ผลิตภัณฑ์มูลค่าเพิ่มจากรำข้าวและถั่วเหลืองโดยปฏิกิริยาไฮโดรไลซิส ในน้ำกึ่งวิกฤติ

นางสาวเกตุมณี วัชรรุจิ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
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VALUE-ADDED PRODUCTS FROM RICE BRAN AND SOYBEAN BY HYDROLYSIS IN SUBCRITICAL WATER

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A Thesis Submitted in Partial Fulfillment of the Requirements
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งานวิจัยนี้ศึกษาการผลิตผลิตภัณฑ์มูลค่าเพิ่มจากผลิตผลทางการเกษตรที่มีราคาขายต่อหน่วยถูก ซึ่งได้แก่ รำข้าว (รำข้าวดิบและรำข้าวสกัด) และถั่วเหลือง (ถั่วเหลืองดิบและถั่วเหลืองสกัด) ด้วยปฏิกิริยาไฮโดรไลซิสที่สภาวะน้ำกึ่งวิกฤติ โดยทดลองการเกิดปฏิกิริยาในระบบถังปฏิกรณ์แบบกะที่สภาวะต่างๆ คือ ศึกษาปฏิกิริยาไฮโดรไลซิสในช่วงอุณหภูมิ 200-220 องศาเซลเซียส ช่วงเวลา 10-30 นาที และ ศึกษาผลของอัตราส่วนของวัตถุดิบต่อน้ำ เท่ากับ 1:5 และ 2:5 ที่จะส่งผลต่อปริมาณผลได้ของโปรตีน กรดอะมิโน และน้ำตาลรีดิวซ์ จากผลการทดลองพบว่าน้ำกึ่งวิกฤตมีประสิทธิภาพที่ดีในการสกัดโปรตีน กรดอะมิโน และน้ำตาลรีดิวส์ อีกทั้งยังสามารถสกัดสารประกอบซึ่งมีคุณสมบัติในการต่อต้านอนุมูลอิสระจากรำข้าว (รำข้าวดิบและรำข้าวสกัด) และถั่วเหลือง (ถั่วเหลืองดิบและถั่วเหลืองสกัด) ได้อีกด้วย สภาวะที่เหมาะสมสำหรับการผลิตโปรตีนและกรดอะมิโนจากรำข้าวและถั่วเหลือง คือที่อัตราส่วน 1:5 และเวลาในการไฮโดรไลซิส 30 นาที ซึ่งอุณหภูมิที่เหมาะสมในการเกิดปฏิกิริยาสำหรับรำข้าว (ทั้งรำดิบและรำสกัด) คือ 220 องศาเซลเซียส และอุณหภูมิที่เหมาะสมสำหรับถั่วเหลืองดิบและถั่วเหลืองสกัดคือ 210 และ 200 องศาเซลเซียส ตามลำดับ นอกจากนี้ผลการทดลองยังแสดงว่า ผลิตภัณฑ์ที่ได้จากการสกัดรำข้าวสกัดที่อุณหภูมิ 200 องศาเซลเซียส เวลา 30 นาที และที่อัตราส่วน 1:5 เหมาะที่จะนำมาใช้เป็นสารอาหารสำหรับเลี้ยงเซลล์ยีสต์และเมื่อนำผลิตภัณฑ์ที่ได้นี้มาทดสอบระบบประสาทสัมผัสด้วยวิธีการเปรียบเทียบตัวอย่างคู่ เพื่อหาความชอบระหว่างเครื่องดื่มนมและกาแฟที่มีการเติมผลิตภัณฑ์กับตัวอย่างควบคุม พบว่าผู้บริโภคมีความชอบในรสชาติโดยรวมของนมที่มีการเติมผลิตภัณฑ์มากกว่านมที่ไม่มีการเติมผลิตภัณฑ์อย่างมีนัยสำคัญ($P \leq 0.5$) และผู้บริโภคมีความชอบไม่แตกต่างกันในรสชาติโดยรวมของกาแฟที่มีการเติมและไม่มีการเติมผลิตภัณฑ์อย่างมีนัยสำคัญ($P > 0.5$) จากงานวิจัยนี้สามารถสรุปได้ว่าการไฮโดรไลซิสที่สภาวะน้ำกึ่งวิกฤติเป็นกระบวนการที่น่าสนใจ เนื่องจากสามารถพัฒนาและเพิ่มมูลค่าให้กับผลิตผลทางการเกษตรที่มีราคาขายต่อหน่วยถูกให้เป็นผลิตภัณฑ์อาหารเสริมที่มีประโยชน์

ภาควิชา.....วิศวกรรมเคมี.....ลายมือชื่อนิสิต.....เกตุมณี วัชรรุจิ.....

สาขาวิชา.....วิศวกรรมเคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

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KEY WORD: HYDROLYSIS / SUBCRITICAL WATER / RICE BRAN / SOY BEAN / PROTEIN / AMINO ACID / ANTIOXIDANT

KETMANEE WACHARARUJI: VALUE-ADDED PRODUCTS FROM RICE BRAN AND SOYBEAN BY HYDROLYSIS IN SUBCRITICAL WATER. THESIS ADVISOR: ASST. PROF. ARTIWAN SHOTIPRUK, Ph.D., 89 pp.

