide dismutase, catalase and peroxidase). These enzymes may help plant cells to avoid oxidative damage during germination.

Pretreatment seed with acid can cause an acidic stress. The acidic stress is defined as the adaptation of plant to growth in a low pH environment. Plants response to acidic stress through different types of defense strategies (e.g. genetic adaptation, enzymatic biosynthesis) in order to maintain the functions of cells. However, the response to acidic stress of plant is depending on its species (Wilkinson, 1994). Acid enhances the germination of seed. The acid-growth hypothesis postulates that the cell wall loosening is induced by cell wall acidification as prerequisite of growth promotion,

resulting in a release of free phenolics from plant cells (Rayle and Cleland, 1992; Obroucheva, 1999). Pretreatment with acid before germination affects physiological and biochemical responses of seed during germination. McCue *et al.* (2000) reported that pretreatment of green pea seeds (*Pisum sativum*) with acidified water (pH 3) for 24 hours before germination caused a 13.59% and 7.69% increase in total phenolic content and protein content, respectively, compared to that of non-treated pea.

Citric acid is a weak organic acid commonly used in food, cosmetic and pharmaceutical industries. The parent base of citric acid, citrate, is a component of the Krebs cycle, and occurs naturally during metabolism in all living organisms (Anastassiadis *et al.*, 2008). Pretreatment with citric acid at low concentration has been shown to enhance germination of seeds from various plant species such as arizona cypress (*Cupressus arizonica*) (Goggans, Jones and Lynch, 1974), montezuma cypress (*Taxodium distichum*) (Denny and Arnold, 2007), wild cherry (*Prunus avium*) (Esen, Günes and Yıldız, 2009), bald-cypress (*Taxodium distichum*) (Popović *et al.*, 2012) and green dammer tree (*Shorea tumbuggai*a ROXB.) (Ankanna and Savithramma, 2012). However, investigation concerning the effect of citric acid on acidic stress of bean has not been reported in the literature.

2.3 Protein hydrolysate

2.3.1 Modification of protein using enzymatic hydrolysis

Rice bean is a potential source of protein as it contains 23.17-25.57% of protein (Katoch, 2013). Opportunities to use proteins in their natural state form often limited by native properties of the proteins. The modification of proteins is an effective way to modify the functional properties, resulting in alternative uses of protein. Compared with acid or alkali hydrolysis, enzymatic hydrolysis of protein using selective proteases provides more moderate condition of the hydrolysis process and fewer or less undesirable side reactions/products. Enzymatic hydrolysis is performed via utilizing proteolytic enzyme to hydrolyse peptide bond. This process changes the conformation and structure of native protein and consequently changes its physicochemical and functional properties including antioxidant activity (Kristinsson and Rasco, 2000).

Proteolytic enzymes maybe classified into 2 groups: endo-proteases and exo-proteases. Endo-proteases hydrolyse internal peptide bond in a polypeptide chain

producing a range of polypeptides, which differs in molecular weight, depending on the extent of hydrolysis. Exoproteases remove amino acid from either the N-terminal or the C-terminal of a peptide chain. A wide variety of commercial proteolytic enzymes are useful in the preparation of protein hydolysates. Commercial sources of enzymes can be obtained from plants, animal tissue, and microorganism. Among of three sources, enzymes from microorganism are preferred because they are cheaper, more controllable with less harmful component (e.g. endogenous enzyme inhibitor, phenolic, etc.) (Araglou, 2009). Examples of commercial proteolytic enzyme commonly used in food industries are Alcalase® from *Bacillus licheniformis*, Neutrase® from *Bacillus amyloliquefaciens* and Flavourzyme® from *Aspergillus oryzae* (Nielsen, 2010).

Protein hydrolysates consist of complex mixtures of free amino acids and peptides with different chain length. The degree which a protein has been hydrolysed is a reflection of the number of peptide bonds broken. The number of peptide bonds broken as a proportion of the total number of peptide bonds present is defined as the degree of hydrolysis (DH). The DH is most commonly assigned to follow the reaction kinetics of enzymatic hydrolysis and to describe the extent of the hydrolytic degradation of food proteins. Several methods have been used for determining DH; the most commonly used methods include the pH-stat, *o*-phthaldialdehyde (OPA), formol titration and trinitrobenzenesulfonic acid (TNBS) method (Rutherfurd, 2010).

The application of modified protein with different DH in food product is dependent on its functional properties and the objective of utilization. The properties of modified protein can be monitored through degree of hydrolysis (DH). Limited proteolysis, commonly at 1-10% DH, causes a change in conformation, molecular weight distribution, stability, flexibility and interfacial properties of modified protein. It has been reported that limited hydrolysis can improve some functional properties by enhancing solubility and associated surface properties related to the feasibility of the formation and stabilization of air-water and oil-water interfaces, which are important properties in food product applications (Guan *et al.*, 2007; Yust *et al.*, 2012). Extensive proteolysis, having higher DH, requires higher concentration of enzyme or longer period of hydrolysis time. The obtained product contains a wide range of peptides with different molecular weight and free amino acids compared to limited proteolysis. The extensive hydrolysis provides nutritional and therapeutic properties are based on the

absorption of peptides. The bioactive peptide derived from protein hydrolysis have shown various biological activities including antioxidant activity, antimicrobial, immunomodulatory, antithrombotic and antihypertensive effects. The extensive proteolysis also lead to a release of phenolic compounds from protein-phenolic complexes, due to changing of hydrophobic behavior of protein, which can enhance the antioxidant activities of product. Moreover, it can be used as function foods, additive supplement with beneficial effect on human health (Hartmann and Meisel, 2007; Malaguti *et al.*, 2014).

2.3.2 Antioxidant activity of protein hydrolysate

In human, oxidative stress usually plays the role of a promoter of chronic diseases. To prevent oxidative stress and its deleterious effects, synthetic and natural antioxidants are commonly used in foods and medicine (Rice-Evans and Diplock, 1993). Enzymatically-modified proteins may serve as natural sources of antioxidants. The extensive protein hydrolysate from bean consists of amino acids, peptides and phenolics which are considered to have high antioxidant activity. The most reactive amino acids tend to be those containing nucleophilic sulfur containing side chains (cysteine, methionine) or aromatic side chains (tryptophan, tyrosine, and phenylalanine) which its hydrogen atom is easy to be removed. Moreover, imidazole-containing side chain of histidine is also oxidatively labile.

Peptides show antioxidant activity through different mechanisms, depending on their amino acid composition and their amino acid sequences (Sarmadi and Ismail, 2010). The different antioxidant mechanism of peptide is shown in Figure 2.1

Phenolic compound (POH) acts as a free radical acceptor and a chain breaker by rapid donating hydrogen and electrons to stabilise free radicals (R·). The phenoxy radical intermediates (PO·) are stable due to resonance delocalization of unpaired electrons around the aromatic ring (equation 2.1). These intermediates also act as the terminator of propagation route by reacting with other free radicals (equation 2.2) (Dai and Mumper, 2010).

$$R\cdot + POH \rightarrow RH + PO\cdot \qquad (2.1)$$

$$PO\cdot + R\cdot \rightarrow POR \qquad (2.2)$$

Oxidation in food is complex and involving different oxidative processes, therefore, evaluating mechanism of an antioxidant requires a variety of analytical techniques. The mechanism of the assay is based on two reactions: (1) hydrogen atom transfer reaction (HAT) and (2) single electron transfer reaction (SET) (Prior, Wu and Schaich, 2005).

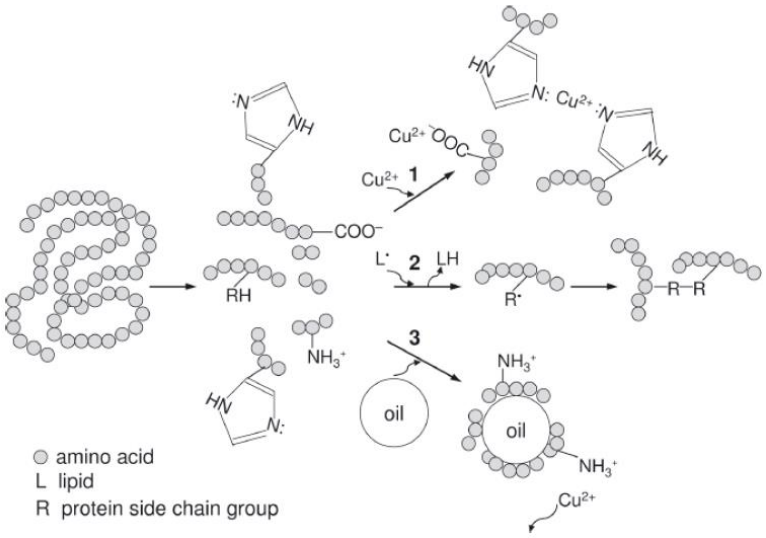

Figure 2.1 Antioxidant mechanism of peptide. (1) metal chelation; (2) radical scavenging; and (3) physical hindrance

Source: Xiong (2010)

The HAT-based assay includes oxygen radical absorbance capacity (ORAC) and total radical-trapping antioxidant parameter (TRAP) assays. These assays measure the capacity of an antioxidant (AH) to quench free radicals ($R^{\cdot}$) by hydrogen atom donation (equation 2.3) (Prior *et al.*, 2005).

$$R\cdot + AH \rightarrow RH + A\cdot \qquad (2.3)$$

SET-based assay includes trolox equivalent antioxidant capacity (TEAC) and ferric ion reducing antioxidant power (FRAP). These assays measure the reducing capacity of the antioxidant (AH) by provides an electron to the free radical ($R^{\cdot}$) as shown in equation 2.4 (Prior *et al.*, 2005).

$$R\cdot + AH \rightarrow R^{-} + AH^{+}\cdot \qquad (2.4)$$

However, some methods such as 1,1-diphenylpicrylhydrazyl (DPPH) radical scavenging activity can exhibit both SET and HAT mechanisms because this indicator radical may be neutralised either by direct reduction via electron transfers or by radical quenching via hydrogen atom transfer (Prior *et al.*, 2005).

In this study, colorimetric method (DPPH radical scavenging activity and FRPP) and electron spin resonance (ESR) method are used for determining antioxidant activity of protein hydrolysate.

DPPH is a stable free radical with one unpaired electron. Its deep violet color, showing maximum absorbance at 515 nm, is used for monitoring the radical scavenging activity of an antioxidant. The decolorization of DPPH radicals during the assay is an indication of antioxidant ability to reduce DPPH radical (Brand-Williams, Cuvelier and Berset, 1995).

FRAP measures the ability of antioxidant to reduce the ferric ion complex $[Fe(III)\text{-}(TPTZ)_2]^{3+}$ to form blue color of ferrous complex $[Fe(II)\text{-}(TPTZ)_2]^{2+}$ which shows maximum absorbance at 593 nm (Benzie and Strain, 1996).

Electron spin resonance (ESR) spectroscopy has become a powerful and direct method to detect free radicals. This method is based on the measurement of transitions of unpaired electrons in a magnetic field, which can detect and identify molecules that have unpaired electrons, such as free radicals. ESR is a technique with high sensitivity and free of interferences from others components such as pigments. ESR allows for detection of low concentration radicals (DPPH, superoxide and hydroxyl) in peptide fractions obtaind from whey protein hydrolysate (Peng, Xiong and Kong, 2009). Mendis, Rajapakse and Kim (2005) evaluated the superoxide, carbon-centered and DPPH scavenging activities of fish skin gelatin hydrolysate using ESR. The results showed a strong radical scavenging activity of extract by quenching all radicals.

2.4 Foam-mat drying

Drying of foods is one of common methods of preservation. Drying is a process of evaporation of moisture from the surface of food materials into the surrounding air. This process can reduce water activity and extend a shelf life of food product. In thermal drying process, both of heat transfer (heat transfer from surrounding environment

evaporates the surface moisture) and mass transfer (transfer of internal moisture to the surface) occur simultaneously (Mujumdar, 2006). The liquid food can be dried in several ways, for example, using a tray dryer, drum dryer and spray dryer. However, these methods may result in some disadvantages such as denaturation of proteins and low rehydration property. Even though freeze-drying yields in high quality product, the high cost associated with investment and operation is its major limitation (Chandrasekar *et al.*, 2015).

2.4.1 Principle of foam-mat drying

Foam-mat drying is a process in which a liquid or semi-solid material is transformed into a stable foam by incorporation of foaming agent into liquid food with subsequent whipping to stiff foam. The foam is then spread as a thin sheet and dried in an air stream. This drying method increases the drying rate by making more porous of materials, thus allowing rapid moisture movement within the materials. The product can be dried rapidly without losing much of its nutritional values (Rajkumar *et al.*, 2007). Kadam *et al.* (2012) studied foam-mat drying on quality of tomato powder prepared from tomato juice. They observed that using carboxy methyl cellulose at 1% (w w^{-1}) and drying temperature at 85 °C gave the best optimum condition for foam-mat drying tomato juice because a decline in ascorbic acid content and lycopene content were lower than other conditions; no fungal and bacterial growth were detected in the final product. Generally foam material can be dried faster than that of non-foamed ones. Foam-mat drying is the method that showed high drying efficiency and low energy consumption. Kudra and Ratti (2006) reported that drying efficiency of foamed apple juice is almost 2 times higher than that of non-foamed juice, while the energy consumption for dry foamed juice just is just 0.2 of that of non-foamed.

2.4.2 Drying kinetics

Drying is normally measured and expressed by moisture content or drying rate over time. The drying kinetics of foam-mat can be described using an appropriate drying model. Because of thin structure of foam, the temperature distribution is easily assumed as uniform, thus thin-layer drying models are suitable for describing its drying kinetics (Venkatachalam, John and Kuppuswamy, 2015). Thin-layer drying models can be grouped into theoretical, semi-theoretical, and empirical models.

Theoretical model is based on diffusion equation of heat or mass transfer which accounts only the internal resistance of moisture movement to the surface. Theoretical model is derived from Fick's model second law of diffusion. The solution for an infinite slab is presented in equation 2.5 (Crank, 1975).

$$MR=\frac{M-M_{eq}}{M_0-M_{eq}}=\frac{8}{\pi^2}\sum_{i=1}^{\infty}\frac{1}{(2i-1)^2}\exp\left(-\frac{(2i-1)^2\pi^2}{4}\frac{D_{eff}t}{Z^2}\right) \quad (2.5)$$

where, M is the instantaneous moisture content (wb); M_e is the equilibrium moisture content (wb); M_o is the initial moisture content (wb); t is the time (min); MR is the moisture ratio; D_{eff} is the effective diffusivity ($mm^2\ min^{-1}$); z is thickness (mm).

Semi-theoretical models is generally classified according to their derivation. These models can divided into two groups as the models that are derived by analogues with Newton's law of cooling, such as Newton and Page model, and the models that are derived from Fick's second law of diffusion. The models derived from Fick's second law of diffusion can be classified into three groups. The first group is single term exponential model and its modified forms such as Henderson and Pabis, logarithmic and Midilli model. The second group is two term exponential model and its modified forms such as two-term model. The third group is three term exponential model such as modified Henderson and Pabis model. The equation of these models are shown in Table 2.1.

The empirical models tends to have similar characteristics to semi-theoretical models. However, they use experimental data and fits them to different mathematical equations. These models strongly depend on the experimental condition (Erbay and Icier, 2010). The examples of empirical models are Wang and Singh, and Weibull distribution. The equation of these models shown in Table 2.1

Wilson *et al.* (2012) studied foam-mat drying characteristics of mango (*Mangifera indica* L.) pulp. They found that among seven thin layer drying equations (Newton, Henderson and Pabis, logarithmic, two-term, two-term exponential, Wang and Singh, and diffusion approach model), Wang and Singh model was found to be the best fit with coefficient of determination (r^2) higher than 0.99 at drying temperatures of 65 and 85 °C. However, the suitable models for describe the drying at 75 °C were

Logarithmic and diffusion approach model. Salahi, Mohebbi and Taghizadeh (2015) reported that Weibull distribution model is the most suitable model to describe drying characteristics of foam-mat dried cantoloupe (*Cucumis melo*) compared to other models (Newton, Page, Henderson and Pabis, two-term, modified Henderson and Pabis, logarithmic, Wang and Sing, approximation of diffusion and Midilli).

Table 2.1 Semi-theoretical and empirical models fitted to the moisture ratio (MR) values

Model	Model equation	References
Semi-theoretical models		
Derived by analogues with Newton's law of cooling		
Newton	MR = exp(-*kt*)	Lewis (1921)
Page	MR = exp(-kt^n)	Page (1949)
Derived from Fick's second law of diffusion		
Henderson and Pabis	MR = *a*exp(-*kt*)	Henderson and Pabis (1961)
Logarithmic	MR = *a*exp(-*kt*) + *c*	Chandra and Singh (1995)
Midilli	MR = *a*exp(-kt^n) + *bt*	Midilli, Kucuk and Yapar (2002)
Two-term	MR = *a*exp(-*bt*) + *c*exp(-*dt*)	Henderson (1974)
Modified Henderson and Pabis	MR = *a*exp(-*kt*) + *b*exp(-*gt*) + *c*exp(-*ht*)	Karathanos (1999)
Empirical models		
Wang and Singh	MR = 1 + *at* + bt^2	Wang and Singh (1978)
Weibull distribution	MR = *a*- *b*exp(-kt^n)	Babalis *et al.* (2006)

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

2.4.3 Quality of foam-mat dried product

Foam-mat drying process affect the physicochemical and microbial characteristics of foam-mat dried product. The amount of foaming agent, whipping time, foam thickness and drying temperature affected the quality of final dried product. Kadam, Wilson and Kaur (2010) studied effect of drying temperature (65, 75 and 85 °C), using milk as foaming agent at different concentration (0%, 10%, 15%, 20% and 25%) on chemical properties of foam-mat dried mango (*Mangifera indica* L.) pulp. The result showed that ascorbic acid and total carotenes tended to decrease with increasing in drying air temperature and concentration of foaming agent. There was no microbial load detected in all foam-mat dried mango. Heat involved during foam mat drying process have some detrimental effect on quality of dried product (Venkatachalam *et al.*, 2015). However, higher drying rate with lesser drying time in foam mat drying compared to normal air drying yielded in foam-mat dried products with high retention of color, flavor, vitamins and sensory characteristics (Kadam and Balasubramanian, 2011).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER III
MATERIALS AND METHODS

3.1 Materials and Equipment

3.1.1 Raw materials

The same lot of rice bean seeds (*Vigna umbellata)* obtained from Choomsin Food Industry Co., Ltd. (Bangkok, Thailand) in the harvest year of 2014, moisture content of approximately 7% dry basis, was used.

3.1.2 Enzymes

α-Amylase (BAN® 240L)**,** Food grade (Sigma, Mülheim, Germany). The specific activity of BAN® 240L is 529 kilo novo units (KNU) g^{-1}, which KNU is defined as the amount of enzyme that hydrolyses 5.26 g of starch per hour at Novo's standard method.

Protease (Flavourzyme® 500L), Food grade (Sigma, Mülheim, Germany). The specific activity of Flavourzyme® is 1,160 leucine aminopeptidase units (LAPU) g^{-1}, which is defined as the amount of enzyme that hydrolyses 1 μmol of leucine-*p*-nitroanilide per minute.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.1.3 Chemicals

AccQ.fluor™ reagent kit	HPLC grade	Water	MA, USA
Acetic acid	HPLC grade	Sigma	Mülheim, Germany
Acetonitrile	HPLC grade	Sigma	Mülheim, Germany
Aluminium chloride	A.R. grade	Sigma	Mülheim, Germany
Amino acid hydrolysate standard	HPLC grade	Thermo scientific	MA, USA
Ammonium molybdate	A.R. grade	Univar	NSW, australia

2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH)	A.R. grade	Sigma	Mülheim, Germany
Bromocresol green	A.R. grade	Carlo Erba	Val de Reuil, France
Caffeic acid	A.R. grade	Sigma	St. Louis, USA
Calcium chloride	A.R. grade	Univar	NSW, Australia
Catechin	A.R. grade	Sigma	St. Louis, USA
Catechol	A.R. grade	Sigma	St. Louis, USA
Trichloroacetic acid	A.R. grade	Carlo Erba	Milan, Italy
trans-Cinnamic acid	A.R. grade	Sigma	St. Louis, USA
Coomassie brilliant blue R-250	A.R. grade	Panreac	Barcelona, Spain
Copper (II) sulfate pentahydrate	A.R. grade	Merck	Darmstadt, Germany
p-Courmaric acid	A.R. grade	Sigma	St. Louis, USA
Epicatechin	A.R. grade	Sigma	St. Louis, USA
Ferulic acid	A.R. grade	Sigma	St. Louis, USA
Folin-Ciocalteu phenol reagent	A.R. grade	Carlo Erba	Milan, Italy
Formic acid	A.R. grade	Sigma	St. Louis, USA
Gallic acid	A.R. grade	Fluka Chemika	Buchs, Switzerland
D-Glucose	A.R. grade	Univar	NSW, Australia
Glycerol	A.R. grade	Univar	NSW, Australia
Glycine	A.R. grade	Vivantis	CA ,USA
Hydrochloric acid	A.R. grade	J.T. Baker	PA, USA

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Hydrocinnamic acid	A.R. grade	Sigma	St. Louis, USA
Hydrogen peroxide	A.R. grade	Sigma	Mülheim, Germany
4-Hydroxybenzoic acid	A.R. grade	ACROS	NJ, USA
6-Hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox®)	A.R. grade	Sigma	Mülheim, Germany
Iron (III) chloride	A.R. grade	Sigma	Mülheim, Germany
Iron (II) chloride tetrahydrate	A.R. grade	Merck	Darmstadt, Germany
Methanol	A.R. grade	Quality Reagent Chemical	Auckland, New Zealand
5,5-dimethyl-1-pyroline-N-oxide (DMPO)	A.R. grade	Sigma	Mülheim, Germany
Tetramethylethylenediamine (TEMED)	A.R. grade	Carlo Erba	Milan, Italy
Methyl red	A.R. grade	Merck	Darmstadt, Germany
Naringenin	A.R. grade	LKT Laboratories	St. Paul, USA
2,4,6-trinitrobenzenesulfonic acid	A.R. grade	Sigma	Mülheim, Germany
Petroleum ether	A.R. grade	Carlo Erba	Milan, Italy
2,2-diphenyl-1-picrylhydrazyl (DPPH)	A.R. grade	Sigma	Mülheim, Germany

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

α-(4-pirydryl-1-oxide)-N-tert-butylnitrone (4-POBN)	A.R. grade	Sigma	Mülheim, Germany
Potassium hydrogen phthalate	A.R. grade	Carlo Erba	Milan, Italy
2,4,6-tripyridyl-s-triazine (TPTZ)	A.R. grade	Sigma	Mülheim, Germany
Rutin	A.R. grade	ACROS	NJ, USA
Selenium reagent mixture	A.R. grade	Merck	Darmstadt, Germany
Sodium acetate	A.R. grade	Univar	NSW, Australia
Sodium carbonate	A.R. grade	Univar	NSW, Australia
Sodium dihydrogen orthophosphate	A.R. grade	Univar	NSW, Australia
Sodium dodecyl sulfate	A.R. grade	Vivantis, USA	CA ,USA
Sodium dodecyl sulphate	A.R. grade	Sigma	Mülheim, Germany
di-Sodium hydrogen arsenate heptahydrate	A.R. grade	Fluka Chemika	Buchs, Switzerland
Sodium hydrogen carbonate	A.R. grade	Merck	Darmstadt, Germany
di-sodium hydrogen orthophosphate	A.R. grade	Univar	NSW, Australia
di-sodium hydrogen phosphate	A.R. grade	Univar	NSW, Australia
Sodium hydroxide	A.R. grade	Univar	NSW, Australia
Sodium sulphate	A.R. grade	Carlo Erba	Val de Reuil, France

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Sulphuric acid 98%	A.R. grade	Quality Reagent Chemical	Auckland, New Zealand
Trisma^{TM} base	A.R. grade	Vivantis	CA ,USA
L-tyrosine	A.R. grade	Univar	NSW, Australia
Quercetin	A.R. grade	Sigma	St. Louis, USA
Vanillin	A.R. grade	Sigma	St. Louis, USA

3.1.4 Equipment

Refrigerated centrifuge (Universal 32 centrifuge, DJB Labcare Ltd., Buckinghamshire, UK)

Electronic balance (2 digit) (Sartorius model BP 310s, Göttingen, Germany)

Electronic balance (4 digit) (Sartorius model BSA 2245, Göttingen, Germany)

Electron spin resonance spectrometer (JES-TE300 model, JEOL Ltd., Tokyo, Japan)

High Performance Liquid Chromatography (HPLC) system for amino acid analysis (Waters, MA, USA)

- Auto sampler (Waters model 717, MA, USA)

- Waters AccQ.Tag amino acid analysis column 5 µm, 3.9 mm × 150 mm (Waters, MA, USA)

- UV/VIS detector (Waters model 2487, MA, USA)

- Pump (Waters 515 HPLC pump, MA, USA)

High Performance Liquid Chromatography (HPLC) system for phenolics analysis (Shimadzu, Kyoto, Japan)

- Auto sampler (SIL-20A, Shimadzu, Kyoto, Japan)

- A Gemini C18 column 5 µm, 4.6 mm × 250 mm (Phenomenex, Macclesfield, Cheshire, UK)

- Photodiode array detector (SPD-20A, Shimadzu, Kyoto, Japan)

- Pump (LC-20AD, Shimadzu, Kyoto, Japan)

Hot air oven (FD 240 Binder, Tuttlingen, Germany)

Liquid Chromatography-Mass Spectrometry (LC-MS)

- Luna C18 column (2.1 x 150 mm, 3 μm) (Phenomenex, CA, USA)
- mass spectrometry (Bruker amaZon SL, IL, USA)

Magnetic stirrer (Steroma G, Steroglass, Perugia, Italy)

pH meter (Cyberscan 1000, Eutech, Singapore)

Protein and nitrogen analyser

- Digestion unit (BUCHI model K-424, Flawil, Switzerland)
- Scrubber unit (BUCHI model B-414, Flawil, Switzerland)
- Distillation unit (BUCHI model B-324, Flawil, Switzerland)

Scanning electron microscope (JEOL, JSM-5410LV, Tokyo, Japan)

Solid phase extraction column (Isolute® C18 end-capped (EC), Biotage, Uppsala Sweden)

Spectrophotometer (Genesys 20 spectrophotometer 4001/4, Thermo Fisher Scientific, MA, USA)

Ultrafiltration membrane 3 kDa molecular weight cutoff (PES) (Vivaflow 50 module, Sartorius AG, Göttingen, Germany).

Vortex mixer (Vortex-Genie 2, Scientific Industries, NY, USA)

Water activity meter (AquaLab, model AquaLink 3.0, WA, USA)

Water bath (Gesellschaft für modell GFL 1092, Burgwedel, Germany)

3.2 Experimental procedures

3.2.1 Effect of pretreatment and germination period on chemical composition of germinated rice bean

Dried rice bean seeds were soaked in tap water (1:10, w:v) containing 1% (w v^{-1}) citric acid, as a pretreatment, at room temperature (30±1 °C) for 6 h. After being

drained for 5 min, seeds were allowed to germinate under a wet muslin cloth at room temperature in the dark. Rice bean seeds soaked in tap water without citric acid and germinated under the same condition were used as the control. Both of the pretreated rice bean seed and the control were randomly selected at 0, 6, 12, 18 and 24 hours of germination, and then steamed for 10 min to stop the germination process before chemical analysis as follows:

3.2.1.1 Proximate analysis of germinated rice bean was conducted as follows:

- Moisture content of germinated rice bean was determined using the oven-dry method, 5 g of ground sample was dried in an oven (Memmert model W350, Schwabach, Germany) at 105 ºC to a constant weight (AOAC, 2006).

- Crude protein of germinated rice bean was calculated from the content of nitrogen determined by Kjeldahl method (AOAC, 2006) with a nitrogen-to-protein conversion of 6.25.

- Crude fat of germinated rice bean was determined using Soxhlet extraction method. Dried ground sample (2 g) was semi-continuously extracted with petroleum ether using Soxhlet extraction apparatus for 6 h. The solvent was evaporated by vacuum condenser. The extracted fat was then dried in an oven at 105 ºC for 30 minutes and allowed to cool to room temperature in a desiccator. The dry weight of the content was used to calculate percentage of fat in the sample (AOAC, 2006).

- Ash content of germinated rice bean was determined according to (AOAC, 2006). Ground sample (2 g) was burned in the furnace (Carbolite, model CWF1200, Sheffield, UK) at 550 ºC for 12-18 h. When the residue turns to white, it is then cooled to room temperature in a desiccator and weighed. If not turn to white, return the residue to the furnace for further ashing.

- Carbohydrate content of germinated rice bean was determined by subtracting the total percentage compositions of protein, fat, and ash contents from 100.

3.2.1.2 Total phenolic content

Germinated rice beans were ground using a Waring blender (Waring Commercial, UK) at high speed for 3 min. The samples were extracted according to the method of Saeed, Khan and Shabbir (2012) by using 95% methanol (1:10, w:v) at 30°C

in a shaking water bath (Gesellschaft fürmodel GFL 1092, Burgwedel, Germany) at 200 rpm for 2 h. After centrifugation at 10,000 × *g* for 10 min, the supernatant was filtered through Whatman No.1 filter paper. The extract was subjected to total phenolic content determination, expressed as mg of gallic acid equivalents per gram of rice bean (dry basis, d.b.), using Folin-Ciocalteu reagent (Waterhouse, 2005) as stated in Appendix A.1.