This study deals with the production of new value-added product derived from agricultural by-products which are raw and deoiled rice bran as well as raw and deoiled soybean meal by means of subcritical water (SW) hydrolysis. The SW hydrolysis reaction was carried out in a closed bath reactor in which the effect of temperature in the range of 200-220 °C, reaction time of 10-30 min, raw material to water weight ratio of 1:5 and 2:5, were determined on the yields of protein, amino acid, and reducing sugars. The hydrolysis products were separated into solid residue, whose dried weight was measured, and the aqueous product, which was analyzed for proteins, amino acids, reducing sugar and its antioxidant activity. The results in the present study suggested that subcritical water could be used to potentially hydrolyze raw and deoiled rice bran and raw and deoiled soybean meal into more valuable products. The suitable hydrolysis time was 30 min and the proper weight ratio of the raw material to water was 1:5. The reaction temperature that was suitable for the production of protein and amino acids was 220 °C for raw and deoiled rice bran, 210 °C for raw soybean meal, and 200 °C for deoiled soybean meal. The products were also found to have antioxidant activity as tested by $ABTS^{\bullet+}$ scavenging assay. In addition, the hydrolysis product of deoiled rice bran obtained at 200 °C after 30 min and ratio of 1:5 was demonstrated for the potential use as nutrients for yeast growth. As a test of possible application of the product in human food, sensory evaluation was conducted on the deoiled rice bran hydrolysis product, in which two pairs of test products were compared: the milk control and the milk added with rice bran product, and the coffee control and the coffee added with the rice bran product. The analysis of the results showed that the number of testers who preferred the milk added with rice bran product was significantly higher than those preferring the milk control ($P \leq 0.05$) and the number of the testers preferring of the coffee control and the coffee added with extraction product were not significantly different ($P > 0.05$). From the results of this study, it was concluded that SW hydrolysis is an interesting technique that could be used to develop new value-added functional foods from low cost agricultural by-products.

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Field of study….Chemical Engineering……Advisor's signature

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CHAPTER I

INTRODUCTION

1.1 Motivation

Rice and soybean are among the most important industrial crops produced as human food sources. Rice (*Oryza sativa*) is a cereal crop which feeds more than half of the world's population. About 617 million metric tons of rice are annually produced (IRRI, 2005) world wide. For Thailand, rice is the most important crop plant and 30.29 million tons of which is produced annually (Office of Agricultural Economics, 2005). This makes Thailand No.6 largest rice producer in the world and the main rice exporter. Due to the consumers' preference of white rice over brown rice, rice bran, which accounts for approximately 8% of milled rice, would be removed during in the milling process, making it one of the most interesting byproducts of rice milling industry (Shih *et al*., 1999). Despite the fact that rice bran is a source of proteins, oil, nutrients, and calories (Barber and Benedito de Barber, 1991), the majority of the rice bran is used as animal feed (Berk.Z, 1992). Only a small proportion is consumed by human, which is usually in the form of rice bran oil.

Similarly, soybean, *Glycine max L.* is considered as an oil plant. It is a legume crop generally cultivated for oil production and protein source. For each ton of crude soybean oil, approximately 4.5 tons of soybean meals are produced, and 44% of protein (Cheftel *et al*., 1985). In Thailand, approximately 0.22 million tons of soybean are produced annually (Office of Agricultural Economics, 2005). Like rice bran, the majority of soy bean meal is used as food source for livestock.

In addition to proteins and amino acids, it is well known that rice bran and soybean contain various antioxidant compounds that impart beneficial effects on human health. Those contained in rice bran include vitamin E (tocopherols and tocotrienols), vitamin C, anthocyanidins, isoflavones, beta-carotene, polyphenols and oryzanol. Among these, the most powerful antioxidative compound in rice bran is oryzanol (Godber and Wells, 1994), which has been reported to be used in cure of nerve imbalance and disorders of menopause (Roger *et al*., 1993). For soybean, the most powerful antioxidants are isoflavones, particulary daidzein and genistein which

have been reported for its ability to fight against breast cancer, prostate cancer, menopausal symptoms, and heart disease (Ruiz-Larrea *et al.*, 1997). Several protein hydrolysates derived from rice bran and soybean meal which cotain amino acids such as tyrosine, methionine, histidine, tryptophan and proline have also been found to exhibit antioxidant activity (Saiga, Tanabe and Nishimura, 2003, Jung and Kim, 1995 and Marcuse, 1960). It is thus the aim of this study to produce value-added products from rice bran and soybean meal for human consumption.

The most common method for the production of protein is by alkali or acid hydrolysis followed by acid precipitation. This method is simple because the agents required for the process is easily available. However, the protein yield is low due to the degradation at extreme pH conditions. Another disadvantage includes use of toxic chemicals, which must be washed thoroughly from the product, leading to a large amount of wastewater. Alternatively, enzymatic process has been studied which produces no toxic chemicals, however it takes a long time and the high cost of enzymes makes the process commercially uneconomical.

Subcritical water or pressurized water at temperature between the boiling point (100 °C) and its critical temperature (374.15 °C) is an interesting alternative. At such condition, water polarity decreases, thus makes it a better solvent for extraction of several organic bioactive compounds (Ibáñez *et al.*, 2003; Basile, Jiménez-Carmona and Clifford, 1998). In addition, at this condition, water ionization constant (K_W) increases. Increased ion product means that water readily ionizes to hydrogen and hydroxide ions, which play an important role in hydrolysis reaction. In our previous study, hydrolysis of deoiled rice bran (subcritical water) for the production of protein was investigated. The amounts of protein and amino acids produced were analyzed using Lowry's and Ninhydrin methods and were found to be higher than those obtained by conventional alkali hydrolysis (Sereewatthanawut and Prapintip *et al.*, 2006).

In the present study, application of subcritical water was extended to the production of value-added products from other agricultural by-products such as raw and deoiled rice bran as well as raw and deoiled soybean meal.

1.2 Objectives

The objectives of this study are as follows:

1. To investigate the subcritical water hydrolysis of agricultural by-products which are raw and deoiled rice bran and raw and deoiled soybean meal to produce useful products containing protein, amino acids, reducing sugar and antioxidants.
2. To investigate the suitable conditions for hydrolysis of protein and amino acids form raw and deoiled rice bran and raw and deoiled soybean meal with subcritical water and examine the antioxidant activity of the soluble hydrolysis product.
3. To examine the possible application of the hydrolysis product as nutrient for yeast growth and as human food.

1.3 Working Scopes

1. Determine the suitable temperature (200-220 °C), time (10-30 min), and solid to water weight ratio (1:5 and 2:5) for hydrolysis of raw and deoiled rice bran and raw and deoiled soybean meal.
2. Determine the amount of protein, amino acids, and reducing sugar in the hydrolysis product using Lowry's, Ninhydrin and DNS methods, and evaluate the antioxidant activity of soluble products using $ABTS^{\bullet+}$ scavenging assay.
3. Preliminarily examine the use of the hydrolysis products derived from deoiled rice bran as culture media for yeast growth.
4. Conduct sensory evaluation of the deoiled rice bran hydrolysis products using a paired preference test.

1.4 Expected Benefits

The results of this study would lead to the development useful of value-added products from available agricultural by-products in the country employing subcritical water processing.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Rice grain

Rice (*Oyaza sativa*) is one of the most staple diets for human especially in Asian countries. For about 617 million metric tons of rice are annually produced in recent years (IRRI, 2005). More than half of the production belongs to Asian countries which are China, India, Indonesia, Bangladesh, Vietnam and Thailand. For Thailand, rice is produced annually 30.29 million tons (Office of Agricultural Economics, 2005). This makes Thailand No.6 largest rice producer in the world and the main rice exporter.

2.1.1 Structure of rice grain

In general, each rice kernel is composed of the hull and bran layers. Hull or husk encloses the brown rice which consists mainly of embryo and endosperm (Figure 2.1). Bran coat is a thin layer which contains fiber, vitamin B, protein and fat. The most nutritious part of rice resides in this layer. Endosperm is made up mostly of starch which is the energy source for the embryo and is what is consumed by human for energy source. The embryo or germ is the innermost part of rice consisting mainly of starch called amylose and amylopectin. This part is removed during the milling process.

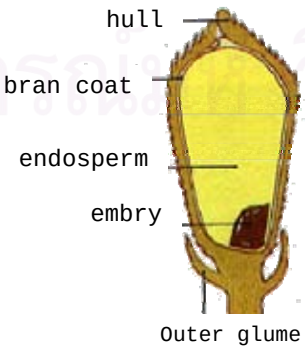


Figure 2.1 Structure of rice grain[1]

[1]Source: Pechsiam Daily Food Co., Ltd., 2006.

After threshing, the rough rice is transported to mills for processing into white rice (polished rice) through a series of operations that free it from the hull, germ, and bran. In many countries, processing of rice for local use is still carried out in one-stage mills. The by-product of this simplest form of processing is a mixture of hulls and bran that seldom reaches the market as it is usually returned to the rice grower. The percentage of by-products from rice milling industry is shown in Table 2.1.

Table 2.1 Percentage of by-products from rice milling industry[1]

Component of By-Product	Weight Percent
Hulls	2
Bran	10
Polishings	3
Broken rice	1 to 17
Polished rice	50 to 66

[1]Source: Smtth (1948).

Rice bran production

Rice bran is the most valuable by-product of the rice milling industry. It is obtained from the outer layers of the brown rice during milling. Rice bran consists of pericarp, aleurone layer, germ and a part of endosperm.

Rice bran can be classified into three groups;

1. Full fatted raw bran (i.e. raw bran) obtained from milling of raw paddy.
2. Full fatted parboiled bran (i.e. parboiled bran) obtained from milling of parboiled paddy.
3. De-fatted/De-Oiled bran obtained after extraction of oil from either raw or parboiled bran.

2.1.2 Rice bran chemical compositions

The compositions of rice bran are reported to vary due to a variety of factors either associated with the rice grain itself and the milling process or the differences in

analytical techniques used by different researchers. The typical range of composition of rice bran is shown in Table 2.2.

Table 2.2 Typical range of variation of the gross chemical composition of rice bran[1]

Constituents (% dry basis)	Minimum	Maximum
Protein	11.5	17.2
Fat	12.8	22.6
Fiber	6.2	14.4
Ash	8.0	17.7
NFE[2]	33.5	53.5

[1]Source: Luh (1991).