3.2.1.3 Reducing sugar content

The ground germinated rice bean was extracted with distilled water (1:10, w:v) at 30 °C in a shaking water bath (Gesellschaft fürmodel GFL 1092, Burgwedel, Germany) at 200 rpm for 2 h. After centrifugation at 10,000 × *g* for 10 min, the supernatant was filtered through Whatman No.1 filter paper. The extract was subjected to reducing sugar content determination, expressed as mg glucose per gram of rice bean (d.b.), according to the method described by Fournier (2005) as stated in Appendix A.2.

3.2.1.4 Protein nitrogen and non-protein nitrogen content

Protein nitrogen (nitrogen from protein and polypeptide) content in all samples was determined according to method of Wongsiri *et al.* (2015). The ground sample (30 g) was solubilised in 180 ml of 0.1 M NaOH solution, stirred for 5 min every 15 min for 2 h, and centrifuged at 12,000×g for 5 min. The precipitate was mixed with 90 ml of 0.1 M NaOH solution and extracted again using the same procedure. This supernatant was pooled with the first supernatant. The collected supernatant was added by 72% (w v^{-1}) trichloroacetic acid (TCA) to give a final concentration of 10% (w v^{-1}) TCA and left for 30 min at room temperature with occasional shaking prior to centrifuge at 12,000×*g* for 20 min. The precipitate was washed with 5 ml ethyl ether and dried for 1 h at 105 °C and weighted. Nitrogen content of the precipitate was then determined using the Kjeldahl method (AOAC, 2006). The non-protein nitrogen (NPN, nitrogen from oligopeptide and free amino acids) in all sample was estimated as the difference between total nitrogen content and protein nitrogen content (Periago *et al.*, 1996).

3.2.1.5 TCA-soluble peptide content

TCA-soluble peptide content of germinated rice bean was determined according to the method of Kudre and Benjakul (2013). Ground germinated rice bean (3 g) was mixed with 27 ml of cold 5% (w v^{-1}) TCA. The mixture was continuously stirred for 2 min before being allowed to stand in ice for 1 h. After centrifugation at 10,000 × *g* for 10 min, the supernatant was then filtered through Whatman no.1 filter paper. TCA soluble peptide content in the supernatant was measured according to the Lowry method (Lowry *et al.*, 1951) and expressed as g tyrosine per 100 g of rice bean (d.b.).

3.2.1.6 Free amino acid content

Free amino acid content of germinated rice bean was analysed using High Performance Liquid Chromatography (HPLC) according to AccQ.Tag method (Astephen, 1993) modified by Wongsiri *et al.* (2015). The extraction of free amino acid of germinated rice bean was measured using the same method as the extraction of TCA-soluble peptide content in Section 3.2.1.5. After centrifugation at 10,000 × *g* for 10 min, the supernatant was adjusted to pH 2.0-3.0 with 1.0 N NaOH to optimize the derivatization reaction. All samples were then filtered through 0.45µm nylon filter and derivatized at 55 ºC with AccQ.Tag solution for 10 min. After derivatization, a 10 µl of derivatized of each sample was injected to the Water AccQ.Tag amino acid analysis column with 3.9 mm i.d. × 150 mm (Waters, MA, USA) equipped with Waters 2487 dual wavelength absorbance detector (Waters, MA, USA) set at 254 nm. The temperature of the column was set at 37 ºC. Mobile phase was the gradient of AccQ.Tag Eluent A solution (AccQ.Tag Eluent A and Milli-Q water at the ratio of 1:10, v:v) (mobile phase A) and 60% acetonitrile (mobile phase B) with the flow rate of 1.0 ml min^{-1}. The gradient condition were as follows; 0-2% B over first 0.5 min, 7% B at 15 min, 10% B at 19 min, 20% B at 28 min, 28% B at 30 min, 32% B at 35 min, 40% B at 40 min, 100% B at 45 min and return to initial condition for 15 min for next analysis. Identification and integration of peaks were performed using Empower software (Water, MA, USA). A known mixture of different amino acids was applied as external standards (Amino Acid Standard H, Thermo Scientific, MA, USA) ranging from 10 to 200 pmol. Preparation of standard amino acids was stated in Appendix A.3.

3.2.1.7 Protein pattern of germinated rice bean

Protein pattern of germinated rice bean was analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). The sample was prepared according to the method of Duangmal and Taluengphol (2010). Three g of ground sample was add to 27 ml of 5% (w v^{-1}) SDS solution. The mixture was then homogenized with a Waring blender for 2 min. The homogenate was incubated at 80°C in a shaking water bath (Gesellschaft fürmodel GFL 1092, Burgwedel, Germany) for 1 h. The sample was then centrifuged at 12,000 × *g* for 30 min. The protein content in supernatant was determined using the Lowry method modified by Peterson (1977). The rice bean protein was mixed at 1:1 (v:v) ratio with the sample treatment buffer (0.5 M Tris-HCL buffer, pH 6.8, containing 4% (w v^{-1}) SDS, 20% (w v^{-1}) glycerol, and 0.1% (w v^{-1}) bromophenol blue) and boiled for 2 min. The molecular weight markers (S8445, Sigma, MO, USA) and samples (7 µg protein) were loaded onto the polyacrylamide gel consisting of a 10% (w v^{-1}) separating gel and 4% (w v^{-1}) stacking gel and subjected to electrophoresis at a content of 20 mA per gel using a Hoefer mini VE gel electrophoresis unit (Amersham Pharmacia Biotech, Uppsala, Sweden). After separation the proteins were exposed to Comassie Brilliant Blue R-250 solution and destained with destaining solution, containing 25% (v v^{-1}) of ethanol and 10% (v v^{-1}) of glacial acetic acid.

3.2.1.8 Antioxidant activities of germinated rice bean

To study the effect of extraction solvents on antioxidant activity of germinated rice bean, ground sample at different germination stages was extracted with water or ethanol. The water extract was prepared by adding 100 ml of 0.1 M phosphate buffer (pH 6.0) to 10 g of ground sample. The mixture was shaken incubated at 30 °C in a shaking water bath (Gesellschaft fürmodel GFL 1092, Burgwedel, Germany) at 200 rpm for 2 h. After being centrifuged at 10,000 × *g* for 10 min, the supernatant was filtered through Whatman No.1 filter paper. For the ethanolic extract of germinated rice bean, the same protocol was repeated with 80% (v v^{-1}) ethanol (Lou, Hsu and Ho, 2014). Both water and ethanolic extract were used to measure antioxidant activity as follows:

DPPH radical scavenging activity of both extracts was evaluated according to method of Brand-Williams *et al.* (1995) as modified by Wongsiri *et al.* (2015). A 1.5 ml of extracted sample was added to 1.5 ml of 0.15 mM DPPH in 80% ethanol solution. The mixture was vigorously vortexed for 1 min and left to stand at room temperature in the dark for 30 min. The absorbance value of resulting solution was measured at 517 nm. The standard curve was prepared using Trolox® in the range of 20-140 µM. The results were calculated and expressed as µmole Trolox® equivalent per gram of rice bean (d.b.).

FRAP of both extract was determined as described by Benzie and Strain (1996). The working FRAP reagent was prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6) with 2.5 ml of 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 40 mM hydrochloric acid solution and with 2.5 ml of 20 mM ferric chloride solution. A 1900 µl of the working FRAP reagent was incubated at 37°C for 30 min. Then 100 µl of the extract was added to the FRAP reagent and kept in the dark at room temperature for 30 min. The absorbance was measured at 593 nm. The standard curve was prepared using Trolox® in the range of 25-300 µM. The results were calculated and expressed as µmole Trolox® equivalent per gram of rice bean (d.b.).

3.2.1.9 Phenolic composition of germinated rice bean

The water and ethanolic extracts obtaining from both pretreated and non-pretreated rice bean at 0 and 18 h of germination from Section 3.2.1.8 were used for determination of phenolic compounds in the germinated rice bean extracts. Phenolic acid and flavonoid compositions were determined using Liquid Chromatography-Mass Spectrometry (LC-MS) (Bruker amaZon SL, IL, USA) equipped with an electrospray ionization (ESI) source. Separation was achieved on a Luna C18 column (2.1 × 150 mm, 3 µm) with flow rate 0.3 ml min^{-1}. The temperature of the column was maintain at 35°C and the injection volume was 10 µl. Mobile phase was the gradient of 1% acetic acid (mobile phase A) and acetonitrile (mobile phase B). The gradient conditions were as follows; the initial condition started with 10% B and was increased to 40% B at 15 min. The solvent gradient was held at 40% for additional 5 min, at 30 min gradient was increased to 60% B and at 60 min gradient was increased to 100% B. It was maintained at 100% B for an additional 10 min, and returning to the initial condition for 15 min for

the next analysis. Identification of phenolic acid and flavonoid compositions were carried out by comparing MS spectra of the unknown peaks with MS data of candidate molecules registered in the MassBank database (http://www.massbank.jp/).

3.2.2 Effect of hydrolysis period on degree of hydrolysis and antioxidant activity of germinated rice bean hydrolysate

The pretreated germinated rice bean at the germination period showing the highest amount of crude protein content and extractable phenolic content from Section 3.2.1 was used as the raw material while non-germinated rice bean without citric acid pretreatment was used as the control. The sample was blended with Waring blender then mixed with 0.1 M phosphate buffer (pH 6.0) containing 0.5 g l^{-1} $CaCl_2$ at a ratio of 1:2.5 (w:v). In order to prevent pasting of the slurry during heating, 1% (by dry weight of germinated rice bean) BAN® 240L was added into slurry. The enzyme-added slurry was heated at 70 °C in a water bath for 2 h., then, heating the slurry up to 85 °C for 20 min to stop enzyme activity. After being cool down to 50 °C, 5% (by dry weight of germinated rice bean) Flavourzyme® was added. The reaction was conducted at 50°C for 0, 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24 h. Then, the enzyme-added slurry was heated at 85 °C for 10 min to inactivate enzyme activity. The sample was then cooled down and centrifuged at 7,000 × g for 10 min. The supernatant was filtered through Whatman No.1 filter paper then analysed as follows:

3.2.2.1 Free amino group and degree of hydrolysis determination

Free amino group calculated according to the method of Adler-Nissen (1979). The 0.125 ml of rice bean hydrolysate solution was mixed with 2 ml of 0.2 M phosphate buffer, pH 8.2, and 2 ml of 0.05% trinitrobenzensluphonic acid (TNBS). The mixture was incubated at 50°C for 60 min. Then 4 ml of 0.1N hydrochloric acid was added. After the solution was cooled down to room temperature, the absorbance was measured at 420 nm. The amount of free amino group was calculated as shown in equation 3.2.

$$\text{Free amino group} = \frac{Absorbance\ 420\ nm \times ml\ of total\ \ reactant\ solution}{\varepsilon\ x\ 1\ cm\ x\ g\ of\ protein\ in\ the\ sample} \times 10^6 \qquad (3.2)$$

Where ε = molar extinction coefficient (20300 M^{-1} cm^{-1})

Degree of hydrolysis (DH) of hydrolysate is based on the measurement of free amino group generated from hydrolysis. The percentage of DH was calculated as shown in equation 3.3.

$$\text{DH}\ (\%) = \frac{(L_t - L_0)}{(L_{max} - L_0)} \times 100 \qquad (3.3)$$

Where L_0 = initial amount of free amino group in the solution

L_t = amount of free amino group in the solution at time t

L_{max} = amount of free amino group in the solution after acid hydrolysis

3.2.2.2 Extractable phenolic content

Extractable phenolic content of rice bean hydrolysate was determined using Folin-Ciocalteu reagent (Waterhouse, 2005) as stated in Section 3.2.1.2.

3.2.2.3 Antioxidant activities

DPPH radical scavenging activity and FRAP of rice bean hydrolysate samples were analysed using the method as described in Section 3.2.1.8.

Two rice bean hydrolysate samples were chosen for further study. The first sample was rice bean hydrolysate prepared from germinated rice bean (GRB) showing the highest amount of total phenolic content and antioxidant activity. The second was non-germinated rice bean hydrolysate without pretreatment (NGRB) subjected to the same hydrolysis period, as a comparative study.

3.2.3 Effect of foam-mat drying temperature and germination on drying kinetics and physico-chemical properties of foam-mat dried rice bean hydrolysate

Rice bean hydrolysate samples prepared from two conditions from Section 3.2.2 were selected for this study. The first sample was germinated rice bean hydrolysate (GRB) showing the highest amount of extractable phenolic content and antioxidant activity. For the second, non-germinated rice bean hydrolysate without citric acid pretreatment (NGRB), as a comparative study.

3.2.3.1 Effect of foam-mat drying temperature and germination on drying kinetics of germinated rice bean hydrolysate

Rice bean hydrolysate foam was achieved using an electric whisk for 2 min with addition of 1% (w v^{-1}) carboxy methyl cellulose (CMC) to obtain a stable foam. Foamed rice bean hydrolysate was spread uniformly with thickness of 1 cm on a stainless steel tray and dried in hot air oven (FD 240 Binder, Tuttlingen, Germany) at two different air temperatures (60 and 70 °C). The moisture content at the designated intervals was determined until the weight of the sample is constant. The obtained data was calculated for drying kinetics. Moisture content of foam during thin-layer drying was expressed in term of moisture ratio (*MR*) using equation 3.4.

$$MR = \frac{M_t - M_e}{M_i - M_e} \tag{3.4}$$

Where M_t = moisture content at a chosen time of drying

M_i = initial moisture content

M_e = equilibrium moisture content

The drying rate of foam-mat dried hydrolysate was calculated from the equation 3.5. The drying rate constant (k) value was determined from the slope values obtained by plotting ln (MR) versus drying time

$$\text{Drying rate} = k\,[\exp(-kt)] \times \text{dry solid} \tag{3.5}$$

Where k = drying constant (h^{-1})

t = drying time (h)

To select a suitable model for describing drying process of rice bean hydrolysate, drying curves were fitted with 9 thin-layer drying mathematic models which divided into 3 groups that were semi-theoretical model derived by analogues with Newton's law of cooling, semi-theoretical model derived from Fick's second law of diffusion and empirical model as shown in Table 2.1. The suitability of the fit of the proposed models was evaluated based on the primary statistic criteria, namely the mean

residual least square (*MRS*), root mean square error (*RMSE*) and correlation coefficient (*r*). The model with lowest values of *MRS* and *RMSE* and highest values of *r* was selected as the best model describing thin-layer drying characteristics of foam-mat dried rice bean protein hydrolysate.

3.2.3.2 Effect of foam-mat drying temperature and germination on physico-chemical properties of foam-mat dried rice bean hydrolysates

Rice bean hydrolysate of GRB prepared from Section 3.2.2 was fractionated into two different fractions using 3 kDa molecular weight cut off membrane ultrafiltration (Vivaflow 50 module, Sartorius AG, Göttingen, Germany). The non-fractionated rice bean hydrolysate (RH), ultrafiltration permeate hydrolysate (UFP) and ultrafiltration retentate hydrolysate (UFR) were then dried at different temperature (60 and 70 °C) using foam-mat drying technique as described in Section 3.2.3.1. Rice bean hydrolysate of NGRB samples subjected to the same process were used as a comparative study. All twelve foam-mat dried rice bean hydrolysate obtained from two drying temperatures were analysed as follows:

(1) Moisture content was determined using the method described in Section 3.2.1.1.

(2) Water activity was measured using a water activity meter (AquaLab, model AquaLink 3.0, WA, USA).

(3) Microstructure of foam-mat dried rice bean hydrolysate (only foam-mat dried RH) was studied using a scanning electron microscope (JEOL, JSM-5410LV, Tokyo, Japan). The sample was covered with gold in a sputter coater (Balzers, model SCD 040, Vaduz, Liechtenstein); then the sample surface was observed in a scanning electron microscope at a voltage of 12 kV with 200× of magnification.

(4) Extractable phenolic content of foam-mat dried rice bean hydrolysate, expressed as mg of gallic acid (GE) equivalents per gram dry weight of rice bean, was analysed using Folin-Ciocalteu method (Waterhouse, 2005) as stated in Appendix A.1.

(5) Total flavonoid content of foam-mat dried rice bean hydrolysate, expressed as mg catechin equivalents (CE) per gram dry weight of rice bean, was

measured based to aluminum chloride colorimetric assay (Zhishen, Mengcheng and Jianming, 1999) as stated in Appendix A.4.

(6) Phenolic composition of foam-mat dried rice bean hydrolysate was determined using High Performance Liquid Chromatography (HPLC) system (Shimadzu, Kyoto, Japan) consisting of an auto-sampler (SIL-20A, Shimadzu, Kyoto, Japan), photodiode array with multiple wavelength (SPD-20A) and solvent delivery module (LC-20AD, Shimadzu, Kyoto, Japan). Separation was achieved on a Gemini C18 column (4.6 mm × 250 mm, 5 µm) (Phenomenex, Cheshire, UK) at flow rate 0.8 ml min^{-1}. The temperature of column was maintained at 35°C and the injection volume was 10 µl. The photodiode array detector was set at wavelengths of 265, 275, 280, 320 and 360 nm. Two-phase gradient system consisting of 0.1% (v v^{-1}) formic acid (mobile phase A) and 70% (v v^{-1}) methanol. (mobile phase B). The gradient condition was as follows: the initial condition started with 5% B and increased to 10% B at 10 min, 12% B at 20 min, 30% B at 40 min, 100% at 60 min, held at 100% for 15 min, and return to initial condition for 5 min before the next analysis. Identification of phenolic compounds in sample was done on comparison with standard phenolic compound running under the same condition.

3.2.3.3 Antioxidant activities of foam-mat dried rice bean hydrolysate and its membrane ultrafiltration fractions

Foam-mat dried rice bean hydrolysate samples prepared from RH, UFP and UFR dried at 60 and 70 °C obtained from Section 3.2.3.2 were analysed as follows:

(1) Antioxidant activities measured using colorimetric method.

Antioxidant activities of foam-mat dried RH, UFP and UFR samples were determined using DPPH radical scavenging activity and FRAP. The solution was prepared from foam-mat dried rice bean hydrolysate at a concentration range of 0.125 to 2.5 mg dried hydrolysate per ml distilled water. The percentage of DPPH scavenging activity was calculated according to equation 3.6.

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A}{A_0} \times 100 \qquad (3.6)$$

where A_0 = absorbance value of DPPH without sample

A = absorbance value of sample and DPPH

The antioxidant activity of foam-mat dried rice bean expressed as the EC_{50}. The EC_{50} of the hydrolysate defined as the concentration of sample that reduces 50% of initial DPPH radical, calculated from the linear regression of plots of percentage of DPPH scavenging activity against the concentration of the samples. The DPPH radical scavenging activity was also expressed as μmole Trolox® equivalent per gram of rice bean (d.b.).

Ferric reducing antioxidant power (FRAP) of foam-mat dried hydrolysates was measured using the method described in Section 3.2.1.8. The measurement was performed using 1 mg dried hydrolysate per ml distilled water. FRAP activity was expressed as μmol Trolox® equivalent per gram sample (d.b.).

(2) Antioxidant activities measured using electron spin resonance (ESR) method

Only foam-mat dried RH samples were used to determine antioxidant activity using DPPH radical scavenging activity, hydroxyl radical scavenging activity and carbon-center radical scavenging activity as described below:

(2.1) The DPPH radical scavenging activity

The DPPH radical scavenging activity of foam-mat dried rice bean hydrolysate was measured following the method of Giri *et al.* (2011) with a slight modification. Foam-mat dried rice bean hydrolysate was prepared at the concentration of 1 mg ml^{-1}. A 50 μl of hydrolysate solution was added to 50 μl of DPPH solution (0.15 mM in 80 %, v v^{-1} ethanol), then mixed thoroughly using a vortex mixer. The reaction mixture was stored at room temperature in the dark for 15 min, then transferred into a 20 μl Pyrex® NMR capillary tube. The ESR spectra of DPPH radical was measured using JES-TE300 electron spin resonance (ESR) spectrometer (JEOL Ltd., Tokyo, Japan). The ESR spectrometry condition used for the DPPH radical scavenging activity assay was as follows: microwave power, 1.2 mW; microwave frequency, 9149.3 MHz; magnetic field, 325.5±25 mT; sweep time, 30 s. DPPH radical scavenging activity was calculated according to equation 3.8 (Peng, Xiong and Kong, 2009)

$$\text{DPPH radical scavenging activity (\%)} = \frac{H_0 - H}{H_0} \times 100 \qquad (3.8)$$

where H_0 = The height of the third resonance peak without hydrolysate

H = The height of the third resonance peak with hydrolysate

(2.2) The hydroxyl radical scavenging activity

Hydroxyl radicals were generated using the Fenton reaction according to the method of Je, Kim and Kim (2005). Foam-mat dried rice bean hydrolysate was prepared at the concentration of 1 mg ml^{-1}. A 20 µl of hydrolysate solution was mixed with 20 µl of 0.3 M of 5,5-dimethyl-1-pyroline-N-oxide (DMPO) and 20 µl of 10 mM $FeSO_4$. 10 mM phosphate buffer (pH 7.4) was used as the control. The reaction started with addition of 20 µl of 10 mM H_2O_2 into the solution, then mixed thoroughly using a vortex mixer, the reaction mixture was then transferred into 20 µl Pyrex® NMR capillary tube. The ESR spectra of DMPO-OH adduct was measured after 2.5 min using JES-TE300 electron spin resonance (ESR) spectrometer (JEOL Ltd., Tokyo, Japan) with the same condition as DPPH radical scavenging activity. Hydroxyl radical scavenging activity was calculated according to equation 3.9 (Peng *et al*., 2009).

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{H_0 - H}{H_0} \times 100 \qquad (3.9)$$

where H_0 = The height of the second resonance peak of mixture without hydrolysate

H = The height of the second resonance peak of mixture with hydrolysate

(2.3) Carbon-centered radicals scavenging activity

Carbon-centered radicals scavenging activity of foam-mat dried rice bean hydrolysate was evaluated. The carbon-centered radicals was generated by 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) according to the method of Giri *et al.* (2011). Foam-mat dried rice bean hydrolysate was prepared at the concentration of 1 mg ml^{-1}. A 20 µl of 1 mg ml^{-1} foam-mat dried hydrolysate solution

was mixed with 20 µl of 40 mM α-(4-pirydryl-1-oxide)-*N*-tert-butylnitrone (4-POBN) and 20 µl of 40 mM AAPH. The reaction mixture was then incubated at 37 °C for 30 min, then transferred to a 20 µl PyrexNMR capillary tube. The spin adduct was measured using JES-TE300 electron spin resonance (ESR) spectrometer (JEOL Ltd., Tokyo, Japan) with the same condition as DPPH radical scavenging activity. Carbon-centered radicals scavenging activity was calculated according to equation 3.10 (Peng *et al.*, 2009).

$$\text{Carbon-center radical scavenging activity (\%)} = \frac{H_0 - H}{H_0} \times 100 \qquad (3.10)$$

where H_0 = The height of the first resonance peak of mixture without hydrolysate

H = The height of the first resonance peak of mixture with hydrolysate

3.2.3.4 Effect of peptides and phenolics on antioxidant activity of foam-mat dried rice bean hydrolysate.

Phenolics and peptide of all twelve foam-mat dried rice bean hydrolysate samples obtained from Section 3.2.3.2 were separated using solid phase extraction (SPE) column (Isolute® C18 end-capped (EC), Biotage, Uppsala Sweden) according to the method of Irakli *et al.* (2012). The sample solution was prepared from foam-mat dried rice bean hydrolysate at concentration 10 mg dried rice bean hydrolysate per ml acidified (1N HCl) deionized water (pH 2.0). The SPE column was preconditioned with 3 ml of methanol and equilibrated with 3 ml of acidified (1N HCl) deionized water (pH 2.0). Then 1 ml sample solution was poured into column. The sample was separate into two fractions as follows:

(1) Unbound fraction (peptides) was eluted from SPE column with 3 ml acidified (1N HCl) deionized water (pH 2.0). This phenolic fraction was subjected to antioxidant determination. The DPPH radical scavenging activity and FRAP of peptide fraction were measured using the method as described in Section 3.2.1.8.

(2) Bound fraction (phenolics) was eluted from SPE column with 3 ml absolute methanol. This phenolic fraction was subjected to antioxidant determination.

The DPPH radical scavenging activity and FRAP of phenolic fraction were measured using the method as described in Section 3.

All experiments were done in triplicates. Analysis of variance (ANOVA) of the experimental data was performed. Duncan's New Multiple Range Test was used for evaluate testing the difference between means at the 95% confidence interval.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of pretreatment and germination period on chemical composition of germinated rice bean

4.1.1 Proximate analysis

Rice beans were pretreated with 1% (w v^{-1}) citric acid for 6 h prior to germination in the dark at room temperature (30±1 °C) for 0, 6, 12, 18 and 24 h then analysed for chemical composition. Non-pretreated rice bean, immersed in tap water for 6 h without pretreatment, was used as a control. As shown in Table 4.1, the length of germination period affected the changes in chemical composition of germinated rice bean. The moisture content of both pretreated rice bean and the control significantly increased during 24 h of germination period ($p \leq 0.05$). This was due to water absorption by seeds during the germination (Bewley and Black, 1983).

Crude protein content of pretreated rice bean and the control tended to increase during 24 h of germination period. However, the content was not significantly different ($p > 0.05$). The highest crude protein content of both pretreated rice bean and the control were observed after 18 h of germination. Kaur (2015) reported that crude protein content of germinated rice bean (after soaking for 12 h) was increased from 25.65% to 26.60% and 26.81% after germination at 37±1 °C for 24 and 48 h, respectively.

Carbohydrate and ash content of both pretreated rice bean and the control were not altered over the 24 h of germination period. However, reducing sugar content of germinated rice bean increased with increasing germination period. Increasing reducing sugar might be due to the degradation of starch into sugars which performed by amylolytic enzymes during germination process (Mayer and Poljakoff-Mayber, 1982). Goyoaga *et al.* (2011) found that reducing sugar content of two cultivars of faba bean, *Vicia faba* cv. Alameda and *Vicia faba* cv. Brocal, increased during 9 days of the germination process ($p \leq 0.05$).

Crude fat content of both pretreated rice bean and the control significantly increased at the first 6 h of germination period. This might result from lipid biosynthesis during early stage of germination. In lipid biosynthesis pathway, sucrose is converted

Table 4.1 Effect of pretreatment and germination period on chemical composition of germinated rice bean

Pretreatment/ Germination period (h)	Moisture (g 100 g^{-1})	Crude protein (g 100 g^{-1}, d.b.)	Carbohydrate (g 100 g^{-1}, d.b.)	Crude fat (g 100 g^{-1}, d.b.)	Ash (g 100 g^{-1},d.b.)	Total phenolic (mg of GAE g^{-1}, d.b.)	Reducing sugar (mg glucose g^{-1}, d.b.)
No pretreatment (the control)							
0	46.94^{a}±0.74	21.62^{a}±1.09	72.37^{b}±1.36	0.55^{b}±0.08	5.46^{ab}±0.23	1.17^{a}±0.09	1.52^{a}±0.15
6	56.72^{b}±0.70	22.84^{ab}±1.69	69.68^{ab}±1.82	1.30^{h}±0.07	6.17^{d}±0.08	1.71^{b}±0.07	1.90^{bc}±0.20
12	57.94^{b}±1.28	23.30^{ab}±1.01	69.57^{a}±0.79	0.94^{f}±0.04	6.19^{d}±0.24	1.74^{b}±0.04	2.20^{c}±0.12
18	57.72^{b}±0.92	24.57^{b}±0.92	68.74^{a}±0.88	0.89^{ef}±0.08	5.80^{bc}±0.20	2.20^{de}±0.12	2.04^{bc}±0.11
24	61.21^{c}±1.53	24.02^{ab}±0.76	69.77^{ab}±0.81	0.77^{d}±0.06	5.44^{ab}±0.11	2.41^{e}±0.21	2.95^{ef}±0.21
1% (w v^{-1}) citric acid							
0	45.57^{a}±1.08	23.04^{ab}±1.71	71.02^{ab}±1.93	0.66^{c}±0.05	5.28^{a}±0.27	1.22^{a}±0.07	1.72^{ab}±0.16
6	59.70^{c}±1.11	22.35^{ab}±0.39	70.28^{ab}±0.54	1.04^{g}±0.04	6.32^{d}±0.27	2.04^{cd}±0.22	2.61^{de}±0.14
12	59.88^{c}±0.42	23.32^{ab}±1.44	69.80^{ab}±1.30	0.88^{ef}±0.06	6.01^{cd}±0.22	1.94^{bc}±0.08	2.59^{d}±0.08
18	60.91^{c}±0.30	24.89^{b}±1.46	68.81^{a}±1.46	0.79^{de}±0.03	5.51^{ab}±0.11	2.83^{f}±0.16	2.85^{def}±0.32
24	60.12^{c}±0.22	24.35^{b}±1.96	69.79^{ab}±2.17	0.20^{a}±0.04	5.53^{ab}±0.25	3.11^{g}±0.24	3.08^{f}±0.34

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).
d.b., dry basis

into hexose phosphates and then to fructose-1,6-bisphosphate which is cleaved into triosphosphates. This triosphosphates is further reduced to glycerol-3-phosphate which serves as the glycerol backbone for triacylglycerol. The accumulation of triacylglycerol leads to an increase in plant lipids (Hildebrand, 2011). However, crude fat content significantly decreased after 6 h of germination ($p \leq 0.05$). Lipid is used as a source of energy during germination of seed. The degradation of lipid involves lipases in seed (Bewley and Black, 1983). Ghavidel and Prakash (2007) reported that germination for 24 h caused a significant ($p \leq 0.05$) increase in crude fat content in green gram (*Phaseolus aureus*), cowpea (*Vigna catjang*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*). Kim *et al.* (2013) also reported that lipid content of soybean (*Glycine max* var.) decreased from 10.1±0.21 to 9.7±0.15 % (w w^{-1}) after germination for 24 h.

4.1.2 Total phenolic content

Germination periods strongly affected total phenolic content of germinated rice bean with pretreated rice bean and the control. At 24 h of germination period, extractable phenolic content of pretreated rice bean and control were 2.06 and 2.55 times higher, respectively, than that of 0 h-germinated rice bean, presumably due to biosynthesis during germination (Randhir *et al.*, 2004). Most plant phenolics are derived from the Shikimate pathway. The biosynthesis of phenolics in this pathway is a sequence of seven metabolic steps beginning with condensation of phosphoenolpyruvate and D-erythrose-4-phosphate. The end product of Shikimate pathway is chorismate, which is the precursor for the synthesis of several aromatic secondary metabolites. Many enzymes involved in the Shikimate pathway and expression of these enzymes are depends on the environment condition (Herrmann and Weaver, 1999). Wongsiri *et al.* (2015) reported that phenolic content of germinated mung bean increased with increasing germination time over 0 - 24 h ($p \leq 0.05$), with germination for 24 h resulted in a 3.48-fold increase in phenolic content. In germinated green pea (*Pisum sativum*), lentil (*Lens culinaris*) and mung bean (*Vigna radiata*), a significant increase of phenolic content was observed after 3 days of germination (Świeca and Gawlik-Dziki, 2015).

Pretreatment with 1% (w v^{-1}) citric acid (pH 2-3) for 6 h before germination resulted in an increase in total phenolic content of germinated rice bean. The increase of total phenolic content of germinated rice bean might be due to adaptation of plant

under acidic stress. Reactions of plants towards environmental stress involve various types of physiological and biochemical responses depending on the genetic and biochemical make-up of the species. One of the defense mechanisms in plants is phenolic biosysthesis (Dubey, 1999; Mazid, Khan and Mohammad, 2011). According to the acid growth theory, external acidification causes cell wall loosening which might result in a release of free phenolics from plant cells during germination (Rayle and Cleland, 1992). McCue *et al.* (2000) reported that total phenolic content of 48 h-germinated pea seeds after pretreatment with 50 µM salicylic acid for 24 h was 13.6% higher than that without pretreatment. Hao *et al.* (2016) also found that pretreatment buckwheat with acidified water, pH 5.83, could promote the accumulation of rutin during 8 days of germination.

The phenolic content determination in plant food extracts might have a shortcoming as the presence of reducing sugar, ascorbic acid, and some amino acid which can react with Folin-Ciocalteu reagent such as cysteine, histidine and tyrosine. It was reported that the sample containing 25 mg of invert sugar per ml of solution was equivalent to an apparent phenol content of 0.005 mg gallic acid equivalent per ml of solution (Waterhouse, 2005). In this experiment, the amount of reducing sugar in each treatment was less than 3.08 mg g^{-1} dry bean (Table 4.1), while only 0.15-0.26 mg g^{-1} dry bean of ascorbic acid was found in 16 rice bean genotypes collected from India (Katoch, 2013) and total amount of cysteine, histidine and tyrosine in this experiment was lower than 0.2 mg g^{-1} dry bean. These small amount of reducing sugar, ascorbic acid and amino acids in rice bean should not contribute a significant interference on total phenolic content analysis. Thus, the increase in total phenolic content during 24 h of bean germination should be mainly from phenolic biosynthesis and/or release of phenolics caused by cell wall loosening.

4.1.3 Total nitrogen, protein nitrogen and non-protein nitrogen content.

Table 4.2 shows that total nitrogen content of germinated beans from both pretreated rice bean and the control tended to increase during 24 h of germination period. There was a significant increase in protein nitrogen during 24 h of germination but no significant change in the amount of non-protein nitrogen. Thus, an increase in the total nitrogen content of both pretreated rice bean and the control might be mainly depends on an increase in protein nitrogen content. Narsih, Yunianta and Harijono

(2012) also found an increase in crude protein content in sorghum during 36 h of germination period while no change in the content of non-protein nitrogen was observed. From our results, pretreatment before germination affected the amount of protein nitrogen in germinated rice bean. It was found that the 24 h-germinated rice beans showed significantly higher protein nitrogen content compared to the control at the same germination period ($p \leq 0.05$).

Table 4.2 Effect of pretreatment and germination period on nitrogen content and TCA-soluble peptide of germinated rice bean

Pretreatment	Germination period (h)	Total nitrogen (%, w w^{-1}, d.b.)	Protein nitrogen (%, w w^{-1}, d.b.)	Non-protein nitrogen^{ns} (%, w w^{-1}, d.b.)	TCA-soluble peptide (g tyrosine 100 g^{-1}, d.b.)	Total free amino acid (g 100 g^{-1}, d.b.)
No pretreatment (water)	0	$3.46^{a}\pm0.17$	$2.21^{a}\pm0.22$	1.25 ± 0.27	$0.40^{d}\pm0.02$	$0.41^{b}\pm0.03$
	6	$3.65^{ab}\pm0.27$	$2.51^{abcd}\pm0.04$	1.14 ± 0.24	$0.32^{b}\pm0.01$	$0.33^{a}\pm0.03$
	12	$3.73^{ab}\pm0.16$	$2.54^{abcd}\pm0.14$	1.19 ± 0.30	$0.37^{c}\pm0.02$	$0.47^{bc}\pm0.01$
	18	$3.93^{b}\pm0.15$	$2.62^{bcd}\pm0.23$	1.31 ± 0.10	$0.24^{a}\pm0.01$	$0.74^{ef}\pm0.02$
	24	$3.84^{ab}\pm0.12$	$2.47^{abc}\pm0.16$	1.37 ± 0.11	$0.25^{a}\pm0.02$	$1.76^{g}\pm0.01$
1% (w v^{-1}) citric acid	0	$3.69^{ab}\pm0.27$	$2.30^{ab}\pm0.26$	1.38 ± 0.07	$0.40^{cd}\pm0.02$	$0.58^{d}\pm0.03$
	6	$3.58^{ab}\pm0.06$	$2.54^{abcd}\pm0.20$	1.04 ± 0.17	$0.38^{cd}\pm0.01$	$0.72^{e}\pm0.02$
	12	$3.73^{ab}\pm0.23$	$2.59^{bcd}\pm0.18$	1.14 ± 0.32	$0.37^{c}\pm0.01$	$0.80^{f}\pm0.06$
	18	$3.98^{b}\pm0.23$	$2.83^{cd}\pm0.26$	1.15 ± 0.18	$0.30^{b}\pm0.01$	$0.56^{d}\pm0.04$
	24	$3.90^{b}\pm0.31$	$2.86^{d}\pm0.04$	1.04 ± 0.28	$0.31^{b}\pm0.02$	$0.53^{cd}\pm0.02$

Chulalongkorn University

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).

^{ns} Mean values not significantly different ($p > 0.05$) in the same column.

d.b., dry basis.

4.1.4 TCA-soluble peptide and free amino acid content

Peptides and amino acids are considered as the main constituents of non-protein nitrogen in legume seeds (Delrot *et al.*, 2001). In this study the total amount of oligopeptide and free amino acid were determined as TCA-soluble peptide. For both pretreated rice bean and the control, TCA-soluble peptides in beans with different periods of germination, shown in Table 4.2, significantly decreased during 24 h of germination period ($p \leq 0.05$), whereas total free amino acid significantly increase with

increasing germination period (Figure 4.1). Since TCA-soluble peptides content is the sum of oligopeptide and free amino acid, a decrease of TCA-soluble peptides content in rice bean was most likely due to a decrease in oligopeptides. Decreasing of oligopeptides during the first phase of germination, initial water uptake of seed, might be because these oligopeptides were substrates for protein synthesis (Bewley, 1997). Müntz *et al.* (2001) reported that increasing in free amino acid during seed germination was mostly from hydrolysis of seed storage protein to free amino acid or biosynthesis of amino acid. Luo *et al.* (2014) reported that after 72 h of germination period, free amino acid content of green and white faba bean (*Vicia faba* L.) were 11.3 and 8.0 times higher than that of 0 h-germinated faba bean, respectively. From our results, pretreatment caused an increase in the amount of free amino acids such as alanine, arginine, aspartic, glutamic, proline and serine including antioxidant amino acids (cysteine, methionine, tyrosine, phenylalanine and histidine) during first 12 h of germination (Figure 4.1). This might be due to stress-induced synthesis of amino acids during seed germination. Other environmental stresses also reported to have an effect on free amino acid composition in plant. Shen *et al.* (2015) reported that free amino acid composition of 10 h-germinated rice (*Oryza sativa* L.) after oxygen-deficit stress was higher than that of untreated germinated rice.

Although free amino acid is one of the constituent of TCA-soluble peptide, it was surprising that the result of total free amino acid in this study was higher than that of TCA-soluble peptide content. This might be due to the different principle of determination technique. TCA-soluble peptide content was determined based on Lowry assay. This assay involves two reactions. First, a protein-Cu^{2+} complex is formed and the change in protein conformation at high pH (pH 13) results in exposure of tyrosine and tryptophan. The second one is a redox reaction with Folin-Ciocaltue, the reaction results in a strong blue color which absorbs strongly at 750 nm. While free amino acid in the sample was determined using HPLC. In this technique, free amino acid was derivatized with AccQ•Tag™. The separation of amino acids was done in Waters AccQ–Taq amino acid analysis column. The total free amino acid was calculated from sum of each free amino acid.

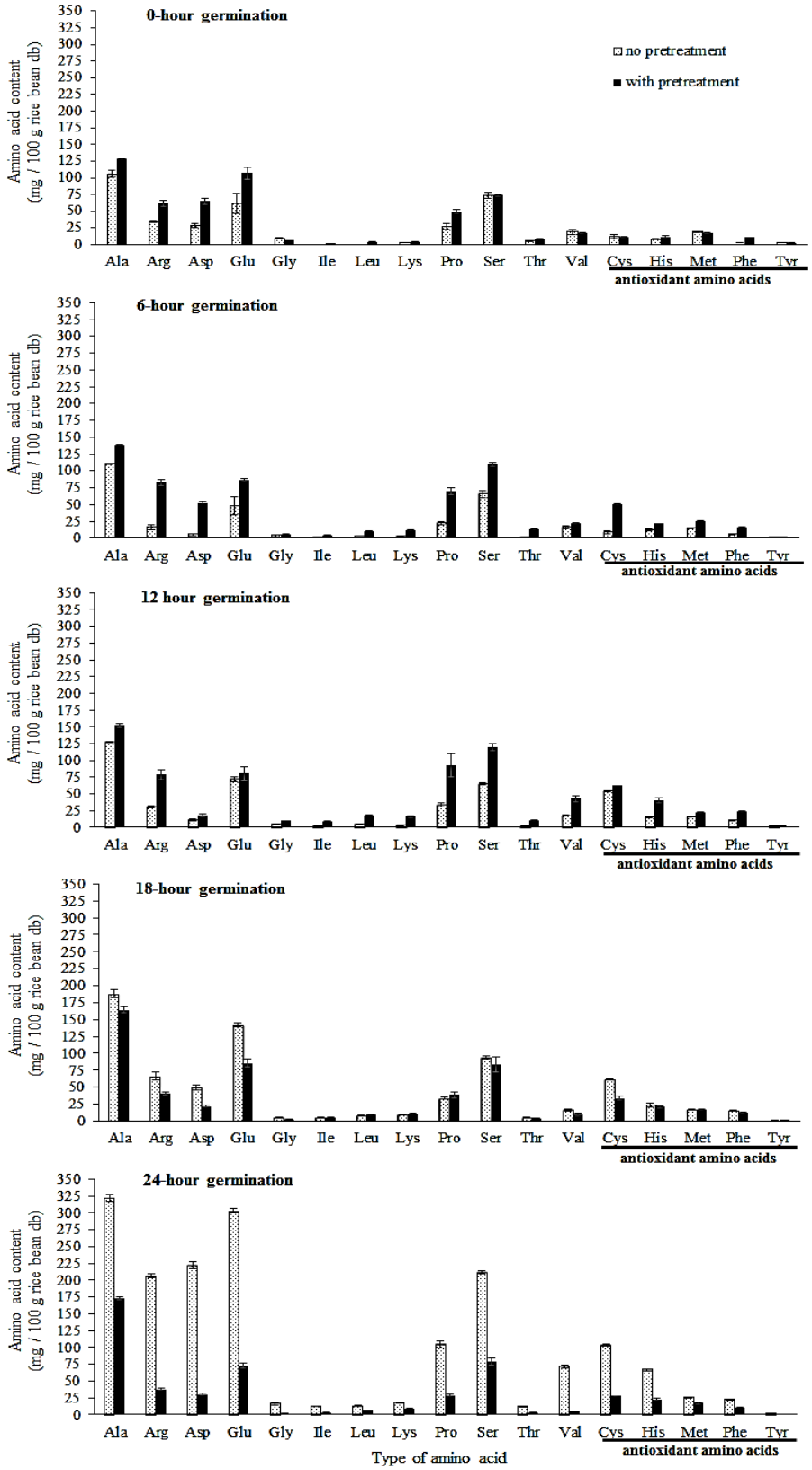

Figure 4.1 Effect of pretreatment and germination period on free amino acid composition of germinated rice bean

4.1.5 Protein pattern

Figure 4.2 shows the SDS-PAGE patterns of protein extracted from germinated rice beans with different periods of germination. The raw volume calculated from the area of intensity peak of non-pretreat and pretreated germinated rice bean determined using GeneTools software (Syngene, Frederick, MD) was shown in Appendix B.1. The results showed that germination period did not significantly affect a change in protein band pattern in non-pretreat germinated rice bean. However, the pretreatment before germination led to a 12.48-30.67% and 7.71-82.28% decrease in the intensity of protein band with MW of 51 and 76 kDa, respectively, in the samples with 12-24 h of germination. A decrease in the intensity of protein band with a MW of 51 and 76 kDa might be because an acidic stress from the citric acid pretreatment caused degradation of storage protein during germination of rice bean. Chehregani and Kavianpour (2007) reported that acidic stress from nitric acid pretreatment (pH 2.0) also resulted in a decrease in the intensity of a protein band with MW of 83 kDa in kidney bean (*Phaseolus vulgaris* L.).

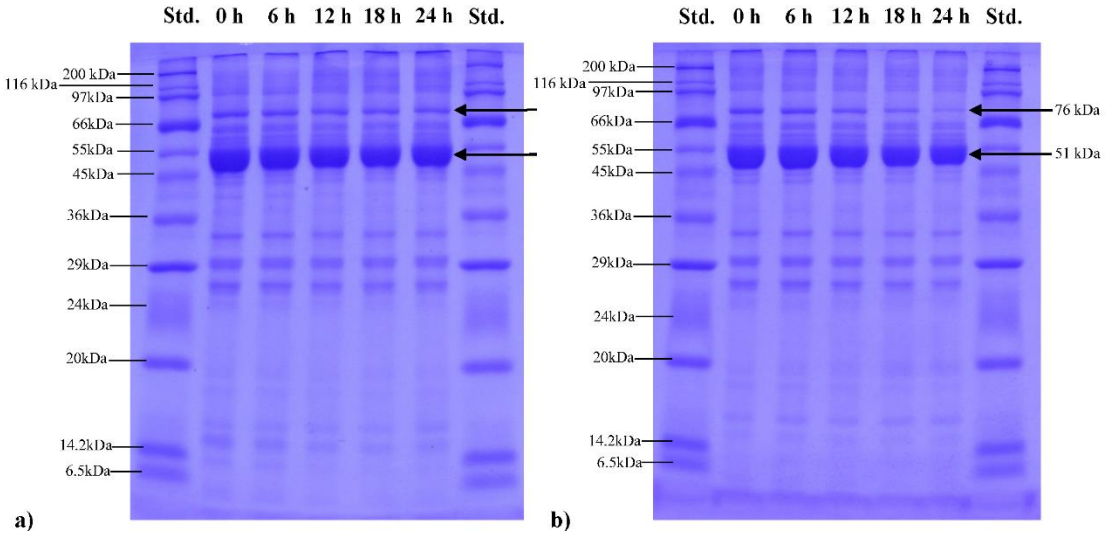

Figure 4.2 The SDS-PAGE patterns of protein extracted from germinated rice bean. Std: standard molecular weight marker; (a) The germinated rice bean under no pretreatment; and (b) The germinated rice bean with pretreatment

The storage proteins of legume are classified into four groups according to their solubility (1) albumin, soluble in water, (2) globulins, soluble in salt solution but insoluble in water, (3) prolamins, soluble in alcohol water mixture but insoluble in water and salt solution, and (4) glutelin, soluble only soluble in dilute acid or alkaline

solutions (Boulter and Derbyshire, 1976). Albumins (6.13-7.47%) and globulin (13.11-15.56%) are the major portion of protein in rice bean proteins (Katoch, 2013). The protein bands with MW of 20-24 kDa and 27-73 kDa are likely to be the basic subunit and the acidic subunit of 11S globulin. The protein bands with MW of 50 and 76 kDa are likely to be β-subunit and α-subunit of 7S globulin (Raut, Kharat and Mendhulkar, 2015).

4.1.6 Antioxidant activity

Table 4.3 presents antioxidant activities of germinated rice bean extracts obtaining from two different extraction solvents. The results pointed that germination period affected antioxidant activity of germinated rice bean extracts. DPPH radical scavenging activity and FRAP of the extract from both treatments significantly increased as germination period increased ($p \leq 0.05$). This trend was found in both water and ethanolic extracts. The effect of pretreatment resulted in a higher increase in antioxidant activities of both extracts.

Table 4.3 Effect of extraction solvent on antioxidant activity of germinated rice bean under no pretreatment and with pretreatment

Pretreatment	Germination period (h)	Antioxidant activity			
		DPPH assay (μmol Trolox g^{-1} dry bean)		FRAP assay (μmol Trolox g^{-1} dry bean)	
		Water extract	Ethanol extract	Water extract	Ethanol extract
No Pretreatment (water)	0	$6.65^{a}\pm0.35$	$9.88^{a}\pm0.28$	$2.69^{a}\pm0.18$	$3.99^{a}\pm0.07$
	6	$9.68^{b}\pm0.53$	$12.85^{b}\pm0.42$	$4.08^{b}\pm0.51$	$6.55^{b}\pm0.18$
	12	$12.45^{d}\pm0.74$	$17.53^{d}\pm0.82$	$4.50^{bc}\pm0.14$	$6.55^{b}\pm0.29$
	18	$13.49^{d}\pm0.54$	$18.38^{de}\pm0.46$	$4.68^{c}\pm0.24$	$6.67^{b}\pm0.23$
	24	$15.00^{e}\pm0.47$	$20.08^{f}\pm1.14$	$4.89^{cd}\pm0.38$	$7.08^{c}\pm0.27$
1% (w v^{-1}) citric acid	0	$6.57^{a}\pm0.38$	$9.32^{a}\pm0.22$	$2.65^{a}\pm0.18$	$4.01^{a}\pm0.19$
	6	$11.44^{c}\pm0.56$	$14.81^{c}\pm0.71$	$4.64^{c}\pm0.26$	$6.87^{bc}\pm0.07$
	12	$13.35^{d}\pm0.84$	$19.01^{e}\pm0.36$	$5.03^{cd}\pm0.30$	$6.91^{bc}\pm0.32$
	18	$15.72^{e}\pm0.89$	$20.60^{f}\pm0.27$	$5.32^{d}\pm0.24$	$7.21^{c}\pm0.18$
	24	$15.47^{e}\pm0.24$	$20.62^{f}\pm0.25$	$5.34^{d}\pm0.18$	$7.22^{c}\pm0.16$

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).
d.b., dry basis.

With increasing germination period, an increase in antioxidant activity of extracts from both pretreated rice bean and the control could be explained through increasing the amount of TPC (Table 4.1), serve as antioxidants due to their free radical scavenging activity or metal chelating ability. Moreover, incresing antioxidant amino acids (cysteine, methionine, tyrosine, phenylalanine and histidine) during germination (Figure 4.1). Cysteine and methionine are sulfur-containing amino acids which potentially oxidizable. Tyrosine and phenylalanine are containing aromatic side chains which hydrogen is easily removed. Moreover, Histidine's imidazole-containing side chain is also oxidatively labile (Elias, Kellerby and Decker, 2008). The higher antioxidant activities (DPPH radical scavenging activity and FRAP) and TPC of germinated seeds compared to raw seeds were also exhibited in germinated seeds of alfalfa (*Medicago sativa* L.), lentil (*Lens sculenta* L.), mung bean (*Vigna radiate* L.), onion (*Allium cepa* L.), broccoli (*Brassica oleraceae* L.), red cabbage (*Brassica oleraceae capitata rubra* L.) and radish (*Raphanus sativus japonicum* L., *Raphanus sativus rambo* L., *Raphanus sativus sinicum rosae* L.) (Aguilera *et al.*, 2015). Fouad and Rehab (2015) also found that germination process caused a significant increase in free amino acid which due to activity of proteolytic enzymes, increase of phenolic which might be due to biosynthesis and bioaccumulation of phenolic compounds as a defensive mechanism, and increase DPPH radical scavenging activity which might be due to increase of polyphenol during germination.

The results demonstrated that the influence of extraction solvents on antioxidant activities of rice bean extract was remarkable. DPPH radical scavenging activity and FRAP of the ethanolic extract at each germination period from both pretreated rice bean and the control were 1.2-1.6 and 1.4-1.6 times higher than those of the water extract, respectively. Since each phenolics and flavonoids, as potential antioxidant, have a different polarity. The different polarity of extraction solvents (water and 80% (v v^{-1}) ethanol) may affect the solubility of these compounds, and consequently affect the antioxidant activities of each extract. The different assays resulted in different antioxidant activity measured in both extracts. This can be explained by the involved mechanism.

Antioxidants can reduce radicals by two main mechanisms. The first is single electron transfer (SET). The SET-based methods detect the ability of an antioxidant to

transfer one electron to reduce radicals. The second is hydrogen atom transfer (HAT). HAT-based methods measure the ability of an antioxidant to quench free radicals by hydrogen donation. DPPH radical scavenging activity is based on both SET and HAT mechanism, while FRAP assay is based on only SET (Balogh, Hegedűs and Stefanovits-Bányai, 2010). Ye *et al.* (2015) also reported that DPPH radical scavenging activity and FRAP of sunflower (*Helianthus annuus* L.) extracts obtained from 90% (v v^{-1}) ethanol were significantly higher than those of water extract ($p \leq 0.05$).

4.1.7 Phenolic composition

Figure 4.3 shows the chromatograms of phenolic compounds in both water and ethanolic extracts obtaining from both pretreated rice beans and the control at 0 and 18 h of germination period. For water extract, it was found that both pretreatment and germination period did not affect the type of extractable phenolic compounds. Five phenolic compounds were found in both pretreated and non-pretreated rice bean at both germination periods. There were one phenolic acids (4-hydroxybenzoic acid) and four flavonoids (robinin, rutin, astragalin and 7-methylquercetin). Pretreatment before germination and germination period also did not affect the type of extractable phenolic compounds in ethanolic extract. However, the number of phenolic compounds found in ethanolic extract was higher than that of water extract. This was because different types of phenolic acids and flavonoids was extracted depending on the polarity of solvents. In the ethanolic extract, seven phenolic compounds were found. There were two phenolic acids (4-hydroxybenzoic acid and sinapic acid) and five flavonoids (rutin, astragalin, epicatechin, 7-methylquercetin and genistein). The results from both extractions solvent showed that flavonoids are the main composition of phenolic compound in germinated rice bean extract. Flavonoids in plant can be divided into flavonoid aglycones (hydrophobic flavonoid or hydrophobic antioxidant) and flavonoid glycosides (hydrophilic flavonoid and hydrophilic antioxidant), in which one or more hydroxyl groups of the aglycones are bound to a sugar (Kumar and Pandey, 2013).

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

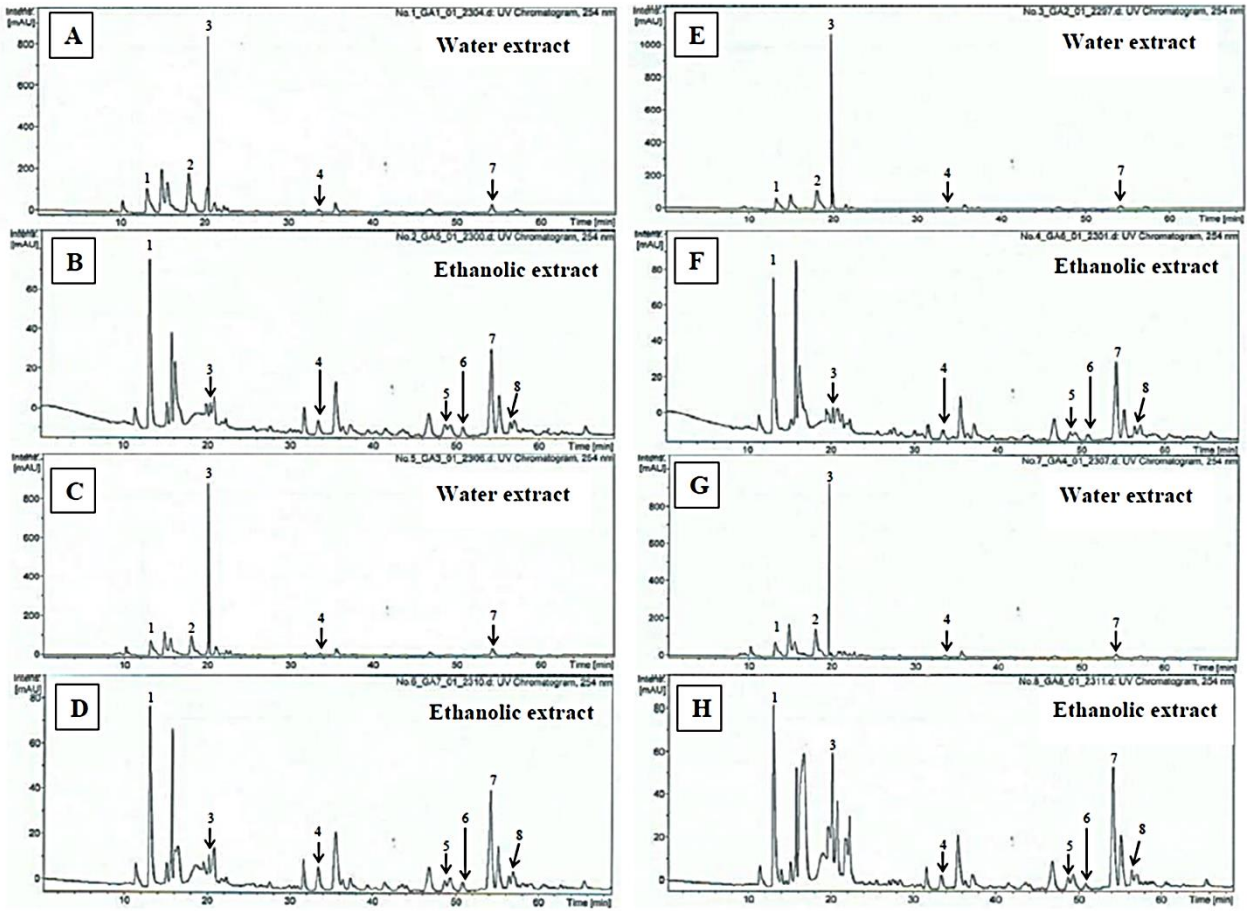

Figure 4.3 HPLC chromatograms of phenolic compounds in germinated rice bean extract: control at 0 h of germination (A)-(B), pretreated rice bean at 0 h of germination (C)-(D), non-pretreated rice bean at 18 h of germination (E)-(F) and pretreated rice bean at 18 h of germination (G)-(H). Peak identifications: (1) 4-hydroxybenzoic acid, (2) robinin, (3) rutin, (4) astragalin, (5) epicatechin, (6) sinapic acid, (7) 7-methylquercetin, (8) genistein

In germinated rice bean extract, epicatechin, 7-methylquercetin and genistein are hydrophobic antioxidant while, robinin, rutin, astragalin are hydrophilic antioxidant. Water and ethanol are both polar solvents. However, ethanol has lower polarity than water. Thus, using 80% (v v^{-1}) ethanol as extraction solvent would give higher efficiency to extract hydrophobic phenolics (epicatechin, 7-methylquercetin and genistein) than water. The different polarity of extraction solvent also affects phenolic composition of Beijing propolis extract (Sun *et al.*, 2015). Twenty-four phenolic compounds, not only nonpolar compounds but also weak-polar and polar compounds, were found in propolis extracts obtained using 75% (v v^{-1}) ethanol. By contrast, in water extract, only fifteen phenolic compounds main polar phenolic acids were found. Some of phenolic compounds found in our result were also similar to rice bean from china. Yao *et al.* (2012) reported that three phenolic acids (*p*-coumaric acid, ferulic acid, and sinapic acid) and five flavonoids (catechin, epicatechin, vitexin, isovitexin and quercetin) were found in ethanolic extract of rice beans from China.