[2]Nitrogen-free extract

Apart from nutritious constituents such as carbohydrates, enzymes, protein and amino acids, several minerals, and vitamins are found in rice bran. The major protein fractions in rice bran are albumin, globulin, glutelin and prolamin. The typical mean weight ratio of albumin: globulin: glutelin: prolamin is 37 : 36 : 22 : 5 (Luh, 1991). Major free amino acids in bran are glutamic acid (7-31%), alanine (11-16%) and serine (5-15%) (Barber and Benedito de Barber, 1991). The average amino acids composition of rice bran is shown in Table 2.3.

Table 2.3 Amino acid composition of rice bran[1]

Amino Acids	Average[2]	Standard Deviation
Lysine	3.88	0.82
Histidine	2.11	0.57
Ammonia	1.72	0.96
Arginine	6.50	1.31
Aspartic Acid	7.62	2.03
Threonine	3.06	0.69
Serine	4.24	0.73
Tryptophan	1.70	0.51
Glutamic Acid	12.84	2.86

Amino Acids	Average[2]	Standard Deviation
Proline	4.10	1.01
Glycine	4.52	0.86
Alanine	5.67	1.22
Cystine	1.63	0.58
Valine	5.45	0.43
Methionine	2.22	0.35
Isoleucine	3.94	0.54
Leucine	6.96	1.44
Tyrosine	3.65	1.28
Phenylalanine	4.47	0.83
%N Recovered	85.60	11.30

[1]Source: Barber and Benedito de Barber (1977).

[2]g / 16.0 gN

2.1.3 Antioxidants in rice bran

Current research has shown that rice bran contains over 100 different antioxidants such as oryzanol, phytosterols, polysaccharides, beta-sitosterol and vitamin E complex (Bidlank, 1999), to name a few. Moreover, rice bran contains over 40 minerals particularly zinc and manganese that enhance the synergistic action of the antioxidants and co-enzymes and ability to control free radicals (Balch, 1993). Among these, the most powerful antioxidative compound in rice bran is oryzanol (Godber and Wells, 1994), which has been reported to be used in cure of nerve imbalance and disorders of menopause (Roger *et al*., 1993). Other minor compounds present in rice bran which are claimed to protect against heart disease are phenolic compounds, ferulic acid, methyl ferulate, coenzyme Q 10, lipoic acid, and saponins (De Decker and Korver, 1996). Saponins in the rice bran was shown to increase the production of Superoxide Dismutase (SOD) and Lipid Peroxide Oxidase (LPO), which help to strengthen the immune system, protect DNA, slow the aging process and suppress tumor formation (Balch, 1993). In addition, several protein hydrolysates derived from rice bran which contain amino acids such as tyrosine, methionine, histidine, tryptophan and proline have also been found to exhibit antioxidant activity (Saiga, Tanabe and Nishimura, 2003, Jung and Kim, 1995 and Marcuse, 1960). These

antioxidants have some benefit individually, but are most effective as part of a system where multiple antioxidants work synergistically to provide a broad range of support for human body.

2.1.4 Utilization of rice bran

Rice bran is widely used in cooking in the form of rice bran oil. The process of oil production involves solvent extraction with hexane to obtain crude rice oil. The crude oil then undergoes several steps such as deacidification, deodorization, winterization, and distillation to obtain refined oil which is ready for use. The deoiled bran is generally used as protein source for livestock.

In spite of the nutritious components in rice bran, rice bran has not yet been widely used for human consumption. However, several researches have been conducted in order to add rice bran into food products which include bread, muffins, pancakes, cookies, cakes, pies, cereals (Lynn, 1969), puddings (Vanossi, 1958), pickles (Sakurai, 1977), koji (ingredients in bean paste and soy sauce manufacture) (Yokochi, 1977). Moreover, Lynn (1969), and Desikachar and Parpia (1970) had studied and proposed the formulation of a bran milk-like beverage as a cow's milk substitution. Table 2.4 shows the comparable nutritive compositions of bran milk to cow's milk. Due to the light-tanned color, virtually odor and flavour free, the beverage is found to be suitable for flavoring and coloring with any flavours.

Table 2.4 Comparison of bran milk-like beverage and cow's milk[1]

Component (% wet basis)	Bran Protein Milk	Cow's Milk
Protein	3.60	3.42
Fat	4.00	3.67
Mineral	0.70	0.73
Carbohydrate	4.81	8.77
Water	87.10	87.30

[1]Source: Lynn (1969).

2.2 Soybean

Soybean *(Glycine max L.)* is originated in Eastern Asia, probably in the north and central China. It is believed that cultivated varieties were introduced into Korea and later into Japan some 2000 years ago. Soybean has been grown as a food crop for thousands of years in China and other countries of East and South East Asia and constitute to this day as an important component of the traditional popular diet in these regions.

Soybeans are, primarily, an industrial crop, cultivated for oil and protein. Despite the relatively low oil content of the seed (about 20% on moisture-free basis), soybeans are the largest single source of edible oil and account for roughly 50% of the total oilseed production of the world. Moreover, it is one of the largest potential sources of dietary protein. With each ton of crude soybean oil, approximately 4.5 tons of soybean meals with a protein content of about 44% are produced. For each ton of soybeans processed, the commercial value of the meal obtained usually exceeds that of the oil.

2.2.1 Structure of soybean

Seed structure of soybean consists of the seed coat (hull) and two cotyledons, plus two additional structures of lesser weight: the hypocotyl and plumule (Figure 2.2). The cotyledons represent 90% of the seed weight and contain practically all the oil and protein in its palisade-like cells. Microscopic examination of these cells reveals the presence of protein bodies (also known as aleuron grains) and lipid bodies (or spherosomes) which constitute storage bodies for proteins and oil, respectively. Protein bodies measure, on the average, 10 microns while the lipid bodies have, typically, 0.2 to 0.5 microns in diameter.