Since crude protein content, TPC and antioxidant activities of pretreated germinated rice bean at 18 and 24 h of germination period were not significantly different ($p>0.05$), the 18 h-germinated rice bean with pretreatment (GRB) was selected as a source for protein hydrolysate for this study while non-germinated rice bean with no pretreatment (NGRB) was used for comparative study.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

4.2 Effect of hydrolysis period on degree of hydrolysis and antioxidant of germinated rice bean protein hydrolysate

The GRB and NGRB were hydrolyzed using 5% (in dried weight of germinated rice bean) Flavourzyme®. The reaction was conducted at 50°C and pH 6.0 for 0, 2, 4, 6, 8, 10, 12, 15, 18, 21 and 24 h as described in Section 3.2.2 The obtained germinated rice bean protein hydrolysates were analysed for free amino group content and degree of hydrolysis, TPC and antioxidant activities.

4.2.1 Free amino group content and degree of hydrolysis

Figure 4.4 showed that free amino group of protein hydrolysis of GRB and NGRB significantly increased with increasing hydrolysis period ($p\leq0.05$). This was

because Flavourzyme® broke down the peptide bonds and generates amino acid groups during enzymatic hydrolysis.

Germination led to an increase in free amino group of hydrolysate. The amount of free amino group of hydrolysate of GRB was 1.23 times higher than that of hydrolysate of NGRB after hydrolysis for 24 h (Figure 4.4). This was due to activity of natural proteolytic enzyme during germination (Müntz, 1996) and also to changes in protein structure which facilitated enzyme access to the protein.

.

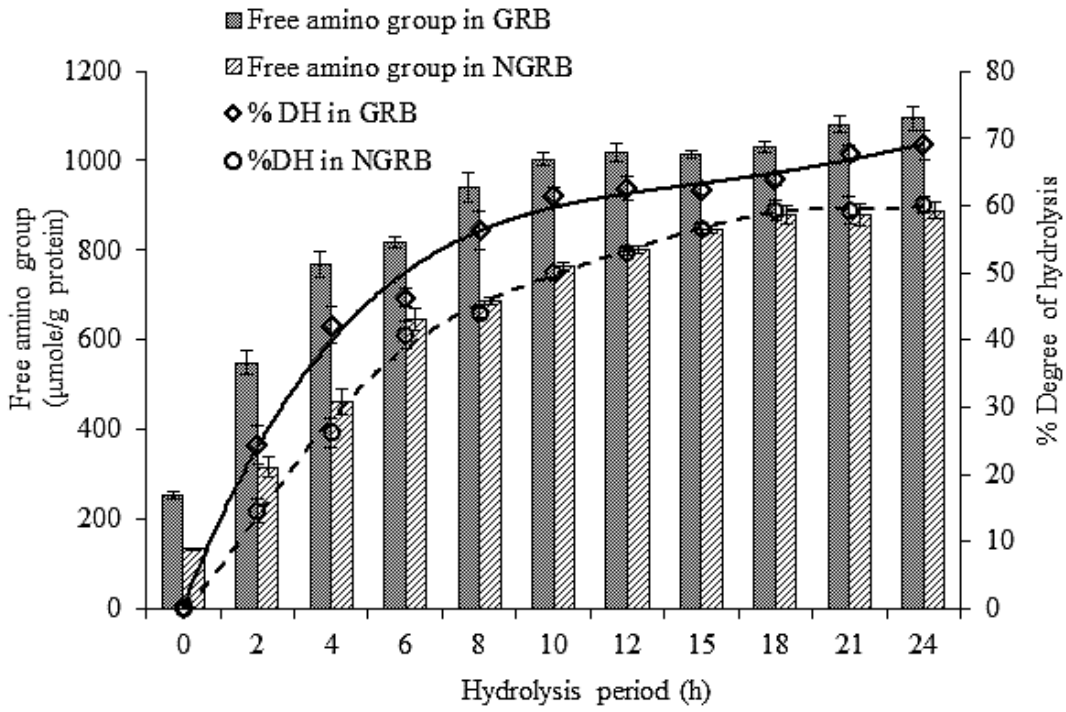

Figure 4.4 Effect of hydrolysis period on free amino groups and degree of hydrolysis of protein hydrolysate of germinated rice bean (GRB) and non-germinated rice bean (NGRB)

Since DH was converted from the amount of free amino group using the equation 3.2 in Section 3.2.2, the greater amount of free amino group, the higher DH of hydrolysate obtained (Figure 4.4). (Monteros *et al.* (2011)) reported that DH of soybean (*Glycine max* var. *Crystalline*) protein hydrolysate, obtained from sequential enzymatic hydrolysis with pepsin and pancreatin for 1 and 2 h, respectively, increased from 79.13% to 82.04% after germination for 6 days. Luis *et al.* (2012) also reported that DH of germinated lima bean (*Phaseolus lunatus*) protein hydrolysate hydrolysed with

Alcalase® 2.4 L for 2 h was 7.66% higher than that of non-germinated lima bean protein hydrolysate

4.2.2 Extractable phenolic content

As shown in Table 4.4, TPC of protein hydrolysate from both GRB and NGRB significantly increased with increasing hydrolysis period ($p \leq 0.05$). An increase of TPC during enzymatic hydrolysis was most likely due to changing of tertiary structure of protein which affects the hydrophobic interaction between polyphenol and protein, resulting in a release of polyphenol from protein-polyphenol complex. Dias *et al.* (2010) reported that protein hydrolysates from common bean (*Phaseolus vulgaris* L. var. *Nanus*) flour, hydrolysed with Trypsin 250 and protease from *Bacillus* sp. (UFLA 817CF) for 5 h, showed a significant increase in TPC compared to non-treated flour. In pinto bean (*Phaseolus vulgaris* L. var. *Pinto*) protein hydrolysate, a significant increase of TPC ($p \leq 0.05$) was also observed after 2 h of enzymatic hydrolysis with Alcalase® 2.4L and Savinase® (Garcia-Mora *et al.*, 2015).

Germination before enzymatic hydrolysis strongly affected TPC of rice bean protein hydrolysate. After 24 h of hydrolysis, TPC of GRB hydrolysate was 1.21 times higher than that of NGRB hydrolysate (Table 4.4). The higher amount of TPC of hydrolysate of GRB was presumably due to biosynthesis during germination (Randhir *et al.*, 2004) and effect of cell wall loosening, according to acid growth theory, leads to release of phenolic compounds.

4.2.3 Antioxidant activities of protein hydrolysate

Table 4.4 shows that antioxidant activities of protein hydrolysate from both GRB and NGRB significantly increased with increasing hydrolysis period ($p \leq 0.05$). Germination led to an increase in antioxidant activity of hydrolysate. The result showed that after 24 h of hydrolysis, DPPH radical scavenging activity and FRAP of hydrolysate of GRB were 1.51-2.00 and 1.93-2.49 times, respectively, higher than that of hydrolysate of NGRB (Table 4.4). Increasing antioxidant activities of hydrolysate of GRB compared to hydrolysate of NGRB can be explained through the increased amount of TPC and free amino acids, play an important role in antioxidant activities, which resulting from germination process (Table 4.1 and Figure 4.1). Sefatie *et al.* (2013) reported that DPPH radical scavenging activity and hydroxyl radical-scavenging

activity of 3-day germinated black soybean (*Glycine max* L. *Merrill*) protein hydrolysate, prepared from protein isolate by sequential enzymatic hydrolysis using pepsin for 2 h followed by pancreatin for 2 h, were significantly higher than those of non-germinated black soybean. Rocha *et al.* (2015) also found that the germination of common bean for 24-72 h considerably enhanced the oxygen radical absorbance capacity of common bean protein hydrolysate prepared from protein concentrate using Alcalase® 2.4L for 1 h.

Table 4.4 Effect of hydrolysis period on extractable phenolic content and antioxidant activities of germinated rice bean (GRB) and non-germinated rice bean (NGRB) hydrolysate

Hydrolysis period (h)	Extractable phenolic content (mg gallic acid g^{-1} dry bean)		Antioxidant activities			
			DPPH radical scavenging activity (µmol Trolox® g^{-1} dry bean)		FRAP (µmol Trolox® g^{-1} dry bean)	
	GRB[1]	NGRB[2]	GRB[3]	NGRB[4]	GRB[3]	NGRB[4]
0	$2.38^{a}\pm0.06$	$1.35^{a}\pm0.06$	$12.19^{a}\pm0.42$	$6.10^{a}\pm0.24$	$5.20^{a}\pm0.05$	$2.11^{a}\pm0.12$
2	$3.06^{b}\pm0.13$	$1.62^{b}\pm0.08$	$13.13^{b}\pm0.29$	$6.62^{b}\pm0.18$	$5.68^{b}\pm0.21$	$2.39^{b}\pm0.09$
4	$3.31^{c}\pm0.04$	$1.86^{c}\pm0.02$	$13.48^{b}\pm0.10$	$7.48^{c}\pm0.29$	$5.73^{b}\pm0.29$	$2.60^{bc}\pm0.11$
6	$3.51^{d}\pm0.11$	$2.02^{d}\pm0.03$	$14.70^{c}\pm0.27$	$8.00^{d}\pm0.16$	$6.67^{c}\pm0.31$	$2.69^{c}\pm0.08$
8	$3.92^{e}\pm0.11$	$2.35^{e}\pm0.06$	$15.75^{d}\pm0.40$	$8.08^{d}\pm0.13$	$7.01^{d}\pm0.13$	$2.81^{c}\pm0.33$
10	$4.18^{f}\pm0.08$	$2.56^{f}\pm0.07$	$16.30^{e}\pm0.03$	$9.02^{e}\pm0.22$	$7.14^{de}\pm0.15$	$3.22^{d}\pm0.11$
12	$4.35^{g}\pm0.04$	$2.66^{fg}\pm0.09$	$16.47^{ef}\pm0.31$	$9.14^{ef}\pm0.26$	$7.19^{de}\pm0.18$	$3.28^{d}\pm0.08$
15	$4.37^{g}\pm0.01$	$2.72^{g}\pm0.07$	$16.56^{ef}\pm0.40$	$9.46^{f}\pm0.30$	$7.47^{e}\pm0.14$	$3.26^{d}\pm0.16$
18	$4.54^{h}\pm0.12$	$2.85^{h}\pm0.10$	$17.00^{fg}\pm0.23$	$10.58^{g}\pm0.20$	$7.39^{ef}\pm0.29$	$3.47^{d}\pm0.12$
21	$4.84^{i}\pm0.05$	$2.95^{hi}\pm0.10$	$17.46^{g}\pm0.14$	$11.54^{h}\pm0.25$	$7.75^{fg}\pm0.08$	$3.83^{e}\pm0.01$
24	$4.86^{i}\pm0.02$	$2.99^{i}\pm0.08$	$17.50^{g}\pm0.35$	$11.62^{h}\pm0.23$	$7.84^{g}\pm0.05$	$3.84^{e}\pm0.04$

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$)

[1] Moisture content of GRB was 60.91%

[2] Moisture content of NGRB was 46.94%

[3] 100 ml of GRB hydrolysate equivalent to 32.96 g dry bean

[4] 100 ml of NGRB hydrolysate equivalent to 44.79 g dry bean

Table 4.4 shows the highest TPC, DPPH radical scavenging activity, and FRAP of both rice bean protein hydrolysate were observed after 24 h of hydrolysis. However, the values were not significantly different from those of 21 h of hydrolysis (p>0.05). To study on the effect of foam-mat drying on properties of rice bean protein hydrolysate in the next step. GRB hydrolyzed for 21 h was chosen for further study while NGRB subjected to the same hydrolysis period was used as comparative study.

4.3 Effect of foam-mat drying temperature on drying kinetics and physico-chemical properties of foam-mat dried rice bean hydrolysate

GRB and NGRB hydrolyzed using 5% (in dried weight of germinated rice bean) Flavourzyme®. The obtained hydrolysate of GRB and NGRB (total soluble solids of 16.2±0.35 and 14.93±0.31% (w w^{-1}), respectively) were then foamed using 1% (w v^{-1}) CMC. Foamed hydrolysate of GRB and NGRB were dried at two different air drying temperature. The moisture content during drying process was determined for calculation of drying kinetics and physico-chemical properties of foam-mat dried samples were determined.

4.3.1 Drying kinetics

Figure 4.5 presents drying curve and drying rate of foam-mat dried rice bean hydrolysate at drying temperatures of 60 and 70 °C. Foam-mat drying of rice bean hydrolysate took about 420 and 300 min to reach moisture equilibrium at 60 and 70 °C, respectively (Figure 4.5a). The results showed that the drying temperature had a significant effect on foam-mat drying time. Increasing drying temperature resulted in decreasing drying time. This is because high temperature caused an increase in mobility of the water molecule which leads to reduce internal resistance to moisture transport and increase the water pressure in foods (Michailidis, 2014). The obtained results were generally in agreement with many literature studies on foam-mat drying of various food, for example, banana (Sankat and Castaigne, 2004), mandarin (Kadam *et al.*, 2011), tomato (Kadam *et al.*, 2012), cherry (Abbasi and Azizpour, 2016), and shrimp (Azizpour, Mohebbi and Khodaparast, 2016).

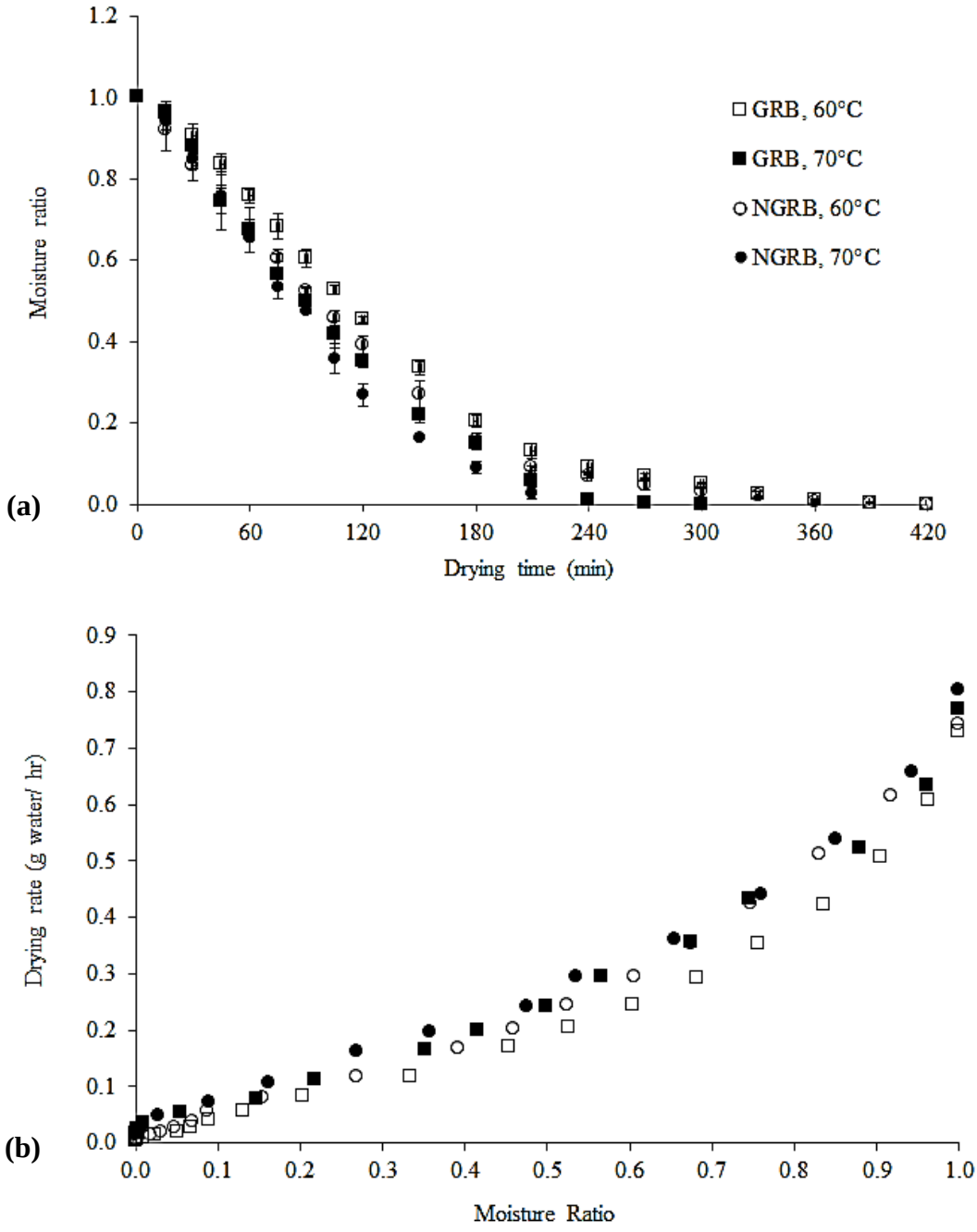

Figure 4.5 Drying curve (a) and drying rate (b) of foam-mat dried rice bean hydrolysate prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) at drying temperature of 60 and 70 °C

The drying rate of both hydrolysate samples is presented in Figure 4.5 b. The drying rate decreased with a decrease in moisture ratio. At the same MR drying rate at drying temperature 70 °C was higher than 60 °C. The falling rate periods can be observed in the whole of drying rate curves according to the change in the drying rate curves. The free water in the sample moves toward the surface during drying and it evaporated at the surface. The falling rate period continues as long as the free water is present in the sample (Okos and Narsimhan, 1992).

It can be seen that drying rate of hydrolysate of GRB was slightly lower than that of hydrolysate of NGRB at both drying temperatures. It is possible that water-soluble compounds in rice bean hydrolysate influence the drying rate of foam-mat drying. Higher amount of total soluble solids in protein hydrolysate of GRB (16.20±0.35 %, w w^{-1}) compared to that in hydrolysate of NGRB (14.93±0.31 %, w w^{-1}) resulted in more bound water in hydrolysate of GRB, which would then retard the rate and amount of moisture loss during drying.

To develop a mathematic model for predicting the foam-mat drying kinetic at different drying temperatures and different treatment of samples. Thin layer drying equations were used to estimate the drying curve for foam-mat dried rice bean protein hydrolysate. The experimental data of the foam-mat drying of rice bean protein hydrolysate were fit into nine mathematical models, divided into three groups according to their derivation, as listed in Table 2.1 in Section 2.4.2. The first group of models derived from Newton's law of cooling which based on heat transfer. The second group of models derived from Fick's second law of diffusion which based on mass transfer. The third group of models is grouped as empirical models which derived based on the experimental data. As shown in Table 4.5, the results showed that all three groups of models have a high potential for describing the drying process of foam-mat drying of rice bean hydrolysate as indicated by *MRS*, *RMSE* and *r*. As the porous structure of foam and the thin layer of the sample (1 cm thickness), the heat and mass transfer occur simultaneously during foam-mat drying process. Thus, foam-mat drying characteristic of rice bean hydrolysate can be predicted using models derived from either Newton's law of cooling or Fick's second law of diffusion.

Table 4.5 The thin-layer drying mathematical model for foam-mat dried hydrolysate of GRB and NGRB

Model	Coefficient								*MRS*	*RMSE*	*r*
	a	*b*	*c*	*d*	*h*	*g*	*k*	*n*			
Semi-theoretical models											
Model derived from Newton's law of cooling:											
Newton											
GRB, 60°C	-	-	-	-	-	-	0.4304	-	7.05×10^{-2}	2.58×10^{-2}	0.8902
GRB, 70°C	-	-	-	-	-	-	0.5309	-	5.65×10^{-3}	7.26×10^{-2}	0.9922
NGRB, 60 °C	-	-	-	-	-	-	0.5007	-	2.86×10^{-3}	5.20×10^{-2}	0.9947
NGRB, 70 °C	-	-	-	-	-	-	0.5820	-	6.08×10^{-3}	7.53×10^{-2}	0.9905
Page											
GRB, 60°C	-	-	-	-	-	-	0.2721	1.5585	7.45×10^{-5}	8.40×10^{-3}	0.9997
GRB, 70°C	-	-	-	-	-	-	0.3884	1.5091	3.22×10^{-4}	1.73×10^{-2}	0.9989
NGRB, 60 °C	-	-	-	-	-	-	0.3864	1.3622	2.79×10^{-4}	1.63×10^{-2}	0.9990
NGRB, 70 °C	-	-	-	-	-	-	0.4264	1.5840	1.45×10^{-4}	1.16×10^{-2}	0.9995
Model derived from Fick's second law of diffusion:											
Henderson and Pabis											
GRB, 60°C	1.1218	-	-	-	-	-	0.4872	-	3.58×10^{-3}	5.82×10^{-2}	0.9888
GRB, 70°C	1.1105	-	-	-	-	-	0.5951	-	3.67×10^{-3}	5.85×10^{-2}	0.9881
NGRB, 60 °C	1.0756	-	-	-	-	-	0.5418	-	2.09×10^{-3}	4.45×10^{-2}	0.9929
NGRB, 70 °C	1.1121	-	-	-	-	-	0.6508	-	4.44×10^{-2}	6.21×10^{-2}	0.9868

GRB, germinated rice bean
NGRB, non-germinated rice bean

Table 4.5 (*continued*) The thin-layer drying mathematical model for foam-mat dried hydrolysate of GRB and NGRB

Model	Coefficient								*MRS*	*RMSE*	*r*
	a	*b*	*c*	*d*	*g*	*h*	*k*	*n*			
Logarithmic											
GRB, 60°C	1.2288	-	-0.1439	-	-	-	0.3648	-	1.79×10^{-3}	4.12×10^{-2}	0.9934
GRB, 70°C	1.3014	-	-0.2357	-	-	-	0.3923	-	1.05×10^{-3}	3.13×10^{-2}	0.9959
NGRB, 60 °C	1.1385	-	-0.0901	-	-	-	0.4417	-	1.06×10^{-3}	3.17×10^{-2}	0.9957
NGRB, 70 °C	1.2532	-	-0.1786	-	-	-	0.4656	-	1.83×10^{-3}	4.14×10^{-2}	0.9930
Midilli											
GRB, 60°C	0.9935	0.0002	-	-	-	-	0.2656	1.5797	7.04×10^{-5}	8.17×10^{-3}	0.9997
GRB, 70°C	1.0064	-0.0078	-	-	-	-	0.3903	1.4199	1.89×10^{-4}	1.32×10^{-2}	0.9993
NGRB, 60 °C	0.9755	-0.0010	-	-	-	-	0.3584	1.4094	2.10×10^{-4}	1.41×10^{-2}	0.9991
NGRB, 70 °C	0.9912	-0.0023	-	-	-	-	0.4142	1.5868	1.12×10^{-4}	1.02×10^{-2}	0.9996
Two-term											
GRB, 60°C	0.5609	0.4872	0.5609	0.4872	-	-	-	-	3.58×10^{-3}	5.82×10^{-2}	0.9888
GRB, 70°C	0.5553	0.5951	0.5553	0.5951	-	-	-	-	5.85×10^{-2}	3.67×10^{-3}	0.9881
NGRB, 60 °C	0.5378	0.5418	0.5378	0.5418	-	-	-	-	2.09×10^{-3}	4.45×10^{-2}	0.9929
NGRB, 70 °C	0.5561	0.6508	0.5561	0.6508	-	-	-	-	4.13×10^{-3}	6.21×10^{-2}	0.9868

GRB, germinated rice bean
NGRB, non-germinated rice bean

Table 4.5 (*continued*) The thin-layer drying mathematical model for foam-mat dried hydrolysate of GRB and NGRB

Model	Coefficient								*MRS*	*RMSE*	*r*
	a	*b*	*c*	*d*	*g*	*h*	*k*	*n*			
Modified Henderson and Pabis											
GRB, 60°C	0.3739	0.3739	0.3739	-	0.4872	0.4872	0.4872	-	3.58×10^{-3}	5.82×10^{-2}	0.9888
GRB, 70°C	0.3702	0.3702	0.3702	-	0.5951	0.5951	0.5951	-	3.67×10^{-3}	5.85×10^{-2}	0.9881
NGRB, 60 °C	0.3586	0.3586	0.3586	-	0.5418	0.5418	0.5418	-	2.09×10^{-3}	4.45×10^{-2}	0.9929
NGRB, 70 °C	0.3707	0.3707	0.3707	-	0.6508	0.6508	0.6508	-	4.13×10^{-3}	6.21×10^{-2}	0.9868
Empirical models:											
Wang and Singh											
GRB, 60°C	-0.3231	0.0259	-	-	-	-	-	-	1.37×10^{-3}	3.61×10^{-2}	0.9966
GRB, 70°C	-0.3975	0.0386	-	-	-	-	-	-	9.00×10^{-4}	2.90×10^{-2}	0.9979
NGRB, 60 °C	-0.3646	0.0326	-	-	-	-	-	-	5.73×10^{-4}	2.33×10^{-2}	0.9977
NGRB, 70 °C	-0.4384	0.0473	-	-	-	-	-	-	1.26×10^{-3}	3.43×10^{-2}	0.9969
Weibull distribution											
GRB, 60°C	0.0016	-0.9920	-	-	-	-	0.2657	1.5818	7.02×10^{-5}	8.16×10^{-3}	0.9997
GRB, 70°C	1.0013	-0.0071	-	-	-	-	0.3850	1.4385	1.97×10^{-4}	1.36×10^{-2}	0.9992
NGRB, 60 °C	-0.0077	-0.9839	-	-	-	-	0.3578	1.4007	2.07×10^{-4}	1.40×10^{-2}	0.9992
NGRB, 70 °C	-0.0118	-1.0035	-	-	-	-	0.4124	1.5763	1.09×10^{-4}	1.01×10^{-2}	0.9996

GRB, germinated rice bean
NGRB, non-germinated rice bean

Weibull distribution model is the best model for predict foam-mat drying kinetics of protein hydrolysate of GRB dried at 60 °C, and protein hydrolysate of NGRB dried at 60 and 70 °C, while Midilli model was the best fit for foam-mat drying of protein hydrolysate of GRB dried at 70 °C. MRS and RMSE of these two models were very low which in range of 7.02×10^{-5}–2.07×10^{-4} and 8.16×10^{-3}–1.40×10^{-2}, respectively. Moreover, the *r* value was in range of 0.9992-0.9997. These above criteria indicated that Weibull distribution and Midilli model were much more suitable to describe the drying characteristics of rice bean protein hydrolysate foam. The obtained models can be used to predicting moisture ratio, drying rate or drying time of foam-mat drying of both protein hydrolysate of GRB and NGRB

4.3.2 Physico-chemical properties

The hydrolysate of GRB and NGRB were fractionated using 3 kDa molecular weight cut off membrane ultrafiltration. Fractionates were named as non-fractionated rice bean hydrolysate (RH), ultrafiltration permeate rice bean hydrolysate (UFP) and ultrafiltration retentate rice bean hydrolysate (UFR). All three fractions prepared from both of GRB and NGRB hydrolysate were then foam-mat dried, with the aid of 1% (w v^{-1}) CMC, at 60 and 70 °C until the moisture content was in the range of 8.0-8.5 %. All twelve foam-mat dried samples were used subjected to extractable phenolic content, total flavonoid content, phenolic composition and antioxidant activity analysis.

4.3.2.1 Moisture content and water activity

Table 4.6 shows some properties of foam-mat dried rice bean hydrolysate prepared from both GRB and NGRB hydrolysate and their peptide fractions. The final moisture content of all foam-mat dried rice bean hydrolysate samples obtained from both drying temperatures were in the range of 8.0-8.5 % (d.b.). All samples showed the same range of a_w (0.406-0.412), indicating that the amount of free water remaining in all samples was the same. The results showed that foam-mat drying method is an effective method to reduce the moisture content and a_w of foamed rice bean hydrolysate to lower value with short drying time. Rajkumar *et al.* (2007) reported that the moisture content of foamed alphonso mango pulp (*Mangifera indica* L.), using 10% (w v^{-1}) egg albumen with 0.5% (w v^{-1}) CMC as foaming agent, was reduced from 79.75±0.05% to 5.56±0.03% (w.b.) after foam-mat drying at 60 °C for 35 minutes.