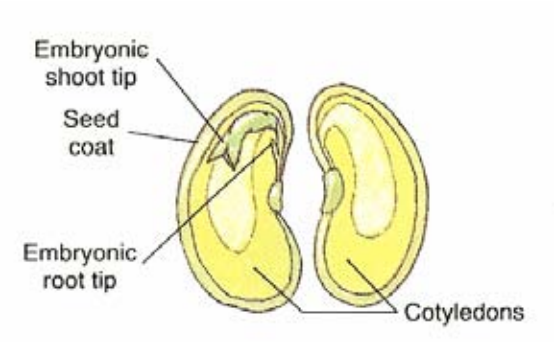


Figure 2.2 Structure of soybean[1]

[1]Source: Biology: the network of life. P.553, 1992.

The hull, which accounts for roughly 8% of the seed weight, holds the two cotyledons together and provides an effective protective layer. It can be removed from the seed by cracking followed by aspiration, as in the process of mechanical dehulling prior to solvent extraction.

2.2.2 Soybean chemical compositions

The composition of soybeans may vary somewhat according to varieties and growing conditions. Through plant breeding it has been possible to obtain protein levels between 40% and 45%, and lipid levels between 18 and 20%.

The proximate composition of soybeans, in fairly representative average figures, is shown in Table 2.5.

Table 2.5 Representative proximate composition[1]

Seed part	% of whole seed weight	% (moisture-free basis)			
		Protein	Lipid	Carbohydrate (incl.fibre)	Ash
Cotyledon	90	43	23	43	5
Hull	8	9	1	86	4.3
Hypocotyl	2	41	11	43	4.4
Whole seed	**100**	**40**	**20**	**35**	**4.9**

[1]Source: Cheftel *et al*. (1985).

The amino acid compositions of soybean are summarized in Table 2.6.

Table 2.6 Amino acid compositions of soybeans [1]

Amino Acids (%)	
I- Essential Amino Acids	
Isoleucine	4.54
Leucine	7.78
Lysine	6.38
Methionine	1.26
Cystine	1.33
Methionine+Cystine	2.59
Phenylalanine	4.94
Tyrosine	3.14
Phenylalanine+Tyrosine	8.08
Threonine	3.86
Tryptophan	1.28
Valine	4.80
II- Non Essential Amino Acids	
Arginine	7.23
Histidine	2.53
Alanine	4.26
Aspartic acid	11.70
Glutamic acid	18.70
Glycine	4.18
Proline	5.49
Serine	5.12

Source: Computed from data in FAO (1970) and FAO/WHO (1973).

2.2.3 Antioxidants in soybean

It has been discovered that soybean contains many antioxidants such as isoflavones, beta-carotene, vitamin C, vitamin A, lycopene and mineral particularly calcium, zinc and selenium (Senck, 2001), which have powerful antioxidant properties and that eating soy can help fight free radical damage to body. Consumption of soybean is believed to reduce both overall cholesterol levels and LDL (low-density lipoprotein or "bad") cholesterol levels, without affecting the levels of beneficial HDL. Most of the health benefits of soybean have been attributed to its content of isoflavones, particularly daidzein and genistein, which resemble natural

estrogens in the body. These isoflavones have been reported for its ability to fight against breast cancer, prostate cancer, menopausal symptoms, and heart disease (Ruiz-Larrea *et al*., 1997). Current research has reported that soy isoflavones alone (Hodgson *et al*., 1996) and in combination with vitamin C (Hwang *et al*., 2000) could reduce the oxidation of plasma LDL cholesterol.

2.2.4 Utilization of soybean

The utilization options of soybeans can be divided into two groups: those based on the utilization of the whole seed and those which start with the fractionation of the soybean into oil and meal. An estimate of the quantitative distribution of the world soybean production according to these main routes of utilization is given in Figure 2.3.