Table 4.6 Properties of foam-mat dried germinated rice bean (GRB) and non-germinated rice bean (NGRB) hydrolysate and its fraction

Sample	Properties			
	Moisture content[ns] (% d.b.)	Water activity[ns]	Extractable phenolic content (mg GAE g^{-1} dry bean)	Extractable flavonoid content (mg CE g^{-1} dry bean)
Rice bean hydrolysate				
GRB, 60 °C	8.51±0.22	0.410±0.005	4.14^{g}±0.04	0.22^{f}±0.01
GRB, 70 °C	8.27±0.28	0.408±0.006	3.83^{f}±0.05	0.19^{e}±0.00
NGRB, 60 °C	8.54±0.45	0.409±0.009	2.84^{e}±0.07	0.22^{f}±0.00
NGRB, 70 °C	8.32±0.32	0.409±0.010	2.63^{d}±0.14	0.18^{d}±0.00
Permeate fraction (<3kDa)				
GRB, 60 °C	8.54±0.23	0.408±0.005	3.97^{fg}±0.04	0.17^{c}±0.00
GRB, 70 °C	8.26±0.26	0.407±0.005	3.85^{f}±0.19	0.16^{c}±0.00
NGRB, 60 °C	8.53±0.24	0.410±0.011	2.68^{de}±0.05	0.16^{c}±0.01
NGRB, 70 °C	8.08±0.77	0.406±0.007	2.39^{c}±0.20	0.16^{c}±0.00
Retentate fraction (≥3kDa)				
GRB, 60 °C	8.58±0.11	0.409± 0.006	1.77^{b}±0.09	0.08^{b}±0.00
GRB, 70 °C	8.27±0.05	0.412± 0.010	1.15^{a}±0.12	0.08^{b}±0.00
NGRB, 60 °C	8.48±0.13	0.411± 0.013	1.75^{b}±0.11	0.07^{a}±0.00
NGRB, 70 °C	8.11±0.68	0.408± 0.016	0.97^{a}±0.04	0.07^{a}±0.01

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).

[ns] Mean values not significantly different ($p > 0.05$) in the same column.

1 g of foam-mat dried GRB hydrolysate equivalent to 14.10 ml of hydrolysate, which 100 ml of GRB hydrolysate equivalent to 32.96 g dry bean.

1 g of foam-mat dried NGRB hydrolysate equivalent to 14.12 ml hydrolysate, which 100 ml of NGRB hydrolysate equivalent to 44.79 g dry bean

Kaushal, Sharma and Sharma (2013) found that the final moisture content of foam-mat dried seabuckthorn pulp (*Hippophae salicifolia*), using 2% (w v^{-1}) CMC as foaming agent, after drying at 55 °C for 4 h was 12.9 % (w.b.). Krasaekoopt and Bhatia (2012) reported that a_w of foam-mat dried yogurt after drying at 50, 60 and 70 °C was 0.355, 0.348 and 0.323, respectively.

4.3.2.2 Microstructure

Figure 4.6 shows microstructure of foam-mat dried rice bean hydrolysate samples observed under a scanning electron microscope (SEM). It was found that drying temperature affects the particle morphology of both foam-mat dried rice bean hydrolysate prepared from GRB and NGRB hydrolysate. The individual irregular particles were observed in foam-mat dried samples after drying at 60 °C, while fused particles were observed after drying at 70 °C (Figure 4.6 a, b, c, d). These might be because the stability of foam during foam-mat drying process was affected by drying time and drying temperature (Fernandes *et al.*, 2012). It was found that the collapse of the foam, at both drying temperature, increases with increasing the drying time. However, increasing drying temperature led to increasing pressure in the air cells. The air cells then erupted and collapsed. The surface of foam-mat dried rice bean hydrolysate samples after drying at 60 °C observed at 500× magnification was slightly different to that of drying at 70 °C (Figure 4.6 e, f, g, h). The difference might be due to different formation of thin layer of collapsed foam covering at the surface of foam-mat dried rice bean particle. Both particles from foam-mat dried rice bean hydrolysate and foam-mat dried yacon (*Smallanthus sonchifolius*) juice after drying at 60 °C (Franco *et al.*, 2016) showed irregular structure. They had similar particle size, about 1.0-1.5 μm.

4.3.2.3 Extractable phenolic content and extractable total flavonoid content

The average concentration of extractable phenolics in rice bean hydrolysate prepared from GRB and NGRB was decreased 14.46% and 3.73%, respectively after drying at 60 °C , while extractable phenolics of GRB and NGRB was decreased 20.87% and 10.85%, respectively after drying at 70 °C (Table 4.4 and 4.6).

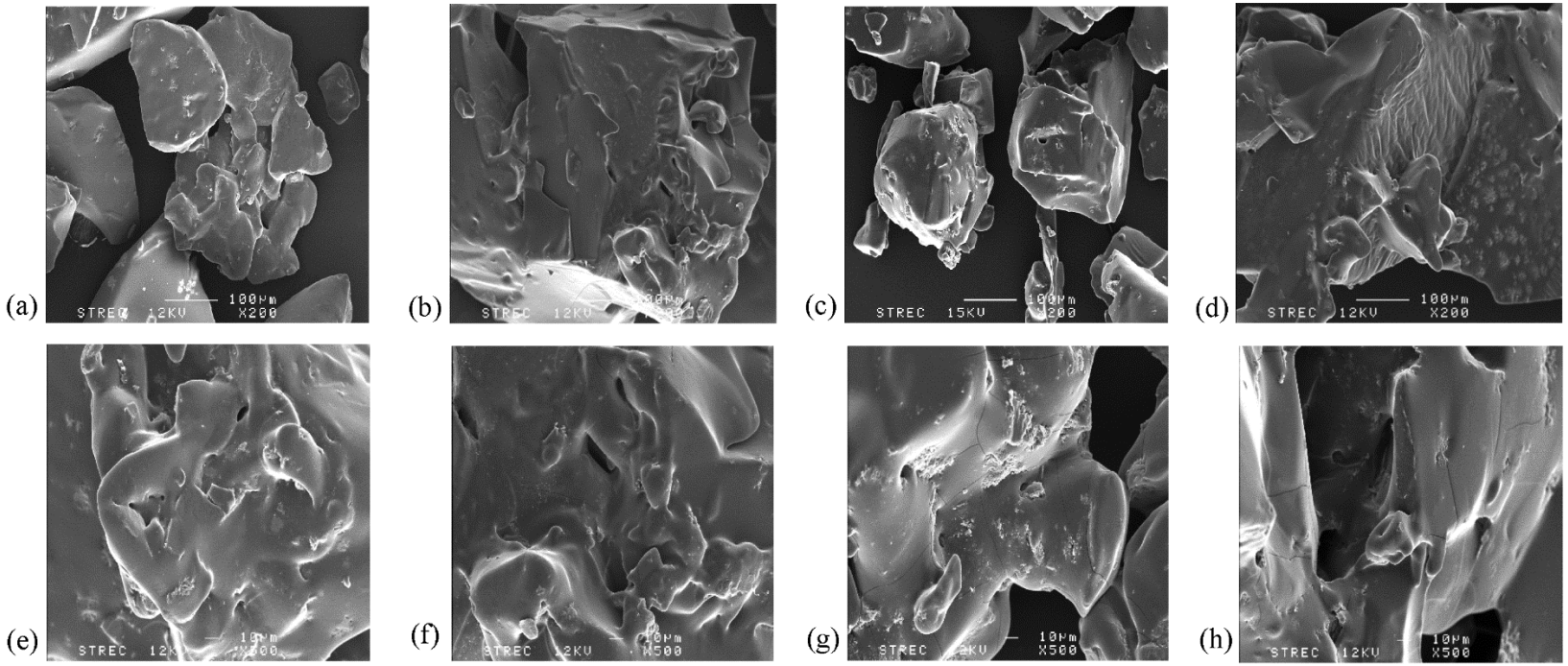

Figure 4.6 Microstructure of foam-mat dried rice bean protein hydrolysate:

(a) Foam-mat dried protein hydrolysate prepared from GRB dried at 60 °C at 200× magnification
(b) Foam-mat dried protein hydrolysate prepared from GRB dried at 70 °C at 200× magnification
(c) Foam-mat dried protein hydrolysate prepared from NGRB dried at 60 °C at 200× magnification
(d) Foam-mat dried protein hydrolysate prepared from NGRB dried at 70 °C at 200× magnification
(e) Foam-mat dried protein hydrolysate prepared from GRB dried at 60 °C at 500× magnification
(f) Foam-mat dried protein hydrolysate prepared from GRB dried at 70 °C at 500× magnification
(g) Foam-mat dried protein hydrolysate prepared from NGRB dried at 60 °C at 500× magnification
(h) Foam-mat dried protein hydrolysate prepared from NGRB dried at 70 °C at 500× magnification

As shown in Table 4.6, extractable phenolics and extractable flavonoid content of foam-mat dried RH decreased with increasing air drying temperature ($p \leq 0.05$). A decrease of extractable phenolics and extractable flavonoid content at higher drying temperature could be due to the binding of phenolic compounds with other components (such as, proteins and peptides) or the changes in the chemical structure of phenolic compounds which cannot be extracted nor determined by photometric methods (Julkunen-Tiitto, 1985). Moreover, Asami *et al.* (2003) reported that drying at temperature higher than 60 °C might cause oxidative condensation or degradation of heat sensitive compound such as (+)-catechin in air dried marionberry, strawberry, and corn. A significant decrease of extractable phenolics and extractable flavonoid content in foam-mat dried product resulting from increasing of drying temperature was also found in foam-mat dried mixed vegetable (bitter gourd, tomato and cucumber) juice (Chandrasekar *et al.*, 2015).

Table 4.6 shows that extractable phenolic in foam-mat dried RH prepared from GRB was significantly higher than that prepared from NGRB ($p \leq 0.05$). Even though extractable flavonoid content of RH prepared from GRB was higher than that prepared from NGRB. The values were not significantly different ($p > 0.05$). This could be due to the fact that extractable phenolic of RH prepared from GRB after enzymatic hydrolysis for 21 h was 1.64 times higher than that of PH prepared from NGRB (Table 4.4), resulting in higher amount of extractable phenolic content and extractable flavonoid content in corresponding foam-mat dried samples.

As shown in Table 4.6, extractable phenolic content and extractable flavonoid content in the peptide fraction collected from UFP were significantly higher than those of UFR ($p \leq 0.05$). This result indicated that foam-mat dried rice bean hydrolysate contained higher amount of low molecular weight phenolic compounds with molecular weight less than 3 kDa compared to those with molecular weight higher than 3 kDa. The samples of low molecular weight phenolic compounds identified in beans are phenolic acids and flavonoids. The highly polymerized polyphenols such as tannin (molecular weights ranging from 500-5000 Da) are commonly found in cell wall. However, extractable phenolic content is depends on the amount of each individual phenolic compound. Therefore, identification and quantification of phenolic compounds in each fraction were carried out in this study. However, it was surprising

that sum of extractable phenolic content in UFP and UFR was higher than extractable phenolic content in RH. This might be due to the higher molecular weight fraction contained some phenolics with molecular weight less than the cut off values of the membrane as result of fouling of membrane ultrafiltration, or due to the fact that some small phenolic compounds may have affinity for some of the large peptide. These results in overestimated levels of extractable phenolic content in UFR fraction.

4.3.2.4 Phenolic composition

Figure 4.7 and Table 4.7 show a HPLC chromatogram of phenolic compound and the contents of individual phenolic compound in foam-mat dried RH. The chromatogram in Figure 4.7 shows twelve phenolic compounds (gallic acid, catechol, 4-hydroxybenzoic acid, (+)-catechin, vanillin, epicatechin, *p*-coumaric acid, ferulic acid, rutin, *trans*-cinnamic acid, naringenin and quercetin) which were found in foam-mat dried PH.

Table 4.7 shows that gallic acid, catechol, epicatechin and rutin were the major compounds present in foam-mat dried RH. Drying temperature did not affect the type of phenolic compounds found in foam-mat dried samples. However, it affected the amount of each phenolic compound in the foam-mat dried samples. The amount of each phenolic compound in foam-mat dried RH tended to decrease with increasing drying temperature (Table 4.7). The decrease in phenolic compound content might be due to chemically converted or decomposition of some phenolic compound as described above. Our results strongly supported the effect of drying temperature on a decrease in TPC found in foam-mat dried rice bean protein hydrolysate (Table 4.6).

The content of most of phenolic compounds (vanillin, epicatechin, *p*-courmaric acid, ferulic acid, rutin and *trans*-cinnamic) in foam-mat dried RH prepared from GRB were significantly higher than those of prepared from NGRB ($p \leq 0.05$) (Table 4.7). This might be due to the higher initial phenolic content of hydrolysate before drying of RH prepared from GRB compared to NGRB (Table 4.4).

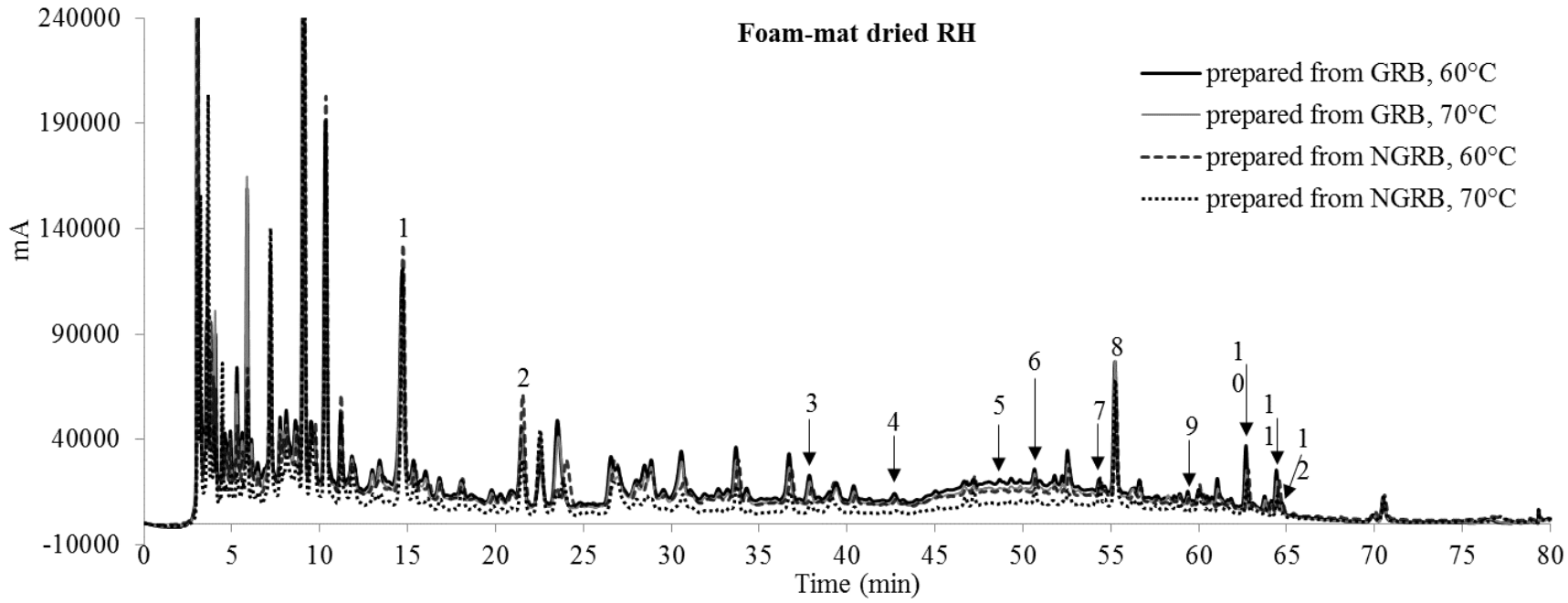

Figure 4.7 HPLC chromatogram of foam-mat dried non-fractionated protein hydrolysate (PH) prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) dried at 60 and 70 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic acid, (11) naringenin, (12) quercetin

Table 4.7 Identification and quantification of phenolic compounds in foam-mat dried non-fractionated rice bean hydrolysate (RH) prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) dried at 60 and 70 °C

Peak no.	Compound	Retention time (min)	λ_{max} (nm)	Phenolic content (mg g^{-1} dry sample)			
				GRB dried at 60 °C	GRB dried at 70 °C	NGRB dried at 60 °C	NGRB dried at 70 °C
1	Gallic acid	14.7	275	2.79^{c}±0.24	2.04^{a}±0.04	2.48^{b}±0.06	1.94^{a}±0.07
2	Catechol	21.5	275	1.91^{c}±0.04	1.76^{b}±0.06	1.80^{bc}±0.08	1.55^{a}±0.05
3	4-Hydroxybenzoic acid	38.1	265	0.04^{b}±0.00	0.03^{a}±0.00	0.03^{a}±0.00	0.03^{a}±0.00
4	(+)-Catechin	42.7	275	0.25^{b}±0.02	0.23^{a}±0.01	0.23^{ab}±0.01	0.21^{a}±0.00
5	Vanillin	48.6	280	0.05^{c}±0.00	0.04^{b}±0.00	0.03^{a}±0.00	0.03^{a}±0.00
6	Epicatechin	50.7	275	1.40^{c}±0.08	1.25^{bc}±0.10	1.21^{ab}±0.10	1.07^{a}±0.06
7	*p*-Coumaric acid	54.0	320	0.13^{c}±0.00	0.10^{b}±0.00	0.10^{ab}±0.01	0.08^{a}±0.01
8	Ferulic acid	55.1	320	0.70^{c}±0.03	0.69^{c}±0.02	0.64^{b}±0.02	0.52^{a}±0.04
9	Rutin	59.4	360	0.89^{d}±0.04	0.58^{a}±0.05	0.73^{c}±0.03	0.65^{b}±0.04
10	*trans*-Cinnamic acid	62.8	275	0.21^{b}±0.01	0.21^{b}±0.01	0.18^{a}±0.01	0.17^{a}±0.00
11	Naringenin	64.1	280	0.63^{c}±0.04	0.55^{ab}±0.03	0.59^{bc}±0.02	0.52^{a}±0.02
12	Quercetin	64.9	360	0.17^{b}±0.03	0.12^{a}±0.01	0.12^{a}±0.01	0.09^{a}±0.00

Mean values followed by different superscript letters in the same row are significantly different ($p \leq 0.05$)

GRB, germinated rice bean

NGRB, non-germinated rice bean

Chulalongkorn University

Figure 4.8 and Table 4.8 show a HPLC chromatogram of phenolic compound and the amount of individual phenolic compound in foam-mat dried UFP. The chromatogram of foam-mat dried UFP was similar to that of prepared from RH (Figure 4.7 and 4.8). This result indicated that most phenolic compounds in foam-mat dried rice bean protein hydrolysate had a molecular weight less than 3 kDa. Gallic acid, catechol, epicatechin and rutin were also the major phenolic compound compounds found in foam-mat dried UFP (Table 4.8). The amount of gallic acid, catechol, *p*-courmaric acid and naringenin in foam-mat dried UFP significantly decreased with an increasing drying temperature ($p \leq 0.05$) (Table 4.8). A decrease in the amount of these phenolic compounds was in the same trend as foam-mat dried RH. The amount of individual phenolic compound in foam-mat dried UFP prepared from GRB was higher than that prepared from NGRB. However, most of these phenolic compounds in both treatments were not significantly different ($p > 0.05$) (Table 4.8). The effect of germination before enzymatic hydrolysis on individual phenolic content after drying was already described above.

Figure 4.9 and Table 4.9 show a HPLC chromatogram of phenolic compound and the contents of individual phenolic compound in foam-mat dried UFR. As shown in Figure 4.9, the chromatogram of foam-mat dried UFR was different from other chromatograms shown earlier in Figure 4.7 and 4.8. It was found that much lower intensity peaks was found in chromatogram of foam-mat dried UFR compared to chromatogram of foam-mat dried RH and UFP. This result indicated that most of phenolics can pass through ultrafiltration membrane with molecular weight cut-off 3 kDa.

Although all twelve phenolic compounds found in foam-mat dried rice bean hydrolysate had molecular weight less than 3 kDa, some phenolic compounds for example, gallic acid, catechol and ferulic acid were found in foam-mat dried UFR (Table 4.9). This most likely due to incomplete separation of phenolic compounds from rice bean hydrolysate by membrane ultrafiltration. As concentration of phenolic compounds in foam-mat dried UFR were very low, the effect of drying temperature and germination before enzymatic hydrolysis on content of each phenolic in foam-mat dried UFR cannot be explained

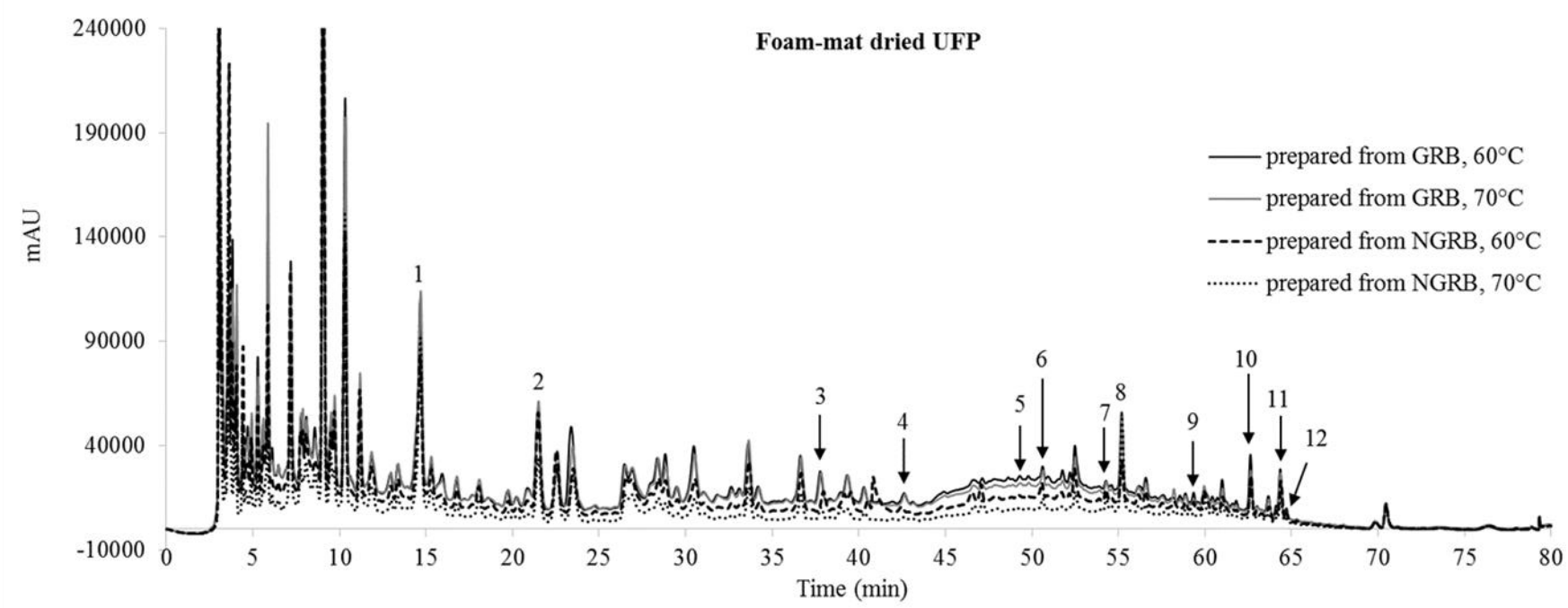

Figure 4.8 HPLC chromatogram of foam-mat dried ultrafiltration permeate rice bean hydrolysate (UFP) prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) dried at 60 and 70 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic acid, (11) naringenin, (12) quercetin

Table 4.8 Identification and quantification of phenolic compounds in foam-mat dried ultrafiltration permeate hydrolysate (UFP) prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) dried at 60 and 70 °C

Peak no.	Compound	Retention time (min)	λ_{max} (nm)	Phenolic content (mg g^{-1} dry sample)			
				GRB dried at 60 °C	GRB dried at 70 °C	NGRB dried at 60 °C	NGRB dried at 70 °C
1	Gallic acid	14.7	275	2.54^{b}±0.16	1.92^{a}±0.09	2.30^{b}± 0.22	1.83^{a}±0.04
2	Catechol	21.5	275	1.82^{b}±0.04	1.61^{a}±0.10	1.79^{b}±0.06	1.53^{a}±0.02
3	4-Hydroxybenzoic acid^{ns}	38.1	265	0.03±0.00	0.03±0.00	0.02±0.02	0.02±0.00
4	(+)-Catechin	42.7	275	0.24^{b}±0.01	0.22^{a}±0.01	0.21^{a}±0.01	0.21^{a}±0.01
5	Vanillin	48.6	280	0.03^{b}±0.00	0.03^{b}±0.00	0.02^{a}±0.00	0.02^{a}±0.00
6	Epicatechin	50.7	275	1.30^{c}±0.06	1.09^{a}±0.08	1.14^{b}±0.04	1.02^{ab}±0.03
7	*p*-Coumaric acid	54.0	320	0.12^{d}±0.01	0.10^{c}±0.00	0.09^{b}±0.00	0.06^{a}±0.01
8	Ferulic acid	55.1	320	0.43^{c}±0.01	0.39^{a}±0.01	0.42^{bc}±0.01	0.40^{ab}±0.01
9	Rutin	59.4	360	0.60^{a}±0.05	0.51^{a}±0.04	0.70^{b}±0.03	0.58^{a}±0.06
10	*trans*-Cinnamic acid	62.8	275	0.18^{b}±0.01	0.17^{b}±0.00	0.18^{b}±0.01	0.14^{a}±0.00
11	Naringenin	64.1	280	0.59^{b}±0.01	0.48^{a}±0.01	0.58^{b}±0.02	0.50^{a}±0.01
12	Quercetin	64.9	360	0.12^{b}±0.01	0.10^{ab}±0.01	0.10^{ab}± 0.01	0.09^{a}±0.01

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Mean values followed by different superscript letters in the same row are significantly different ($p \leq 0.05$)

GRB, germinated rice bean

NGRB, non-germinated rice bean

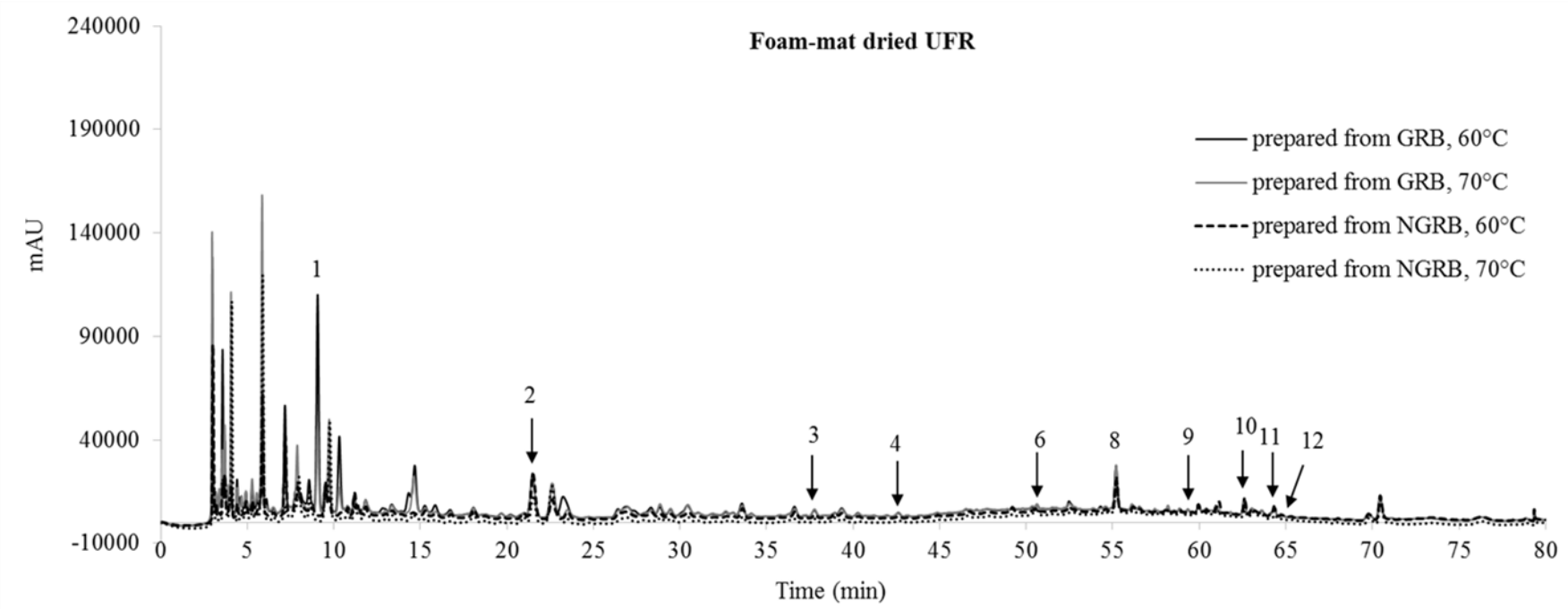

Figure 4.9 HPLC chromatogram of foam-mat dried ultrafiltration retentate rice bean hydrolysate (UFR) prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) dried at 60 and 70 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic acid, (11) naringenin, (12) quercetin

Table 4.9 Identification and quantification of phenolic compounds in foam-mat dried ultrafiltration retentate protein hydrolysate (UFR) prepared from germinated rice bean (GRB) and non-germinated rice bean (NGRB) dried at 60 and 70 °C

Peak no.	Compound	Retention time (min)	λ_{max} (nm)	Phenolic content (mg g^{-1} dry sample)			
				GRB dried at 60 °C	GRB dried at 70 °C	NGRB dried at 60 °C	NGRB dried at 70 °C
1	Gallic acid	14.7	275	0.52^{b}±0.02	0.43^{a}±0.03	0.49^{b}±0.01	0.42^{a}±0.02
2	Catechol	21.5	275	0.16^{b}±0.00	0.12^{a}±0.00	0.15^{b}±0.01	0.11^{a}±0.00
3	4-Hydroxybenzoic acid^{ns}	38.1	265	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00
4	(+)-Catechin^{ns}	42.7	275	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00
5	Vanillin^{ns}	48.6	280	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
6	Epicatechin	50.7	275	0.05^{c}±0.00	0.03^{a}±0.00	0.04^{b}±0.00	0.04^{b} ±0.00
7	*p*-Coumaric acid^{ns}	54.0	320	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
8	Ferulic acid	55.1	320	0.21^{b}±0.01	0.19^{ab}±0.00	0.20^{b}±0.01	0.18^{a}±0.01
9	Rutin	59.4	360	0.06^{b}±0.01	0.04^{a}±0.01	0.04^{a}±0.00	0.03^{a}±0.00
10	*trans*-Cinnamic acid^{ns}	62.8	275	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00
11	Naringenin	64.1	280	0.02^{b}±0.00	0.01^{a}±0.00	0.02^{b}±0.00	0.01^{a}±0.00
12	Quercetin	64.9	360	0.01^{b}±0.00	0.01^{b}±0.00	0.01^{b}±0.00	0.00^{a}±0.00

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Mean values followed by different superscript letters in the same row are significantly different ($p \leq 0.05$)

GRB, germinated rice bean

NGRB, non-germinated rice bean

4.3.3 Antioxidant activities of foam-mat dried rice bean hydrolysate and its membrane ultrafiltration fractions

Foam-mat dried RH, UFP and UFR, dried at 60 °C and 70 °C prepared from GRB and NGRB were determined for antioxidant activities using colorimetric and electron spin resonance (ESR) method.