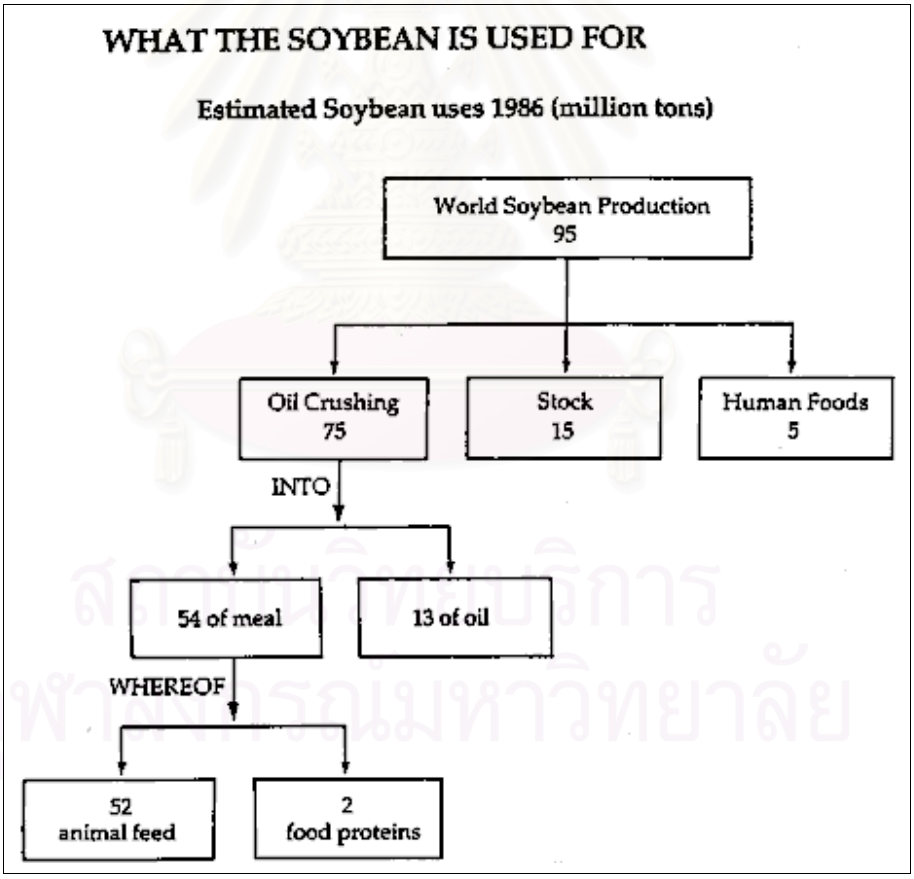


Figure 2.3 Main Routes of Soybean Utilization[1]

[1]Source: Soya Technology Systems, 1987

2.3 Isolation of proteins and amino acids from rice bran

Although the utilization of rice bran is still limited to animal feed as mentioned previously, various processing methods have been developed through many years for isolation of proteins from rice bran either in the forms of full-fat or deoiled. The existed methods can be classified into two categories; (i) physical method and (ii) chemical method. Both methods are described below.

Physical method

This method is also called “Dry Fractionation Method”. In principle, the bran was fractionated by the separated collection of the individual bran streams from the different whitening cones. Although this method induces less unnatural alterations, it has not been proven to be successful since the differences among bran streams do not justify the individual segregation (Barber and Benedito de Barber, 1977). The increments of fraction of protein before and after processing this method have proven to be relatively low which were only 15% protein as compared with 10.6% in the original deoiled bran (Houston and Mohammad, 1966), and 14.7% from 13.9% in the original deoiled bran (Anderson and Guraya, 2001). As a result, chemical method is more popular among industries.

Chemical method

Existing chemical methods can be classified into three groups; (i) Alkali Extraction, (ii) Enzyme-Assisted Water Extraction and (iii) Organic Solvent Sedimentation.

Alkali extraction

This method is consisted of alkali extraction followed by solid-liquid separation and isoelectric precipitation at pH 4.5. Sodium hydroxide has been found to be the most effective and economical alkali agents (Lynn, 1969). The range of pH used in the extraction process is typically 9 to 11. According to Barber and Maquieira, 1977, the extractability of protein increases with the increase of pH where the relation between pH and extractability of raw and stabilized bran is shown in Figure 2.4.

Alkali extraction was proven to be effective in solubilizing and extracting protein from rice bran, and with this method, 71-73% protein concentration could be

obtained from the extraction of unstabilized rice bran (Prakash and Ramanatham, 1994 and Gnanasambandam and Hettiarachchy, 1995). In recent years, various researches (Connor *et al.*, 1976, Mitsuo *et al.*, 1981, and Jiamyangyuen *et al.*, 2005) have also studied the extractability of deoiled rice bran using this method. The protein contents were reported in the high range of 70-73% and the yields were reported to vary from 10- 20%. Table 2.7 shows the protein yields of deoiled rice bran from alkali extraction process of various research studies.

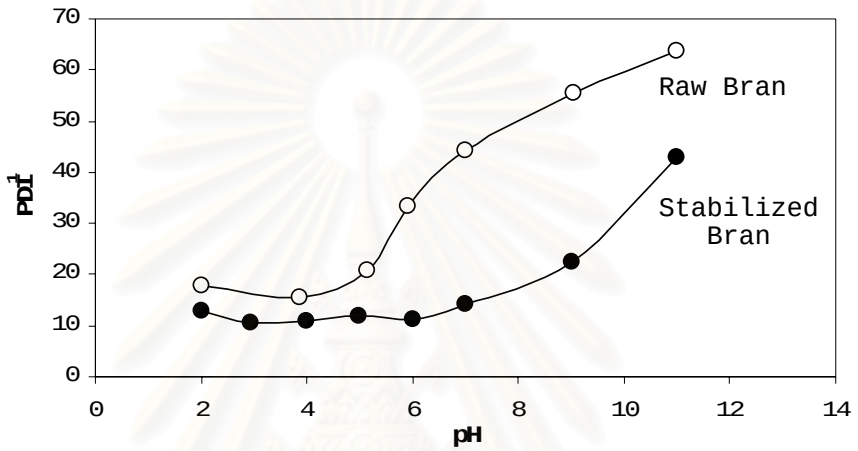


Figure 2.4 Effects of pH of extraction of raw and stabilized rice bran[2]

[1]PDI: Protein dispersibility index (%water dispersible protein / %total protein x 100)

[2]Source: Barber and Maquieira (1977), redrawn by the authors.