4.3.3.1 Antioxidant activities measured using colorimetric method

Table 4.10 shows that EC_{50} of foam-mat dried PH significantly increased with increasing air-drying temperature ($p \leq 0.05$). This trend can be observed

in both foam-mat dried RH prepared from GRB and NGRB. In order to compare antioxidant activities of these fractions with other standard antioxidant compounds, EC_{50} was converted to ascorbic acid equivalent antioxidant capacity (AEAC). From Table 4.10, it can be seen that AEAC of foam-mat dried PH prepared from GRB and NGRB were decrease from 2.52 to 2.25 and 2.43 to 2.22 mg ascorbic acid equivalent per g foam-mat dried rice bean hydrolysate, respectively, after increase drying temperature from 60 to 70 °C. The decrease of FRAP with increasing drying temperature also found in foam-mat dried RH samples (Table 4.10). The reduction of antioxidant activities of foam-mat dried samples in all fractions after increasing of drying temperature was related with the decrease of extractable phenolic content and extractable flavonoid content which exhibit antioxidant activity, as shown Table 4.6. Auisakchaiyoung and Rojanakorn (2015) similarly showed that an increase in drying temperature from 60 to 80 °C resulted in a significantly decrease in phenolic content and antioxidant activities, ABTS and DPPH radical scavenging activity, of foam-mat dried gac fruit (*Momordica cochinchinensis)*. They therefore concluded that heat treatment was the main reason for degradation of TPC, lycopene and β-carotene which affect antioxidant activity of dried product.

The germination before enzymatic hydrolysis was found to have a positive effect on antioxidant activity of foam-mat dried rice bean hydrolysate. DPPH radical scavenging activity and FRAP of foam-mat dried RH prepared from GRB was slightly higher than those of foam-mat dried rice bean hydrolysate prepared from NGRB. This might be because extractable phenolic content after 21 h enzymatic hydrolysis of GRB was higher than that shown in NGRB (Table 4.4). The higher amount of initial extractable phenolic content of protein hydrolysate of GRB may contribute to higher extractable phenolic content and antioxidant activities of foam-mat dried hydrolysate of GRB compared with foam-mat dried hydrolysate of NGRB.

DPPH radical scavenging activity and FRAP of foam-mat dried UFP were significantly higher than those of foam-mat dried UFR ($p \leq 0.05$). These results indicated that both peptides size and amount of phenolics contribute to antioxidant activity of foam-mat dried rice bean hydrolysate. The separation of peptides from phenolics was further explained in Section 4.3.4. This was to investigate whether

peptides or phenolics was the main contribution to antioxidant activity in foam-mat dried RH.

Table 4.10 DPPH radical scavenging activity and FRAP of foam-mat dried germinated rice bean (GRB) and non-germinated rice bean (NGRB) hydrolysate and its fraction

Sample	DPPH assay		FRAP (μmol Trolox g^{-1} dry bean)
	EC_{50} (mg ml^{-1})	DPPH radical scavenging activity (μmol Trolox® g^{-1} dry bean)	
Protein hydrolysate			
GRB, 60 °C	1.54^{cd}±0.02	12.50^{e}±0.55	3.54^{e}±0.13
GRB, 70 °C	1.72^{e}±0.01	10.74^{c}±0.29	3.31^{de}±0.12
NGRB, 60 °C	1.59^{cd}±0.03	8.24^{ab}±0.10	2.53^{ab}±0.16
NGRB, 70 °C	1.74^{e}±0.03	7.93^{a}±0.32	2.34^{a}±0.11
Permeate fraction (<3kDa)			
GRB, 60 °C	1.40^{a}±0.05	12.51^{e}±0.35	4.00^{f}±0.11
GRB, 70 °C	1.56^{cd}±0.06	11.44^{d}±0.41	3.87^{f}±0.08
NGRB, 60 °C	1.44^{ab}±0.06	8.86^{b}±0.06	2.84^{c}±0.15
NGRB, 70 °C	1.61^{d}±0.03	8.22^{ab}±0.92	2.75^{bc}±0.16
Retentate fraction (≥3kDa)			
GRB, 60 °C	1.50^{bc}±0.09	12.62^{e}±0.38	3.50^{e}±0.04
GRB, 70 °C	1.62^{d}±0.05	11.66^{d}±0.23	3.14^{d}±0.39
NGRB, 60 °C	1.58^{cd}±0.09	8.67^{b}± 0.25	2.51^{ab}±0.09
NGRB, 70 °C	1.75^{e}±0.02	7.81^{a}±0.10	2.31^{a}±0.14

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).

1 g of foam-mat dried GRB hydrolysate equivalent to 14.10 ml of hydrolysate, which 100 ml of GRB hydrolysate equivalent to 32.96 g dry bean.

1 g of foam-mat dried NGRB hydrolysate equivalent to 14.12 ml hydrolysate, which 100 ml of NGRB hydrolysate equivalent to 44.79 g dry bean.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

4.3.3.2 Antioxidant activities measured using ESR method

DPPH radical scavenging activity of foam-mat dried sample was determined using both colorimetric and ESR method (Table 4.11). A sample of ESR spectrum of DPPH radical was shown in Figure 4.10 a. The height of the third resonance peak was evaluated. The results showed that DPPH radical scavenging activity of hydrolysate of GRB and NGRB at the same concentration (1.5 mg ml^{-1}) determined using both colorimetric and ESR method tend to decrease as increasing drying temperature. This might result from the reduction of extractable content which exhibit antioxidant activity. There was a slight difference in value between the DPPH radical scavenging activity obtained from colorimetric and ESR method. This difference was probably because of its different measurement principle. Determination of DPPH radical scavenging activity using colorimetric method is based on monitoring a decrease in deep-violet color of DPPH radical at 517 nm. The absorbance of DPPH radical might be interfered by other interferences. Teow *et al.* (2007) reported that anthocyanins in the sample led to under-estimation of antioxidant activity using colorimetric method. ESR method is based on the measurement the transition of unpaired electron under magnetic field. ESR method investigates the fulfillment of the unpaired electron in DPPH by measuring a decrease in signal intensity of the free radicals with no interference from color pigment is observed. In this experiment the value obtained from ESR method was 1.20-1.32 times higher than that of colorimetric method. Therefore, it is important to consider that slight under-estimation of antioxidant activity might occur when sample contains some interference.

Different mechanisms of foam-mat dried rice bean hydrolysate in scavenging of free radical were studied via hydroxyl radicals and carbon-centered radical. Since hydroxyl radical is powerful oxidation agent, hydroxyl radical is the most reactive free radicals (Fontmorin *et al.*, 2016). To scavenging hydroxyl radical, foam-mat dried rice bean hydrolysate should have ability to be hydrogen donor to fulfill the lone pair electron in hydroxyl radical and convert it to stable form.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 4.11 Antioxidant activities of foam-mat dried rice bean protein hydrolysate prepared from GRB and NGRB measured using colorimetric method and electron spin resonance (ESR) method at concentration 1.5 mg ml^{-1} of protein hydrolysate

Treatments	DPPH radical scavenging activity (%)		Hydroxyl radical scavenging activity (%)	Carbon-centered radical scavenging activity (%)
	colorimetric method	ESR method		
GRB, 60 °C	52.99^{b}±2.83	63.77^{b}±1.41	27.97^{b}±0.39	70.60^{c}±0.69
GRB, 70 °C	43.97^{a}±1.48	58.19^{a}±2.34	17.57^{a}±1.06	67.23^{b}±1.34
NGRB, 60 °C	46.36^{a}±0.71	57.99^{a}±2.06	27.81^{b}±2.14	69.08^{bc}±1.36
NGRB, 70 °C	44.23^{a}±2.21	56.53^{a}±1.48	16.74^{a}±0.74	63.18^{a}±1.75

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).
GRB, germinated rice bean
NGRB, non-germinated rice bean

Carbon-centered radical is one of the major radical in food which can react with molecular oxygen to give peroxyl radicals that stimulate lipid peroxidation, converting lipid to lipid hydroperoxide (Wright, Shadnia and Chepelev, 2009). Foam-mat dried rice bean hydrolysate can act as carbon-centered radical scavenger by transfer hydrogen atom to this radical. This will convert carbon-centered radical into stable compounds.

In this study both hydroxyl radical and carbon-centered radical in foam-mat dried rice bean hydrolysate were investigated using ESR. To determine hydroxyl and carbon-centered radical scavenging activity, the height of the second resonance peak and the height of the of the first resonance peak were evaluated, respectively. The samples of both ESR spectrum were shown in Figure 4.10 b and c. Table 4.11 shows that hydroxyl radical scavenging activity and carbon-centered radical scavenging activity significantly decreased with increasing drying temperature ($p \leq 0.05$). This might result from the reduction of extractable phenolic content due to increasing drying temperature as described above. Foam-mat dried rice bean hydrolysate showed high carbon-centered radical scavenging activity (63.18-70.60%), which displayed the greatest capability to prevent lipid oxidation via peroxyl radical scavenging.

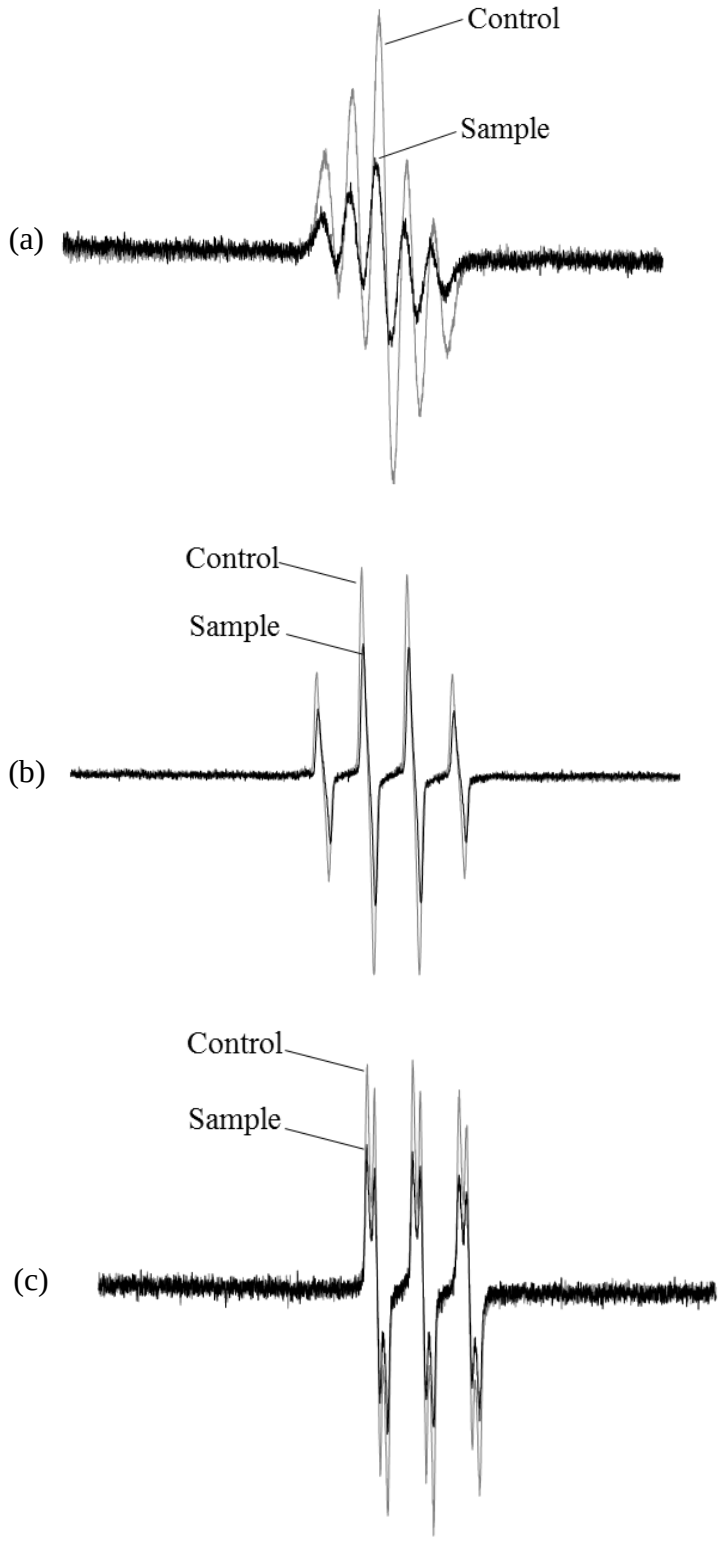

Figure 4.10 ESR spectrum of (a) DPPH radical signal, (b) hydroxyl radical signal, and (c) carbon-centered radical signal in the absence of protein hydrolysate (control) or with protein hydrolysate (sample)

The hydroxyl radical scavenging activity and carbon-centered radical scavenging activity of foam-mat dried hydrolysate prepared from GRB was higher than those from NGRB. These results shared similar trend with both DPPH radical scavenging activity and FRAP determined using colorimetric method (Table 4.10).

4.3.4 Antioxidant activities of peptide and phenolic fraction separated from foam-mat dried rice bean hydrolysate

Peptides and phenolics were separated from foam-mat dried RH, UFP and UFR obtained from both GRB and NGRB using a solid phase extraction (SPE) column. Figure 4.11-4.14 show chromatogram of phenolic compound which contained in peptide and phenolic fraction from foam-mat dried GRB and NGRB at 60 and 70 ºC. It was found that most phenolics can be separated from foam-mat dried sample using C18 SPE column. However, gallic acid and catechol still remained in the peptide fraction. This might be due to low hydrophobicity of gallic acid and catechol, which cannot be separated using C18 SPE column.

The antioxidant activities of the obtained phenolics and peptides fraction were shown in Table 4.12. The results showed that % DPPH radical scavenging activity and FRAP of phenolic fraction obtained from foam-mat dried RH were 1.71-2.50 and 0.91-1.49 times higher than those of peptide fraction. The results indicated that phenolics were the main components responsible for the antioxidant activities of foam-mat dried rice bean hydrolysate. Both lower molecular weight phenolic compounds, such as phenolic acids and flavonoids, and the larger molecular weight phenolic compound, such as lignin can act as antioxidant (Faustino *et al.*, 2010). Over results showed that antioxidant activity of phenolic compounds with molecular weight lower than 3 kDa was significantly higher than that of phenolic compounds with higher molecular weight ($p \leq 0.05$) (Table 4.12).

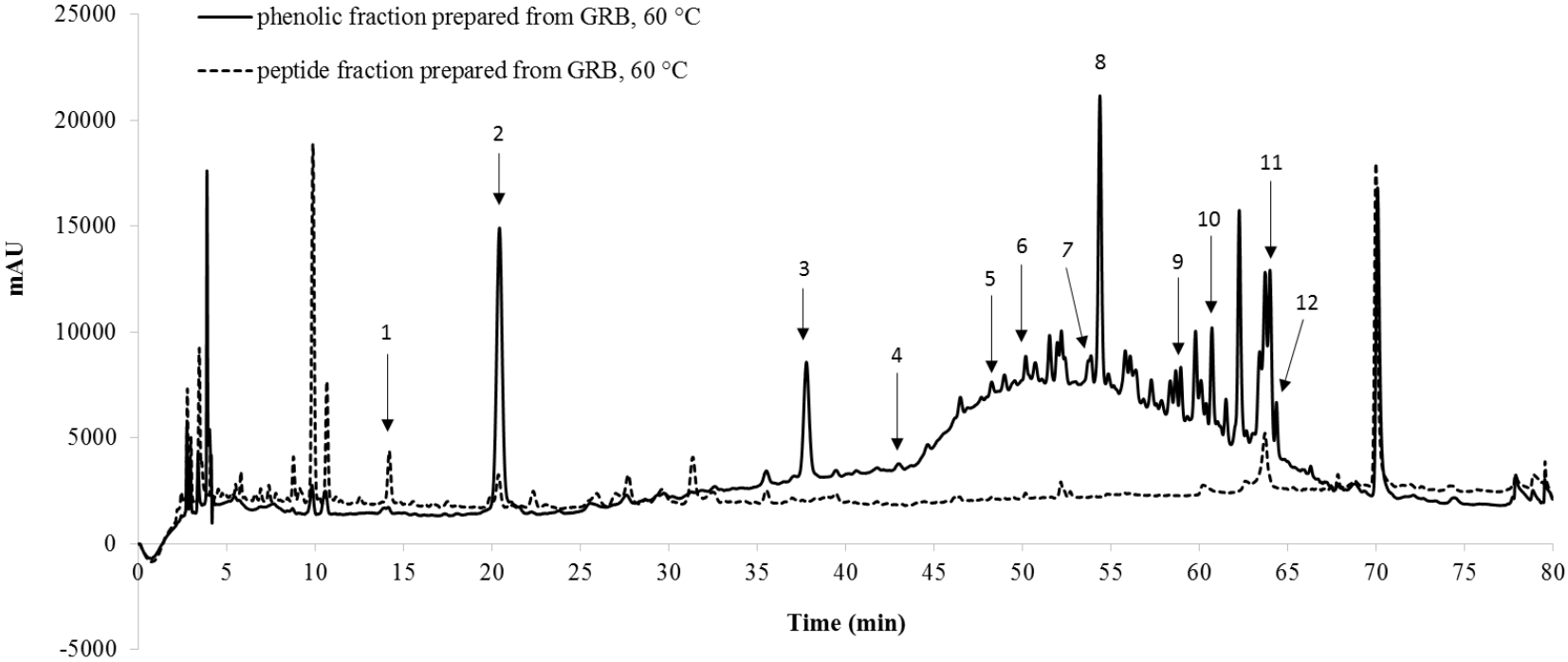

Figure 4.11 HPLC chromatogram of phenolic and peptide fraction prepared from germinated rice bean (GRB) hydrolysate dried at 60 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic acid, (11) naringenin, (12) quercetin

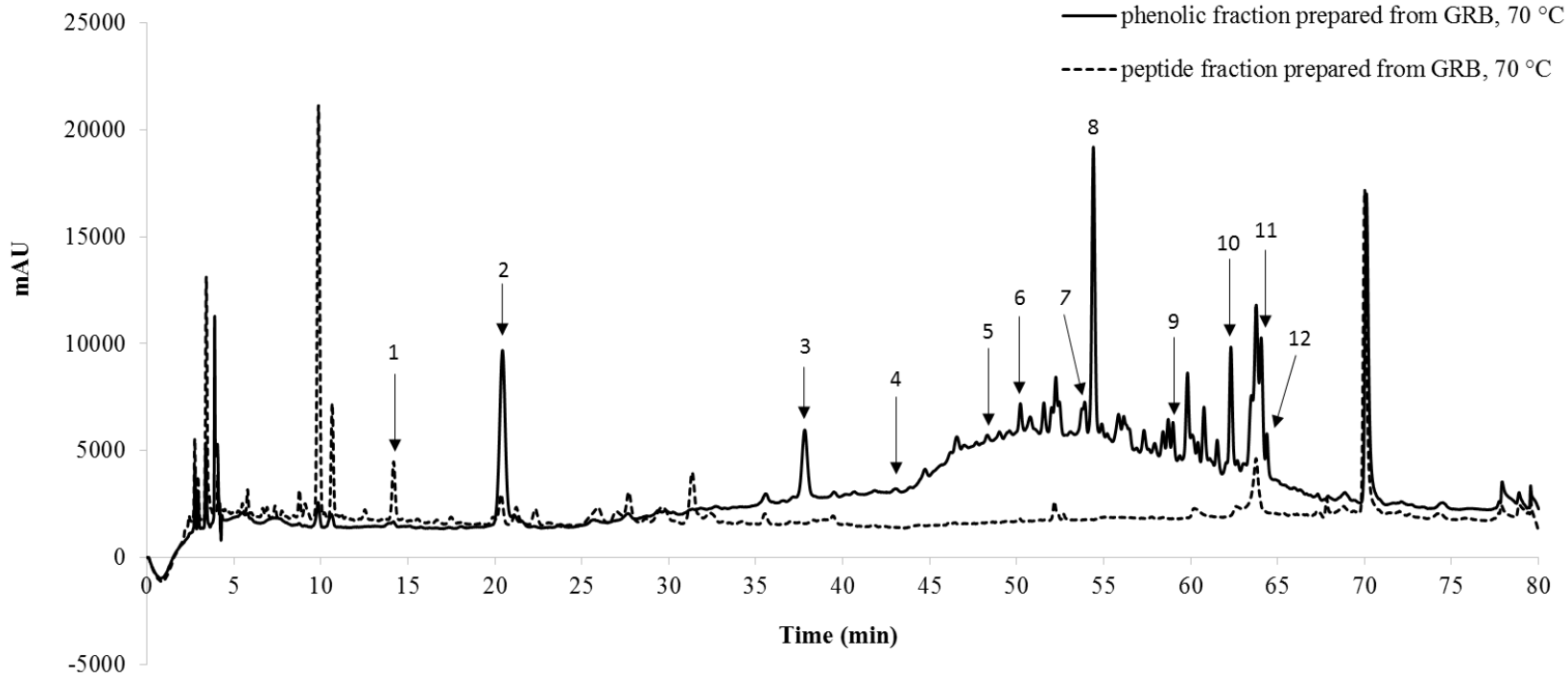

Figure 4.12 HPLC chromatogram of phenolic and peptide fraction prepared from non-germinated rice bean (NGRB) hydrolysate dried at 70 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic ad, (11) naringenin

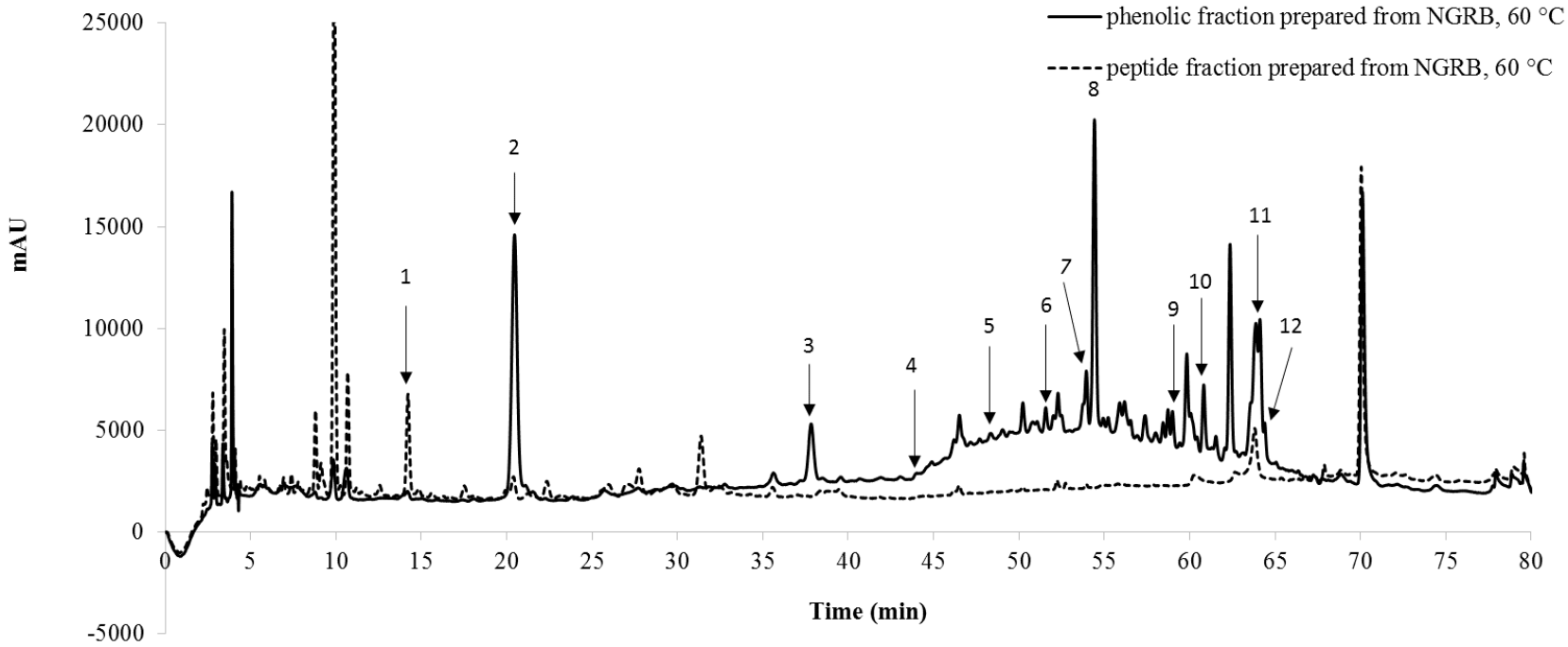

Figure 4.13 HPLC chromatogram of phenolic and peptide fraction prepared from germinated rice bean (GRB) hydrolysate dried at 60 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic acid, (11) naringenin, (12) quercetin

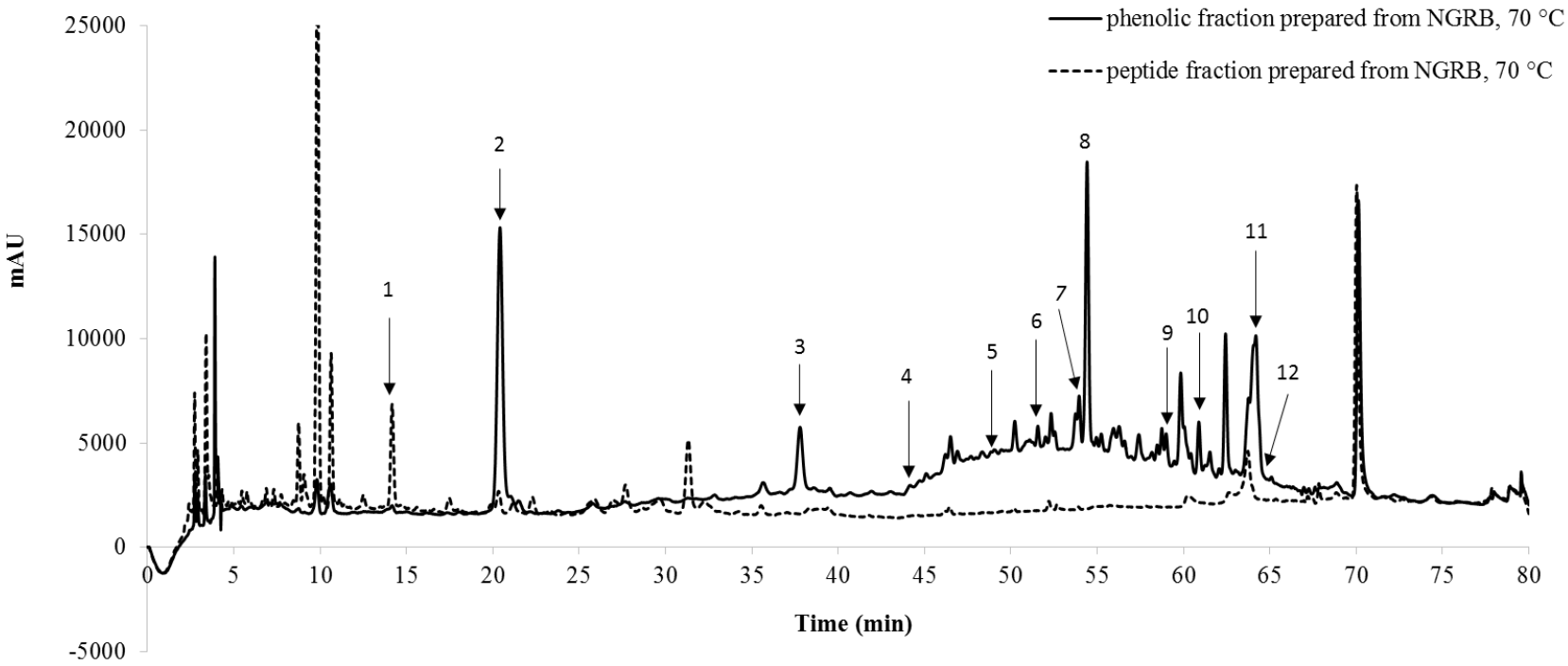

Figure 4.14 HPLC chromatogram of phenolic and peptide fraction prepared from germinated rice bean (GRB) hydrolysate dried at 70 °C. Peak identifications: (1) gallic acid, (2) catechol, (3) 4-hydroxybenzoic acid, (4) (+)-catechin, (5) vanillin, (6) epicatechin, (7) p-coumaric acid, (8) ferulic acid, (9) rutin, (10) trans-cinnamic acid, (11) naringenin, (12) quercetin

Table 4.12 DPPH radical scavenging activity and FRAP of peptide and phenolic fraction of foam-mat dried germinate rice bean (GRB) and non-germinated rice bean (NGRB) hydrolysate

Sample	DPPH scavenging activity (μmol Trolox® g^{-1} dry bean)		FRAP (μmol Trolox® g^{-1} dry bean)	
	Peptide fraction	Phenolic fraction	Peptide fraction	Phenolic fraction
Protein hydrolysate				
GRB, 60 ºC	4.05^{e}±0.09	7.49^{f}±0.11	4.78^{f}±0.15	6.67^{g}±0.11
GRB, 70 ºC	3.92^{e}±0.15	7.29^{e}±0.13	4.48^{e}±0.17	6.01^{f}±0.19
NGRB, 60 ºC	2.67^{c}±0.09	5.45^{c}±0.10	2.83^{cd}±0.17	4.17^{de}±0.13
NGRB, 70 ºC	2.61^{c}±0.12	5.20^{b}±0.15	2.74^{bc}±0.05	4.02^{d}±0.24
Permeate fraction (<3kDa)				
GRB, 60 ºC	4.53^{f}±0.12	7.74^{g}±0.19	4.83^{f}±0.23	6.99^{g}±0.29
GRB, 70 ºC	4.37^{f}±0.06	7.57^{fg}±0.09	4.46^{e}±0.17	6.20^{f}±0.39
NGRB, 60 ºC	2.97^{d}±0.07	5.62^{cd}±0.08	3.04^{d}±0.14	4.38^{e}±0.08
NGRB, 70 ºC	2.91^{d}±0.06	5.47^{c}±0.07	3.02^{d}±0.11	4.49^{e}±0.08
Retentate fraction (≥3kDa)				
GRB, 60 ºC	2.37^{b}±0.16	5.75^{d}±0.11	2.61^{bc}±0.17	2.96^{c}±0.27
GRB, 70 ºC	2.27^{b}±0.11	5.42^{c}±0.08	2.50^{b}±0.14	2.59^{b}±0.18
NGRB, 60 ºC	1.68^{a}±0.06	4.18^{a}±0.09	1.57^{a}±0.12	1.52^{a}±0.15
NGRB, 70 ºC	1.61^{a}±0.11	4.03^{a}±0.09	1.50^{a}±0.11	1.37^{a}±0.13

Mean values followed by different superscript letters in the same column are significantly different ($p \leq 0.05$).

1 g of foam-mat dried GRB hydrolysate equivalent to 14.10 ml of hydrolysate, which 100 ml of GRB hydrolysate equivalent to 32.96 g dry bean.

1 g of foam-mat dried NGRB hydrolysate equivalent to 14.12 ml hydrolysate, which 100 ml of NGRB hydrolysate equivalent to 44.79 g dry bean.

The antioxidant activities of hydrolysate largely depend on peptide structure, amino acid composition of the peptide and molecular weight of peptide (Tang *et al.*, 2009; Udenigwe and Aluko, 2011). It was found that the lower molecular weight (<3kDa) showed significantly greater DPPH radical scavenging activity and FRAP than the higher molecular weight fraction (Table 4.12). The reason was most likely due to accessibility of small peptides and amino acids to the redox reaction system are greater than large peptides and proteins (Chaléa *et al*., 2014). Ajibola *et al.* (2011) also reported that peptide with molecular weight lower than 1 kDa exhibited significantly higher DPPH radical scavenging activity, FRAP and hydroxyl radical scavenging activities compared to higher molecular weight peptide fraction ($p \leq 0.05$). They suggest that the high antioxidant activities of peptide with molecular weight lower than 1 kDa might be due to the highest contents of total hydrophobic amino acids with strong antioxidant activity. Lin *et al.* (2013) reported % DPPH radical scavenging activity of peptide fraction obtained from soybean protein hydrolysate using membrane ultrafiltration with molecular weight lower between 1-3 kDa was the highest, while the lowest was found in peptide with molecular weight between 10-30 kDa.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER V
CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

Germination led to an increase in crude protein content, total phenolic content and reducing sugar content of rice bean. Pretreatment with 1% (w v^{-1}) citric acid resulted in an increase in total phenolic content of germinated rice bean during germination. The increase in crude protein content was due to an increase in protein nitrogen. However, pretreatment changed some of the storage protein during germination. Pretreatment and germination also caused an increase in DPPH radical scavenging activity and FRAP. However, ethanolic extract possessed higher antioxidant activities than the water extracts.

18 h-germinated rice bean with pretreatment (GRB) was selected to be hydrolyzed using Flavourzyme® while non-germinated rice bean with no pretreatment (NGRB) was used as control. Enzymatic hydrolysis led to an increase in free amino group, degree of hydrolysis, extractable phenolic content, and antioxidant activities of both hydrolysate from GRB and NGRB.

Dying rate of both treatments at 70 ºC was higher than 60 ºC. At both drying temperatures, drying rate of hydrolysate of GRB was slightly lower than that of hydrolysate of NGRB. The foam-mat drying of rice bean hydrolysate can be predicted using model derived based on mass and heat transfer. Weibull distribution and Midilli model were suitable to describe the drying characteristic of hydrolysate foam prepared from GRB and NGRB. Higher drying temperature leads to melting between foam-mat dried rice bean hydrolysate particles and led to a decrease in extractable phenolic content, and extractable flavonoid content of foam-mat dried hydrolysate prepared from both GRB and NGRB. Gallic acid, catechol, epicatechin and rutin were the major compounds present in both foam-mat dried rice bean hydrolysates.

Antioxidant activities, measured using both colorimetric method and electron spin resonance (ESR) method, of foam-mat dried rice bean hydrolysate decreased when drying temperature increased. Lower DPPH radical scavenging activity was found

when measurement using colorimetric method compared to that measurement using ESR method. This trend was observed in both foam-mat dried hydrolysate of GRB and NGRB. Higher antioxidant activity was found in foam-mat dried hydrolysate of GRB compared with that of NGRB. Antioxidant activities of foam-mat dried rice bean hydrolysate were from both peptides and phenolics. The low molecular weight peptide (lower than 3 kDa) showed the higher antioxidant activities than high molecular weight peptide (higher than 3 kDa). However, the main components responsible for the antioxidant activity of foam-mat dried rice bean hydrolysate was phenolics.

5.2 Suggestions

The present study on foam-mat dried rice bean hydrolysate provides limited information on further applications. Thus, further research should focus on:

1) The solubility study of foam-mat dried rice bean hydrolysate for food applications.

2) The sensory evaluation of the foam-mat dried rice bean hydrolysate for food applications.

3) The *in vivo* antioxidant activity of foam-mat dried rice bean hydrolysate.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

REFERENCES

Abbasi, E., and Azizpour, M. (2016). Evaluation of physicochemical properties of foam mat dried sour cherry powder. *LWT-Food Science and Technology* **68**, 105-110.

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry* **27**, 1256-1262.

Aguilera, Y., Herrera, T., Benítez, V., Arribas, S. M., de Pablo, A. L. L., Esteban, R. M., and Martín-Cabrejas, M. A. (2015). Estimation of scavenging capacity of melatonin and other antioxidants: Contribution and evaluation in germinated seeds. *Food chemistry* **170**, 203-211.

Ajibola, C. F., Fashakin, J. B., Fagbemi, T. N., and Aluko, R. E. (2011). Effect of peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. *International Journal of Molecular Sciences* **12**, 6685-6702.

Anastassiadis, S., Morgunov, I. G., Kamzolova, S. V., and Finogenova, T. V. (2008). Citric acid production patent review. *Recent patents on biotechnology* **2**, 107-123.

Andersen, P. (2007). Report on the nutritive value of ricebean. Available from http://www.ricebean.org/publication.htm [2016, June 14].

Ankanna, S., and Savithramma, N. (2012). Study on habitat survey and seed germination of Shorea tumbuggaia ROXB. A globally threatened medicinal tree of seshachalam biosphere researve of India. *International Journal of Research in BioScience* **1**, 63-70.

AOAC (2006). "Official Methods of Analysis (18th ed.)," Washington, DC.

Araglou, D. L., Labropoulos, A. E., and Varzakus, T. H. (2009). Enzyme applied in food technology. *In* "Advances in food biochemistry" (F. Yildiz, ed.), pp. 101-129. CRC press, florida.

Asami, D. K., Hong, Y.-J., Barrett, D. M., and Mitchell, A. E. (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and

sustainable agricultural practices. *Journal of agricultural and food chemistry* **51**, 1237-1241.

Auisakchaiyoung, T., and Rojanakorn, T. (2015). Effect of foam-mat drying conditions on quality of dried Gac fruit (*Momordica cochinchinensis*) aril. *International Food Research Journal* **22**, 2025-2031.

Azizpour, M., Mohebbi, M., and Khodaparast, M. H. H. (2016). Effects of foam-mat drying temperature on physico-chemical and microstructural properties of shrimp powder. *Innovative Food Science & Emerging Technologies* **34**, 122-126.

Babalis, S. J., Papanicolaou, E., Kyriakis, N., and Belessiotis, V. G. (2006). Evaluation of thin-layer drying models for describing drying kinetics of figs (*Ficus carica*). *Journal of Food Engineering* **75**, 205-214.

Balogh, E., Hegedűs, A., and Stefanovits-Bányai, É. (2010). Application of and correlation among antioxidant and antiradical assays for characterizing antioxidant capacity of berries. *Scientia horticulturae* **125**, 332-336.

Benzie, I. F., and Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry* **239**, 70-76.

Berlyn, G. P. (1972). Seed germination and morphogenesis. *In* "Seed biology " (T. Kozlowski, T ed.), pp. 223-312. Academic Press, New York.

Bewley, J. D. (1997). Seed germination and dormancy. *The plant cell* **9**, 1055.

Bewley, J. D., and Black, M. (1983). "Physiology and biochemistry of seeds in relation to germination," Springer-Verlag Berlin Heideberg, New York.

Brand-Williams, W., Cuvelier, M.-E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology* **28**, 25-30.

Chandra, P. K., and Singh, R. P. (1995). "Least Squares Approximation," CRC Press, Boca Raton.

Chandrasekar, V., Gabriela, J. S., Kannan, K., and Sangamithra, A. (2015). Effect of foaming agent concentration and drying temperature on physiochemical and antimicrobial properties of foam mat dried powder. *Asian Journal of Dairy and Food Research* **34**, 39-43.

Chehregani, A., and Kavianpour, F. (2007). Effects of acid rain on the developmental stages of ovules and seed proteins in Bean plants (*Phaseolus vulgaris L.*). *Am J Plant Physiol* **2**, 367-372.

Crank, J. (1975). "The Mathematics of Diffusion," Oxford University Press, England.

Dai, J., and Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* **15**, 7313-7352.

Delrot, S., Rochat, C., Tegeder, M., and Frommer, W. (2001). Amino acid transport. *In* "Plant Nitrogen" (P. J. Lea and J.-F. Morot-Gaudry, eds.), pp. 213-235. Springer Berlin Heidelberg, Berlin.

Denny, G. C., and Arnold, M. A. (2007). Pre-germination seed treatments and stratification affect germination of montezuma cypress. *Journal of Environmental Horticulture* **25**, 1-3.

Dias, D. R., Abreu, C. M. P. d., Silvestre, M. P. C., and Schwan, R. F. (2010). In vitro protein digestibility of enzymatically pre-treated bean (*Phaseolus vulgaris L.*) flour using commercial protease and Bacillus sp. protease. *Food Science and Technology (Campinas)* **30**, 94-99.

Duangmal, K., and Taluengphol, A. (2010). Effect of protein additives, sodium ascorbate, and microbial transglutaminase on the texture and colour of red tilapia surimi gel. *International journal of food science & technology* **45**, 48-55.

Dubey, R. (1999). Protein synthesis by plants under stressful conditions. *In* "Handbook of plant and crop stress" (M. Pessarakli, ed.), pp. 365-397. Marcel Decker, Newyork.

Elias, R. J., Kellerby, S. S., and Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical reviews in food science and nutrition* **48**, 430-441.

Erbay, Z., and Icier, F. (2010). A review of thin layer drying of foods: theory, modeling, and experimental results. *Critical Reviews in Food Science and Nutrition* **50**, 441-464.

Esen, A. H. S., Özgür, R., Uzilday, B., Özgecan, Z., and Dinc, A. (2012). The response of the xerophytic plant *Gypsophila aucheri* to salt and drought stresses: the role of the antioxidant defence system. *Turkish Journal of Botany* **36**, 697-706.

Esen, D., Günes, N., and Yıldız, O. (2009). Effects of citric acid presoaking and stratification on germination behavior of *Prunus avium L*. seed. *Pakistan Journal of Botany* **41**, 2529-2535.

Faustino, H., Gil, N., Baptista, C., and Duarte, A. P. (2010). Antioxidant activity of lignin phenolic compounds extracted from kraft and sulphite black liquors. *Molecules* **15**, 9308-9322.

Fernandes, R. V. d. B., Queiroz, F., Botrel, D. A., Rocha, V. V. R., Lima, C. F. d., and Souza, V. R. d. (2012). Foam mat drying of tomato pulp.

Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M. K., Kozlowska, H., and Vidal-Valverde, C. (2008). Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. *emmerald*, *Glycinemax* cv. *jutro* and *Glycine max* cv. *merit*. *Food Chemistry* **111**, 622-630.

Fontmorin, J., Castillo, R. B., Tang, W., and Sillanpää, M. (2016). Stability of 5, 5-dimethyl-1-pyrroline-N-oxide as a spin-trap for quantification of hydroxyl radicals in processes based on Fenton reaction. *Water research* **99**, 24-32.

Fouad, A. A., and Rehab, F. (2015). Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil (*Lens culinaris Medik*.) sprouts. *Acta Scientiarum Polonorum Technologia Alimentaria* **14**, 233-246.

Fournier, E. (2005). Mono-and oligosaccharides. *In* "Handbook of food analytical chemistry " (R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. Smith and P. Sporns, eds.), pp. 651-660. Wiley Interscience, New York.

Franco, T. S., Perussello, C. A., Ellendersen, L. N., and Masson, M. L. (2016). Effects of foam mat drying on physicochemical and microstructural properties of yacon juice powder. *LWT-Food Science and Technology* **66**, 503-513.

Garcia-Mora, P., Frias, J., Peñas, E., Zieliński, H., Giménez-Bastida, J. A., Wiczkowski, W., Zielińska, D., and Martínez-Villaluenga, C. (2015). Simultaneous release of peptides and phenolics with antioxidant, ACE-inhibitory and anti-inflammatory activities from pinto bean (*Phaseolus vulgaris L. var. pinto*) proteins by subtilisins. *Journal of Functional Foods* **18**, 319-332.

Ghavidel, R. A., and Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro

starch and protein digestibility of some legume seeds. *LWT-Food Science and Technology* **40**, 1292-1299.

Gill, P. K., Sharma, A. D., Singh, P., and Bhullar, S. S. (2002). Osmotic stress-induced changes in germination, growth and soluble sugar content of *Sorghum bicolor* (L.) Moench seeds. *Bulgarian Journal of Plant Physiology* **28**, 12-25.

Giri, A., Osako, K., Okamoto, A., Okazaki, E., and Ohshima, T. (2011). Antioxidative properties of aqueous and aroma extracts of squid miso prepared with Aspergillus oryzae-inoculated koji. *Food Research International* **44**, 317-325.

Goggans, J., Jones, L., and Lynch, K. (1974). Germination rate of Arizona Cypress improved by better cone collection techniques and seed pregermination treatments. *Tree Planters' Notes* **25**, 3-4.

Goyoaga, C., Burbano, C., Cuadrado, C., Romero, C., Guillamón, E., Varela, A., Pedrosa, M. M., and Muzquiz, M. (2011). Content and distribution of protein, sugars and inositol phosphates during the germination and seedling growth of two cultivars of *Vicia faba*. *Journal of food composition and analysis* **24**, 391-397.

Guan, X., Yao, H., Chen, Z., Shan, L., and Zhang, M. (2007). Some functional properties of oat bran protein concentrate modified by trypsin. *Food Chemistry* **101**, 163-170.

Hao, J., Wu, T., Li, H., Wang, W., and Liu, H. (2016). Dual effects of slightly acidic electrolyzed water (SAEW) treatment on the accumulation of γ-aminobutyric acid (GABA) and rutin in germinated buckwheat. *Food chemistry* **201**, 87-93.

Hartmann, R., and Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. *Current opinion in biotechnology* **18**, 163-169.

Henderson, S. (1974). Progress in developing the thin layer drying equation. *Transactions of the ASAE* **17**, 1167-1168.

Henderson, S., and Pabis, S. (1961). Grain drying theory I. Temperature effect on drying coefficient. *Journal of Agricultural Engineering Research* **6**, 169-174.

Herrmann, K. M., and Weaver, L. M. (1999). The shikimate pathway. *Annual review of plant biology* **50**, 473-503.

Irakli, M. N., Samanidou, V. F., Biliaderis, C. G., and Papadoyannis, I. N. (2012). Simultaneous determination of phenolic acids and flavonoids in rice using solid- phase extraction and RP- HPLC with photodiode array detection. *Journal of separation science* **35**, 1603-1611.

Je, J.-Y., Kim, S.-Y., and Kim, S.-K. (2005). Preparation and antioxidative activity of hoki frame protein hydrolysate using ultrafiltration membranes. *European Food Research and Technology* **221**, 157-162.

Julkunen-Tiitto, R. (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* **33**, 213-217.

Kadam, D. M., and Balasubramanian, S. (2011). Foam mat drying of tomato juice. *Journal of food processing and preservation* **35**, 488-495.

Kadam, D. M., Rai, D. R., Patil, R., Wilson, R. A., Kaur, S., and Kumar, R. (2011). Quality of fresh and stored foam mat dried Mandarin powder. *International Journal of Food Science & Technology* **46**, 793-799.

Kadam, D. M., Wilson, R. A., and Kaur, S. (2010). Determination of biochemical properties of foam- mat dried mango powder. *International journal of food science & technology* **45**, 1626-1632.

Kadam, D. M., Wilson, R. A., Kaur, S., and Manisha (2012). Influence of foam mat drying on quality of tomato powder. *International Journal of Food Properties* **15**, 211-220.

Karathanos, V. T. (1999). Determination of water content of dried fruits by drying kinetics. *Journal of Food Engineering* **39**, 337-344.

Katoch, R. (2013). Nutritional potential of rice bean (*Vigna umbellata*): an underutilized legume. *Journal of food science* **78**, C8-C16.

Kaur, M. (2015). Chemical composition of ricebean (*Vigna umbellata*): Effect of Domestic Processing. *Indian journal of applied research* **5**, 311-313.

Kaushal, M., Sharma, P., and Sharma, R. (2013). Formulation and acceptability of foam mat dried seabuckthorn (*Hippophae salicifolia*) leather. *Journal of food science and technology* **50**, 78-85.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Kim, S. L., Lee, J. E., Kwon, Y. U., Kim, W. H., Jung, G. H., Kim, D. W., Lee, C. K., Lee, Y. Y., Kim, M. J., and Kim, Y. H. (2013). Introduction and nutritional evaluation of germinated soy germ. *Food chemistry* **136**, 491-500.

Krasaekoopt, W., and Bhatia, S. (2012). Production of yogurt powder using foam-mat drying. *AU Journal of Technology* **15**, 166-171.

Kristinsson, H. G., and Rasco, B. A. (2000). Fish protein hydrolysates: production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition* **40**, 43-81.

Kudra, T., and Ratti, C. (2006). Foam-mat drying: Energy and cost analyses. *Canadian Biosystems Engineering* **48**, 3.

Kudre, T., and Benjakul, S. (2013). Effect of legume seed protein isolates on autolysis and gel properties of surimi from sardine *(Sardinella albella*). *International Journal of Chemical, Environmental & Biological Sciences* **1**, 2320-4087.

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal* **2013**.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature* **227**, 680-685.

Lewis, W. K. (1921). The Rate of Drying of Solid Materials. *Industrial & Engineering Chemistry* **13**, 427-432.

Lin, S., Wang, J., Zhao, P., Pang, Y., Ye, H., Yuan, Y., Liu, J., and Jones, G. (2013). Optimized antioxidant peptides fractions preparation and secondary structure analysis by MIR. *International journal of biological macromolecules* **59**, 151-157.

Lou, S.-N., Hsu, Y.-S., and Ho, C.-T. (2014). Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. *Journal of Food and Drug Analysis* **22**, 290-295.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.

Luis, C.-G., Mario, D.-M., Alma, M.-A., Gloria, D.-O., and David, B.-A. (2012). Lima bean (*Phaseolus lunatus*) protein hydrolysates with ACE-I inhibitory activity. *Food and Nutrition Sciences* **3**, 511-521.

Luo, Y., Xie, W., Hao, Z., Jin, X., and Wang, Q. (2014). The impact of processing on in vitro bioactive compounds bioavailability and antioxidant activities in faba bean(*Vicia faba* L.) and azuki bean (*Vigna angularis* L.). *International Food Research Journal* **21**, 1031-1037.

Malaguti, M., Dinelli, G., Leoncini, E., Bregola, V., Bosi, S., Cicero, A. F., and Hrelia, S. (2014). Bioactive peptides in cereals and legumes: Agronomical, biochemical and clinical aspects. *International journal of molecular sciences* **15**, 21120-21135.

Mayer, A. M., and Poljakoff-Mayber, A. (1982). "The Germination of Seeds: Pergamon International Library of Science, Technology, Engineering and Social Studies," Elsevier, Oxford.

Mazid, M., Khan, T., and Mohammad, F. (2011). Role of secondary metabolites in defense mechanisms of plants. *Biology and medicine* **3**, 232-249.

McCue, P., Zheng, Z., Pinkham, J. L., and Shetty, K. (2000). A model for enhanced pea seedling vigour following low pH and salicylic acid treatments. *Process Biochemistry* **35**, 603-613.

Mendis, E., Rajapakse, N., and Kim, S.-K. (2005). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. *Journal of agricultural and food chemistry* **53**, 581-587.

Michailidis, P. A., and Krokida, M. K. (2014). Drying of Foods. *In* "Food Engineering Handbook Food Process Engineering" (T. VarZakas and C. Tzia, eds.), Vol. 2, pp. 395-435. CRC Press, Boca Raton.

Midilli, A., Kucuk, H., and Yapar, Z. (2002). A new model for single-layer drying. *Drying technology* **20**, 1503-1513.

Monteros, L. A. G. D. L., Ramón-Gallegos, E., Torres-Torres, N., and Mora-Escobedo, R. (2011). Effect of germinated soybean protein hydrolysates on adipogenesis and adipolysis in 3T3-L1 cells. *Plant foods for human nutrition* **66**, 355-362.

Mujumdar, A. S. (2006). "Principle, classification and selection of dryer," CRC Press, New York.

Mukherjee, A., and Sarkar, A. (1991). Rice bean–a new crop for sustaining pulse production in India. *Environment and Ecology* **9**, 207-211.

Müntz, K. (1996). Proteases and proteolytic cleavage of storage proteins in developing and germinating dicotyledonous seeds. *Journal of Experimental Botany* **47**, 605-622.

Müntz, K., Belozersky, M., Dunaevsky, Y., Schlereth, A., and Tiedemann, J. (2001). Stored proteinases and the initiation of storage protein mobilization in seeds during germination and seedling growth. *Journal of Experimental Botany* **52**, 1741-1752.

Narsih, Yunianta, and Harijono (2012). The study of germination and soaking time to improve nutritional quality of sorghum seed. *International Food Research Journal* **19**, 1429-1432.

Nielsen, P. M. (2010). Enzymes in protein modification. *In* "Enzymes in food technology" (R. J. Whitehurst and M. Van Oort, eds.), pp. 292-319. Wiley-Blackwell.

Obroucheva, N. V. (1999). "Seed germination: a guide to the early stages," Backhuys, Leiden.

Okos, M., and Narsimhan, G. (1992). Singh. RK i Weitnauer, AC (1992). Food dehydration. *In* "DR Heldman, & DB Lund, Handbook of food engineering" (D. R. Heldman and D. B. Lund, eds.), pp. 43-562. Marcel Dekker, New York.

Page, G. E. (1949). Factors Influencing the Maximum Rates of Air Drying Shelled Corn in Thin layers, Perdue, West Lafayette, Indiana.

Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., and Fortuna, T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food chemistry* **143**, 300-306.

Peng, X., Xiong, Y. L., and Kong, B. (2009). Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. *Food Chemistry* **113**, 196-201.

Periago, M., Ros, G., Martínez, C., and Rincón, F. (1996). Variations of non-protein nitrogen in six Spanish legumes according to the extraction method used. *Food research international* **29**, 489-494.

Popović, V., Ivetić, V., Šijačić-Nikolić, M., Knežević, R., Matović, B., and Lavadinović, V. (2012). Effect o fPre-treatments on Seed Germination Rate

from Difference Bald Cypress (*Taxodium distichum* Rich.) trees. *Forestry Ideas* **18**, 163-168.

Prior, R. L., Wu, X., and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry* **53**, 4290-4302.

Rajkumar, P., Kailappan, R., Viswanathan, R., and Raghavan, G. (2007). Drying characteristics of foamed alphonso mango pulp in a continuous type foam mat dryer. *Journal of Food Engineering* **79**, 1452-1459.

Randhir, R., Lin, Y.-T., and Shetty, K. (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry* **39**, 637-646.

Raut, V. G., Kharat, S. N., and Mendhulkar, V. D. (2015). Electrophoretic Protein profile of few Pulses consumed locally in Mumbai region. *International Journal of Pure and Applied Bioscience* **3**, 156-161.

Rayle, D. L., and Cleland, R. E. (1992). The Acid Growth Theory of auxin-induced cell elongation is alive and well. *Plant physiology* **99**, 1271-1274.

Rice-Evans, C. A., and Diplock, A. T. (1993). Current status of antioxidant therapy. *Free Radical Biology and Medicine* **15**, 77-96.

Rocha, T. D. S., Hernandez, L. M. R., Mojica, L., Johnson, M. H., Chang, Y. K., and Mejía, E. G. (2015). Germination of *Phaseolus vulgaris* and alcalase hydrolysis of its proteins produced bioactive peptides capable of improving markers related to type-2 diabetes in vitro. *Food Research International* **76**, 150-159.

Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: a review. *Journal of AOAC International* **93**, 1515-1522.

Saeed, N., Khan, M. R., and Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC complementary and alternative medicine* **12**, 1.

Salahi, M. R., Mohebbi, M., and Taghizadeh, M. (2015). Foam- Mat Drying of Cantaloupe (*Cucumis melo*): Optimization of Foaming Parameters and Investigating Drying Characteristics. *Journal of Food Processing and Preservation* **39**, 1798-1808.

Sankat, C. K., and Castaigne, F. (2004). Foaming and drying behaviour of ripe bananas. *LWT-Food Science and Technology* **37**, 517-525.

Sarmadi, B. H., and Ismail, A. (2010). Antioxidative peptides from food proteins: a review. *Peptides* **31**, 1949-1956.

Sefatie, R., Fatoumata, T., Eric, K., Shi, Y., and Guo-Wei, L. (2013). In vitro antioxidant activities of protein hydrolysate from germinated black soybean (*Glycine max* L.). *Advanve Journal of Food Science and Technology* **5**, 453-459.

Shen, S., Wang, Y., Li, M., Xu, F., Chai, L., and Bao, J. (2015). The effect of anaerobic treatment on polyphenols, antioxidant properties, tocols and free amino acids in white, red, and black germinated rice (*Oryza sativa* L.). *Journal of Functional Foods* **19**, 641-648.

Sun, C., Wu, Z., Wang, Z., and Zhang, H. (2015). Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. *Evidence-Based Complementary and Alternative Medicine.* http://dx.doi.org/10.1155/2015/595393.

Świeca, M., and Gawlik-Dziki, U. (2015). Effects of sprouting and postharvest storage under cool temperature conditions on starch content and antioxidant capacity of green pea, lentil and young mung bean sprouts. *Food chemistry* **185**, 99-105.