Table 2.7 Yield of protein of deoiled rice bran from alkali extraction process

Researcher (Year)	pH	Protein Yield (%)
Connor *et al.* (1976)	n/a	14-20
Mitsuo *et al.* (1981)	8-9	21
Jiamyangyuen *et al.* (2005)	9.5 and 12	9.58-9.60 and 12.20

Alkali extraction method is popular among industries and researches due to the availability of agents and simplicity of the process. However, operating under high pH condition could lead to undesirable results including molecular cross-linking and rearrangements of proteins resulting in decrease in nutritive value and formation of toxic compounds such as lysinoalanine (DeGroot and Slump, 1969, Cheftel *et al.*, 1985, and Otterburn *et al.*, 1989). Moreover, the exposure of proteins to alkali may cause severe protein degradation and undesirable flavor (Lynn, 1969). For the amino

acid component, the concentration does not much differ substantially from that of the original bran (Connor *et al*., 1977).

Enzyme-assisted water extraction

This method is widely used for food-use high-protein products. Various food grade enzymes have been used to enhance the protein extractability. Generally, alkali proteases were found to be more effective than carbohydrases (Hanmuangjai *et al.,* 2002). According to Wang *et al.*, 1999, the successful result of this method was accounted for 92% protein concentration was obtained from deoiled rice bran using phylase and xylanase. However, the enzyme dose not produces toxic but it will take a long reaction time and cost of enzymes is high and the process is uneconomical. The extraction of protein with water is recently found to be one of the most popular methods and will be concentrated in this present study.

Organic solvent sedimentation

In organic solvent sedimentation, n-Hexane is usually used to disintegrate rice bran into brown fraction and white fraction. The white fraction is separated from brown fraction by sedimentation and continuous centrifugation of the supernatant. The white fraction amounts to 35-40% of total original bran and contains around 22% protein (Mitsuda *et al.*, 1977). Although this method does not result in significant protein concentration, it is widely used since it intends to be attached to the conventional rice bran production. This method however has some drawbacks. n-Haxane can be emitted into the atmosphere where it may produce ozone and photochemical oxidant by reacting with other pollutants, which can adversely impact with the environment (Rosenthal, 1996). Furthermore, the adulteration of n-hexane in protein product may reduce its food-use functional property and quality of the protein.

2.4 Isolation of proteins and amino acids from soybean

Most of soybean meal is used as a high-protein component of animal feeds. However, some of it is also processed into products for human consumption. These include defatted soybean flours and grits, soybean protein concentrates, and soybean protein isolates which can be incorporated into a wide range of food products.

Edible soybean protein concentrates are relatively new products. Their availability as commercial products dates from 1959. In the last 30 years or so, these versatile products have become important ingredients, well accepted by many food industries. In many applications, they simply replace soy flours. In others, they have specific functions which can not be performed by soy flours.

Soybean protein concentrates normally cost 2 to 2.5 times more than defatted soy flour. Considering the relative protein contents of these two products, the cost per unit weight of protein is about 80% higher in the concentrate.

The starting material for the production of soy protein concentrates is dehulled, defatted soybean meal with high protein solubility (white flakes). The concentration of protein is increased by removing most of the soluble non-protein constituents. These constituents are primarily soluble carbohydrates (mono, di and oligosaccharides), but also some low molecular weight nitrogenous substances and minerals. Normally, 750 kilograms of soybean protein concentrate are obtained from one metric ton of defatted soybean flakes. Soybean protein concentrates have basically the following proximate composition, on a moisture-free basis:

Table 2.8 Representative proximate composition of soybean protein concentrates[1]

Component	Weight Percent
Protein	70
Total carbohydrate	20
Ash	5 to 8
Lipids	1

[1]Source: Campbell *et al.* (1985).

Table 2.9 Amino acid composition of SCP and soy flour (grams per 16g nitrogen)[1]

Amino Acids	Soy flour	Soy protein concentrate (SCP)	
		Alcohol wash	Acid wash
Alanine	4.00	4.86	4.03
Arginine	6.95	7.98	6.46
Aspartic acid	11.26	12.84	11.28
Half-cystine	1.45	1.40	1.36
Glutamic acid	17.18	20.20	18.52
Glycine	3.99	4.60	4.60
Histidine	2.60	2.64	2.59
Isoleucine	4.80	4.80	5.26
Leucine	6.50	7.90	8.13
Lysine	5.70	6.40	6.67
Methionine	1.34	1.40	1.40
Phenylalanine	4.72	5.20	5.61
Proline	4.72	6.00	5.32
Serine	5.00	5.70	5.97
Threonine	4.27	4.46	3.93
Tryptophan	1.80	1.60	1.35
Tyrosine	3.40	3.70	4.37
Valine	4.60	5.00	5.57

[1]Source: Campbell *et al*. (1985)

Isolated soybean proteins (ISP), or soybean protein isolates as they are also called, are the most concentrated form of commercially available soybean protein products. They contain over 90% protein, on a moisture free basis.

Soy protein isolates have been known and produced for industrial purposes, mainly as adhesives for the paper coating industry, well before World War II. ISP's for food use; however, have been developed only in the early fifties.

The basic principles of ISP production are simple. Using defatted soy flour or flakes as the starting material, the protein is first solubilized in water. The solution is separated from the solid residue. Finally, the protein is precipitated from the solution, separated and dried. In the production of ISP for food use, in contrast to ISP for

industrial use, care is taken to minimize chemical modification of the proteins during processing. Obviously, the sanitary requirements are also much more demanding.