Tang, C.-H., Peng, J., Zhen, D.-W., and Chen, Z. (2009). Physicochemical and antioxidant properties of buckwheat (*Fagopyrum esculentum Moench*) protein hydrolysates. *Food Chemistry* **115**, 672-678.

Teow, C. C., Truong, V.-D., McFeeters, R. F., Thompson, R. L., Pecota, K. V., and Yencho, G. C. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chemistry* **103**, 829-838.

Udenigwe, C. C., and Aluko, R. E. (2011). Chemometric analysis of the amino acid requirements of antioxidant food protein hydrolysates. *International Journal of Molecular Sciences* **12**, 3148-3161.

Venkatachalam, S., John, S. G., and Kuppuswamy, K. (2015). Foam Mat Drying of Food Materials: A Review. *Journal of Food Processing and Preservation* **39**, 3165-3174.

Wang, C., and Singh, R. (1978). "A single layer drying equation for rough rice." St Joseph MI: ASAE Paper.

Waterhouse, A. L. (2005). Polyphenolics. *In* "Handbook of Food Analytical Chemistry, Volume 1: Water, Proteins, Enzymes, Lipids, and Carbohydrates" (R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. M. Smith and P. Sporns, eds.), pp. 461-470. John Wiley & Sons, London.

Wilkinson, R. (1994). "Acid soil stress and plant growth," Marcel Dekker, New York.

Wilson, R. A., Kadam, D. M., Chadha, S., and Sharma, M. (2012). Foam mat drying characteristics of mango pulp. *International Journal of Food Science and Nutrition Engineering* **2**, 63-69.

Wongsiri, S., Ohshima, T., and Duangmal, K. (2015). Chemical Composition, Amino Acid Profile and Antioxidant Activities of Germinated Mung Beans (*Vigna radiata*). *Journal of Food Processing and Preservation* **39**, 1956-1964.

Wright, J. S., Shadnia, H., and Chepelev, L. L. (2009). Stability of carbon- centered radicals: Effect of functional groups on the energetics of addition of molecular oxygen. *Journal of computational chemistry* **30**, 1016-1026.

Xiong, Y. L. (2010). Antioxidant peptides. *In* "Bioactive proteins and peptides as functional foods and nutraceuticals" (Y. Mine, E. Li-Chan and B. Jiang, eds.), pp. 29-42. Blackwell Publishing Ltd.

Yao, Y., Cheng, X.-Z., Wang, L.-X., Wang, S.-H., and Ren, G. (2012). Major phenolic compounds, antioxidant capacity and antidiabetic potential of rice bean (*Vigna umbellata* L.) in China. *International journal of molecular sciences* **13**, 2707-2716.

Ye, F., Liang, Q., Li, H., and Zhao, G. (2015). Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (*Helianthus annuus* L.). *Industrial Crops and Products* **76**, 574-581.

Yust, M. d. M., Millán- Linares, M. d. C., Alcaide- Hidalgo, J. M., Millán, F., and Pedroche, J. (2012). Hypocholesterolaemic and antioxidant activities of chickpea (*Cicer arietinum* L.) protein hydrolysates. *Journal of the Science of Food and Agriculture* **92**, 1994-2001.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry* **64**, 555-559.

APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

APPENDIX A
ASSAY TECHNIQUES

Appendix A.1 Total phenolics content (Waterhouse, 2005)

1. Prepare the sodium carbonate solution by dissolving 200 g of sodium carbonate in 800 ml of distilled water and boil for 10 min. After cooling, a few crystals of sodium carbonate was added to the solution and allow to stand for 24 h. Then the solution was filtered through Whatman No. 1 filter paper. The volume is adjusted to 1000 ml with distilled water.

2. 1 ml of sample or standard solution of gallic acid at different concentration was added into a 100 ml volumetric flask. 1 ml of distilled water was used as a blank. 70 ml of distilled water and 5 ml Folin-Ciocalteau reagent were then added. The reaction mixture was then incubated at room temperature for 8 min.

3. 15 ml of sodium carbonates solution was added and made up the volume to 100 ml with distilled water and incubated at room temperature for 2 h.

4. The obtained solution was placed in a plastic cuvette. Its absorbance was measured at 765 nm.

5. The absorbance of blank was subtracted from the absorbance of all samples and standard.

6. The standard curve prepared from gallic acid was shown in Figure A.1. This standard curve was used for estimation of the phenolic content (gallic acid equivalents) in the sample.

Appendix A.2 Reducing sugar content (Fournier, 2005)

1. Prepare low-alkalinity copper reagent by combining the two solutions. The first solution was prepared by dissolving 12 g of sodium potassium tartrate, 24 g of anhydrous sodium carbonate, 4 g of copper sulfate, and 16 g of sodium hydrogen carbonate in 450 ml of distilled water. The second solution was prepared by dissolving 180 g of anhydrous sodium sulfate in 500 ml of boiling water. These two solutions

were added to 1000-ml volumetric flask and made up the volume to 1000 ml with distilled water.

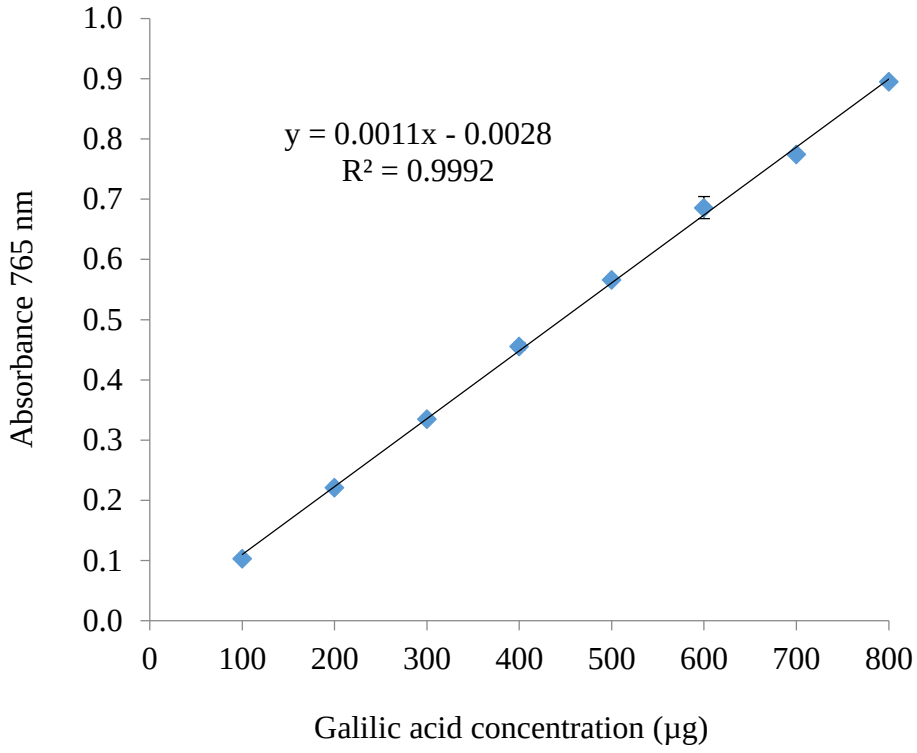

Figure A.1 Gallic acid standard curve for total phenolics content determination

2. Prepare arsenomolybdate reagent by dissolving 25 g of ammonium molybdate in 450 ml distilled water, then 21 ml of concentrated sulfuric acid and 25 ml of distilled water containing 3 g of disodium hydrogen arsenate heptahydrate were added. The mixture was stirred at room temperature for 24 h. This solution should be stored in a brown bottle.

3. Prepare a standard curve of glucose using glucose concentration ranging from 5 to 100 µg ml^{-1}.

4. 1 ml of sample, standard glucose solution, or blank was transferred in a test tube and mixed with 1 ml of low-alkalinity copper reagent.

5. Heat the test tube containing reaction mixture in boiling water for 10 min.

6. Let the tubes cool down, then 1 ml of arsenomolybolic acid reagent was added.

7. The solution was transferred to a plastic cuvette and measured its absorbance at 500 nm.

8. The standard curve prepared from glucose was shown in Figure A.2. This standard curve was used for estimation of reducing sugar content (glucose equivalents) in the sample.

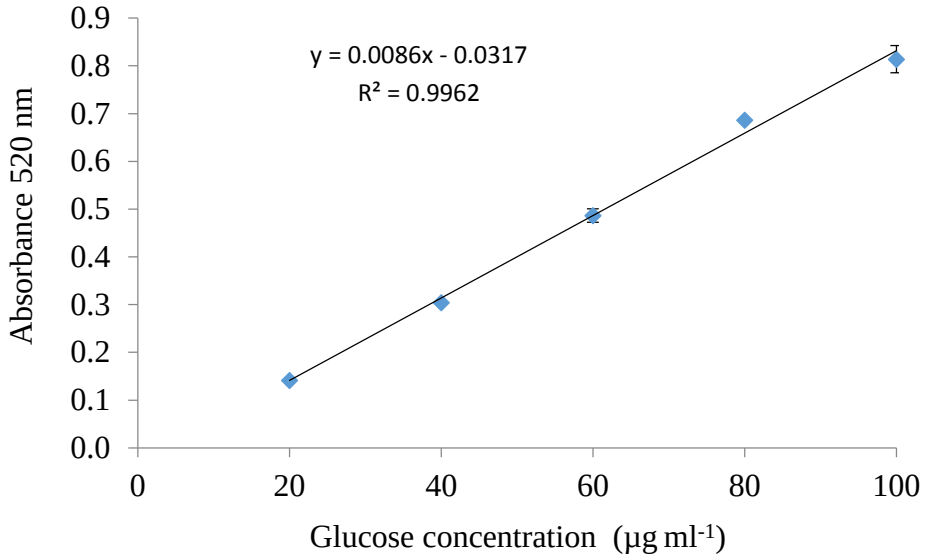

Figure A.2 Glucose standard curve for reducing sugar content determination

Appendix A.3 Preparation of standard amino acids

1. Prepare Waters AccQ. reagent, used for derivatization of amino acid, by adding 1 ml of Waters AccQ. fluor reagent diluent into the vial containing Waters AccQ. fluor reagent powder.

2. The solution was mixed thoroughly using a vortex mixer and incubated at 55 °C for 10 min.

3. Prepare the standard amino acid by pipette 40 µl of mixed 17 amino acid stock solution (Amino acid standard H, Pierce, MA) into 6×50 mm test tube and dilute with 960 µl of Milli-Q water. The concentration of each amino acid containing in the solution was 100 pmol ml^{-1}.

4. Pipette 1 to 20 µl of the standard amino acid into test tube and made up to 1 ml with Waters AccQ. fluor borate buffer. The obtained standard amino acid solutions contained 10 to 200 pmol of each amino acid.

5. The amino acid in the solution was derivertized by mixing 1 ml of standard amino acid solution with 20 µl of Waters AccQ. Reagent and then the mixture was incubated 55 ºC for 10 min.

Appendix A.4 Total flavonoid content (Zhishen, Mengcheng, and Jianming, 1999)

1. 1 ml of sample or standard solution of catechin at different concentration was added to 10-ml volumetric flasks containing 4 ml of water.
2. 0.3 ml of 5% (w v^{-1}) sodium nitrite was added into the flask to start the reaction.
3. After 5 min, 0.3 mL of 10% (w v^{-1}) aluminium chloride was added into the mixture. 1 min later, 2 ml of 1 M sodium hydroxide was added into the mixture.
4. The volume was made up to 10 ml with distilled water.
5. 2 ml of the solution was transferred into a plastic cuvette and its absorbance was measured at 510 nm.
6. The standard curve prepared from standard solution of catechin was shown in Figure A.3. This standard curve was for estimation of flavonoid content (catechin equivalents) in the sample.

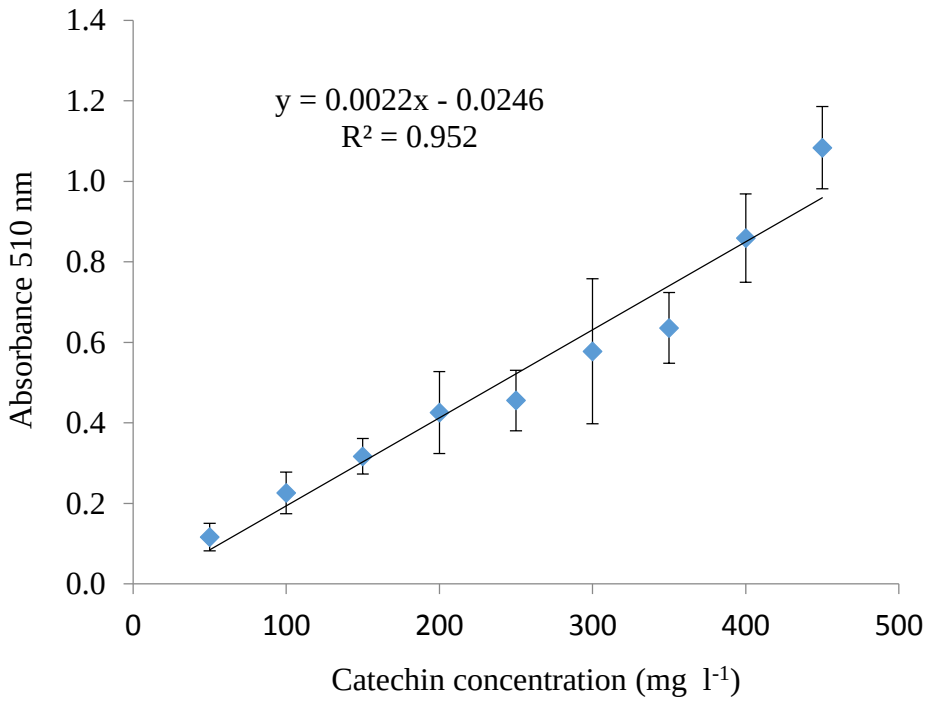

Figure A.3 Catechin strandard curve for total flavonoid content determination

APPENDIX B

RAW DATA

Appendix B.1 Band intensity on SDS–PAGE gel using GeneTools software

Table B.1 Raw volume of each protein band of germinated rice bean under no pretreatment calculated from the area of the intensity peak

Molecular weight (kDa)	Raw volume at different germination period				
	0 h	6 h	12 h	18 h	24 h
169.85	607,703.50	1,106,541.00	1,693,110.38	2,061,140.50	2,032,426.25
103.20	1,727,897.38	1,949,228.63	858,717.81	1,023,787.50	1,266,893.50
76.01	1,847,637.25	2,364,517.00	1,833,789.25	1,742,992.25	1,704,642.75
64.66	2,003,592.63	2,118,310.00	1,838,474.50	1,631,958.75	1,491,842.00
60.48	1,076,876.63	1,026,408.38	777,105.13	908,459.50	780,389.81
51.01	10,018,361.00	9,332,681.50	9,413,262.00	9,432,737.00	8,506,253.00
40.76	843,725.31	791,164.13	444,443.69	396,809.25	387,965.31
33.61	1,776,063.50	1,703,618.50	1,825,117.75	2,004,551.63	2,071,333.63
29.57	1,997,690.88	2,617,278.25	2,261,007.25	2,342,438.75	2,400,767.31
27.06	1,709,871.00	1,876,424.00	1,865,252.88	2,160,366.75	2,318,264.00
19.73	56,436.61	58,370.09	54,713.38	47,440.72	33,028.45
18.21	106,579.95	23,229.59	24,769.03	55,094.50	80,112.92
15.84	190,634.56	188,014.08	142,868.13	90,262.13	79,208.25
14.85	191,188.75	81,652.50	69,181.91	65,725.69	9,989.44

Table B.2 Raw volume of each protein band of germinated rice bean with pretreatment calculated from the area of the intensity peak

Molecular weight (kDa)	Raw volume at different germination period				
	0 h	6 h	12 h	18 h	24 h
169.85	634,270.50	1,133,210.69	1,543,181.50	1,533,307.13	1,429,578.13
103.20	1,654,662.31	1,480,513.97	1,370,086.84	1,077,908.13	1,174,580.48
76.01	1,373,526.63	1,846,759.75	1,267,609.75	591,287.88	243,349.77
64.66	2,090,810.38	2,118,616.88	1,908,283.13	1,650,387.63	833,985.63
60.48	543,584.19	644,955.13	591,054.38	563,907.19	487,051.50
51.01	10,006,567.00	8,977,106.00	8,757,625.25	7,909,652.50	6,937,854.50
40.76	646,966.11	478,017.81	422,619.00	258,380.86	173,642.28
33.61	1,732,639.00	1,929,127.19	2,388,513.88	2,154,077.88	2,282,926.72
29.57	2,093,067.63	2,374,554.38	2,501,234.13	2,724,354.00	2,774,516.63
27.06	2,082,841.00	2,133,754.50	2,409,610.00	2,420,313.00	2,530,653.75
19.73	57,733.88	57,578.00	44,816.05	28,220.31	25,114.44
18.21	52,179.81	29,176.61	121,807.77	34,113.53	58,315.70
15.84	110,978.53	112,291.41	97,884.34	45,542.55	26,151.19
14.85	182,035.41	76,992.36	66,426.06	36,178.63	9,167.59

APPENDIX C
STATISTIC ANALYSIS

Table C.1 The ANOVA table showing the effect of pretreatment and germination period on chemical composition of germinated rice bean

Chemical composition	SOV	df	MS	F	Sig
Moisture	Treatment	9	96.907	113.075	0.000
	Error	20	0.857		
	Total	30			
Crude protein	Treatment	9	3.210	1.818	0.127
	Error	20	1.7766		
	Total	30			
Carbohydrate	Treatment	9	3.371	1.704	0.154
	Error	20	1.979		
	Total	30			
Crude fat	Treatment	9	0.265	82.025	0.000
	Error	20	0.003		
	Total	30			
Ash	Treatment	9	0.422	9.651	0.000
	Error	20	0.044		
	Total	30			
Total phenolic	Treatment	9	1.191	55.725	0.000
	Error	20	0.021		
	Total	30			
Reducing sugar	Treatment	9	0.894	22.483	0.000
	Error	20	0.040		
	Total	30			

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table C.2 The ANOVA table showing the effect of pretreatment and germination period on nitrogen content and TCA-soluble peptide of germinated rice bean

Nitrogen content	SOV	df	MS	F	Sig
Total nitrogen	Treatment	9	0.082	1.813	0.128
	Error	20	0.045		
	Total	30			
Protein nitrogen	Treatment	9	0.122	3.413	0.011
	Error	20	0.036		
	Total	30			
Non-protein nitrogen	Treatment	9	0.047	0.972	0.491
	Error	20	0.048		
	Total	30			
TCA-soluble peptide	Treatment	9	0.011	39.631	0.000
	Error	20	0.000		
	Total	30			

Table C.3 The ANOVA table showing the effect of extraction solvent on antioxidant activity of germinated rice bean under no pretreatment and with pretreatment

Antioxidant activity	Extract	SOV	df	MS	F	Sig
DPPH assay	Water	Treatment	9	34.314	98.937	0.000
		Error	20	0.347		
		Total	30			
DPPH assay	Ethanol	Treatment	9	56.251	172.372	0.000
		Error	20	0.326		
		Total	30			
FRAP assay	Water	Treatment	9	2.857	35.910	0.000
		Error	20	0.080		
		Total	30			
FRAP assay	Ethanol	Treatment	9	4.608	103.459	0.000
		Error	20	0.045		
		Total	30			

Table C.4 The ANOVA table showing the effect of hydrolysis period on total phenolic content and antioxidant activities of GRB and NGRB hydrolysate

Properties	SOV	df	MS	F	Sig
Total phenolic of GRB	Hydrolysis period	10	1.874	278.731	0.000
	Error	22	0.007		
	Total	32			
Total phenolic of NGRB	Hydrolysis period	10	0.959	172.190	0.000
	Error	22	0.006		
	Total	32			
% DPPH radical scavenging activity of GRB	Hydrolysis period	10	10.262	116.918	0.000
	Error	22	0.088		
	Total	32			
% DPPH radical scavenging activity of NGRB	Hydrolysis period	10	10.214	192.352	0.000
	Error	22	0.053		
	Total	32			
FRAP of GRB	Hydrolysis period	10	2.425	64.394	0.000
	Error	22	0.038		
	Total	32			
FRAP of NGRB	Hydrolysis period	10	0.975	52.044	0.000
	Error	22	0.019		
	Total	32			

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table C.5 The ANOVA table showing effect of drying temperature and peptide size on some properties of foam-mat dried rice bean hydrolysate

Properties	SOV	df	MS	F	Sig
Moisture content	Hydrolysis period	11	0.095	0.677	0.746
	Error	24	0.140		
	Total	36			
Water activity	Hydrolysis period	11	9.354×10^{-6}	0.108	1.000
	Error	24	8.631×10^{-5}		
	Total	36			
Total phenolic content	Hydrolysis period	11	3.693	299.150	0.000
	Error	24	0.012		
	Total	36			
Total flavonoid content	Hydrolysis period	11	0.010	592.773	0.000
	Error	24	0.000		
	Total	36			

Table C.6 The ANOVA table showing effect of germination and drying temperature on phenolic compounds of foam-mat dried non-fractionated hydrolysate (RH*)*

Phenolic acids	SOV	df	MS	F	Sig
Gallic acid	Treatment	3	0.468	26.625	0.000
	Error	8	0.018		
	Total	12			
Catechol	Treatment	3	0.067	18.372	0.001
	Error	8	0.004		
	Total	12			
4-Hydroxybenzoic acid	Treatment	3	6.6667×10^{-5}	8.000	0.009
	Error	8	8.333×10^{-6}		
	Total	12			
(+)-Catechin	Treatment	3	0.001	5.9055	0.020
	Error	8	0.000		
	Total	12			

Table C.6 (continue) The ANOVA table showing effect of germination and drying temperature on phenolic compounds of foam-mat dried non-fractionated hydrolysate (PH).

Phenolic acids	SOV	df	MS	F	Sig
Vanillin	Treatment	3	0.000		
	Error	8	8.333×10^{-6}	24.000	0.000
	Total	12			
Epicatechin	Treatment	3	0.057	7.240	0.011
	Error	8	0.008		
	Total	12			
p-Courmaric acid	Treatment	3	0.001	25.067	0.000
	Error	8	4.167×10^{-5}		
	Total	12			
Ferulic acid	Treatment	3	0.020	31.105	0.000
	Error	8	0.001		
	Total	12			
Rutin	Treatment	3	0.053	36.127	0.000
	Error	8	0.001		
	Total	12			
Trans-Cinnamic acid	Treatment	3	0.001	12.9000	0.002
	Error	8	8.333×10^{-5}		
	Total	12			
Naringenin	Treatment	3	0.007	9.619	0.005
	Error	8	0.001		
	Total	12			
Quercetin	Treatment	3	0.004	18.486	0.001
	Error	8	0.000		
	Total	12			

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table C.7 The ANOVA table showing effect of germination and drying temperature on phenolic compounds of foam-mat dried ultrafiltration permeate hydrolysate (UFP)

Phenolic acids	SOV	df	MS	F	Sig
Gallic acid	Treatment	3	0.329	16.327	0.001
	Error	8	0.020		
	Total	12			
Catechol	Treatment	3	0.0660	14.852	0.001
	Error	8	0.004		
	Total	12			
4-Hydroxybenzoic acid	Treatment	3	0.000	1.333	0.330
	Error	8	7.500×10^{-5}		
	Total	12			
(+)-Catechin	Treatment	3	0.001	10.185	0.004
	Error	8	7.500×10^{-5}		
	Total	12			
Vanillin	Treatment	3	1.608×10^{-5}	14.846	0.001
	Error	8	1.083×10^{-5}		
	Total	12			
Epicatechin	Treatment	3	0.044	14.1882	0.001
	Error	8	0.003		
	Total	12			
p-Courmaric acid	Treatment	3	0.001	89.833	0.000
	Error	8	1.667×10^{-5}		
	Total	12			
Ferulic acid	Treatment	3	0.001	10.185	0.004
	Error	8	7.500×10^{-5}		
	Total	12			
Rutin	Treatment	3	0.019	8.022	0.009
	Error	8	0.002		
	Total	12			

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table C.7 (continue) The ANOVA table showing effect of germination and drying temperature on phenolic compounds of foam-mat dried ultrafiltration permeate hydrolysate (UFP)

Phenolic acids	Treatment	df	MS	F	Sig
trans-Cinnamic acid	Treatment	3	0.001	17.792	0.001
	Error	8	6.667×10^{-5}		
	Total	12			
Naringenin	Treatment	3	0.010	48.333	0.000
	Error	8	0.000		
	Total	12			
Quercetin	Treatment	3	0.000	3.619	0.065
	Error	8	5.833×10^{-5}		
	Total	12			

Table C.8 The ANOVA table showing effect of germination and drying temperature on phenolic compounds of foam-mat dried ultrafiltration retentate hydrolysate (UFR*)*

Phenolic acids	SOV	df	MS	F	Sig
Gallic acid	Treatment	3	0.006	13.476	0.002
	Error	8	0.000		
	Total	12			
Catechol	Treatment	3	0.001	36.779	0.000
	Error	8	4.033×10^{-5}		
	Total	12			
4-Hydroxybenzoic acid	Treatment	3	3.056×10^{-7}	1.222	0.363
	Error	8	2.500×10^{-7}		
	Total	12			
(+)-Catechin	Treatment	3	7.778×10^{-6}	4.667	0.036
	Error	8	1.667×10^{-6}		
	Total	12			
Vanillin	Treatment	3	9.417×10^{-8}	12.556	0.002
	Error	8	7.500×10^{-9}		
	Total	12			

Table C.8 (continue) The ANOVA table showing effect of germination and drying temperature on phenolic compounds of foam-mat dried ultrafiltration retentate hydrolysate (UFR)

Phenolic acids	SOV	df	MS	F	Sig
Epicatechin	Treatment	3	0.000		
	Error	8	3.083×10^{-6}	56.027	0.000
	Total	12			
p-Courmaric acid	Treatment	3	8.333×10^{-8}	0.333	0.802
	Error	8	2.500×10^{-7}		
	Total	12			
Ferulic acid	Treatment	3	0.001	5.219	0.027
	Error	8	0.000		
	Total	12			
Rutin	Treatment	3	0.000	15.194	0.001
	Error	8	2.770×10^{-5}		
	Total	12			
trans-Cinnamic acid	Treatment	3	1.861×10^{-6}	2.030	0.188
	Error	8	9.167×10^{-7}		
	Total	12			
Naringenin	Treatment	3	4.044×10^{-5}	10.551	0.004
	Error	8	3.833×10^{-6}		
	Total	12			
Quercetin	Treatment	3	3.333×10^{-5}	4.000	0.052
	Error	8	8.333×10^{-6}		
	Total	12			

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table C.9 The ANOVA table showing effect of drying temperature and peptides size on antioxidant activity of foam-mat dried rice bean hydrolysate

Antioxidant activity	SOV	df	MS	F	Sig
EC_{50}	Treatment	11	0.037	13.994	0.000
	Error	24	0.003		
	Total	36			
DPPH assay	Treatment	11	11.777	74.185	0.000
	Error	24	0.159		
	Total	36			
FRAP assay	Treatment	11	1.044	39.379	0.000
	Error	244	0.027		
	Total	36			

Table C.10 The ANOVA table showing effect of germination and drying temperature on antioxidant activities measured using colorimetric method and electron spin resonance (ESR) method at concentration 1.5 mg ml^{-1} of hydrolysate

Antioxidant activity	SOV	df	MS	F	Sig
% DPPH scavenging activity (colorimetric method)	Treatment	3	53.136	13.66	0.002
	Error	8	3.902		
	Total	12			
% DPPH scavenging activity (ESR method)	Treatment	3	30.4475	8.761	0.007
	Error	8	3.478		
	Total	12			
% Hydroxyl radical scavenging activity	Treatment	3	115.563	72.350	0.000
	Error	8	1.597		
	Total	12			
% Carbon-centered radical scavenging activity	Treatment	3	30.815	17.174	0.001
	Error	8	1.794		
	Total	12			

Table C.11 The ANOVA table showing effect of molecular weight fraction on antioxidant activity of peptide and phenolic fraction of foam-mat dried rice bean hydrolysate

Antioxidant activity	fraction	SOV	df	MS	F	Sig
DPPH scavenging activity	Peptide	Treatment	11	3.009	268.749	0.000
		Error	24	0.011		
		Total	36			
DPPH scavenging activity	Phenolic	Treatment	11	4.992	387.156	0.000
		Error	24	0.013		
		Total	36			
FRAP	Peptide	Treatment	11	4.131	184.602	0.000
		Error	24	0.022		
		Total	36			
FRAP	Phenolic	Treatment	11	11.043	257.586	0.000
		Error	24	0.043		
		Total	36			

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

VITA

Mr. Burachat Sritongtae was born on October 26th, 1982, in Bangkok, Thailand. He obtained a Bachelor of Science (Microbiology), Faculty of Science, Kasetsart University in academic year 2005. In 2005, he went to study at Department of Food Technology, Faculty of Science, Chulalongkorn University and received Master of Science (Food Technology) in 2009. In 2010, he enrolled in the doctoral degree program in Food Technology at Department of Food Technology, Faculty of Science, Chulalongkorn University.