The cost of isolated soybean proteins is five to seven times higher than that of defatted soy flour. On an equal protein weight basis the cost ratio of these two products is nearly 3:1

Table 2.10 Typical composition of ISP (Moisture-free basis)[1]

Component	Weight Percent
Protein	90
Fat	0.5
Ash	4.2
Total carbohydrate	0.3

[1]Source: Kolar *et al*. (1985)

Methods of protein extraction from soybean

The methods of protein extraction of soybean are similar to those employed for extraction of rice bran protein. These methods are summarized here.

The aqueous alcohols wash process

The process is based on the ability of aqueous solutions of lower aliphatic alcohols (methanol, ethanol and isopropyl alcohol) to extract the soluble sugar fraction of defatted soy flour without solubilizing its proteins. The optimal concentration of alcohol for this process is about 60% by weight. Starting with defatted white flakes as raw material, the process consists of the following steps: liquid-solid extraction, removal and recovery of the solvent from the liquid extract, removal and recovery of the solvent from the extracted flakes, drying and grinding of the flakes.

However, in the case of alcohol extraction, the solvents are quite volatile and flammable. Adequate precautions for the prevention of fire and explosion are necessary.

The acid-wash process

This process is based on the pH-dependence of the solubility of soybean proteins. It is recalled that the majority of soybean proteins exhibit minimum solubility at pH 4.2 to 4.5 (isoelectric region). Therefore, it is possible to extract the sugars, without solubilizing the majority of the proteins, using, as a solvent, water to which an acid has been added so as to keep the pH at the isoelectric region.

The acid-wash process has the obvious advantage of using a non-flammable, non-explosive, non-toxic and inexpensive solvent: water. To a certain extent, this is also the disadvantage of the process. Separation of the solid from the solvent is more difficult and less complete.

Alkali Extraction

More protein can be extracted at higher pH. However, the extracted proteins may undergo undesirable chemical modifications in strongly alkaline solutions. These include protein denaturation and chemical changes in amino acids. Excessively high pH also favours protein-carbohydrate interaction (Maillard reaction) which results in the formation of dark pigments and loss of nutritive value. Furthermore, proteins precipitated from highly alkaline media tend to retain too much water, and do not settle well. In addition, during the alkali extraction process, undesirable chemical reactions could occur. For example, dehydroalanine can react with free epsilon-amino groups of lysine, to produce lysinoalanine. This compound has been found to cause kidney lesions in rats under certain experimental conditions.

2.5 Subcritical water

Subcritical water is hot water at temperature between its boiling point (100 °C) and its critical temperature (374.15 °C) under pressure sufficient to maintain water in the liquid state (Figure 2.5). At a very high temperature, H_2O exhibits hydrocarbon-like properties and can be used for the destruction of organic matter. An increase in the temperature of water results in a decrease in its dielectric constant and an increase in ionization constant (K_W). The significance of increased ionization constant is that the hydrogen and hydroxide ions increase which play an important role in hydrolysis reaction.

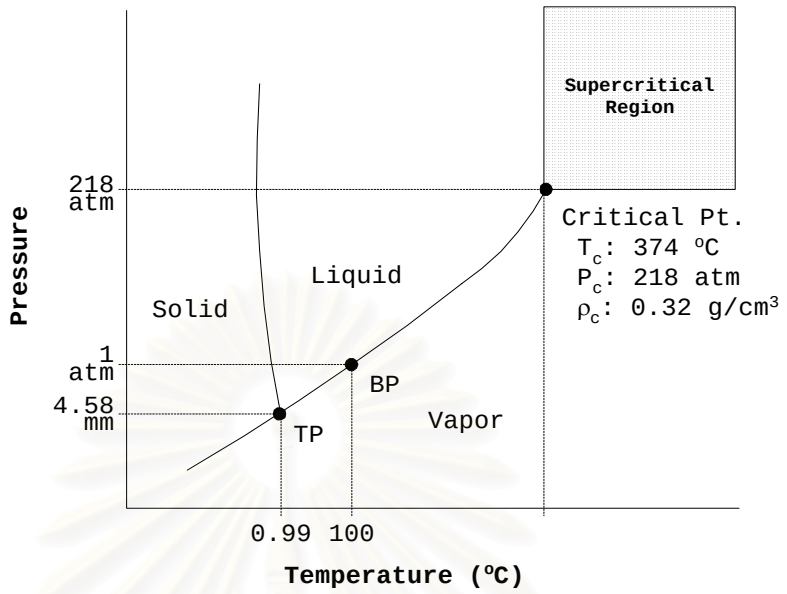


Figure 2.5 Phase diagram of water[1]

[1]Source: Chaplin (2006), redrawn by the authors.

Dielectric constant

At an ambient temperature, the high degree of association in the liquid water and the strong intermolecular hydrogen bonding causes its dielectric constant (permittivity relative to vacuum) to be as high as around 80. As the temperature rises the dielectric constant falls, as shown in Figure 2.6. Consequently, subcritical water can be a good solvent for larger organic compounds, and the solubility of an organic compound in subcritical water is often many orders of magnitude higher than its solubility in water at ambient temperature. From Figure 2.6, at approximately 200 °C, water dielectric constant is equal to that for methanol (i.e. 33) at 25 °C.

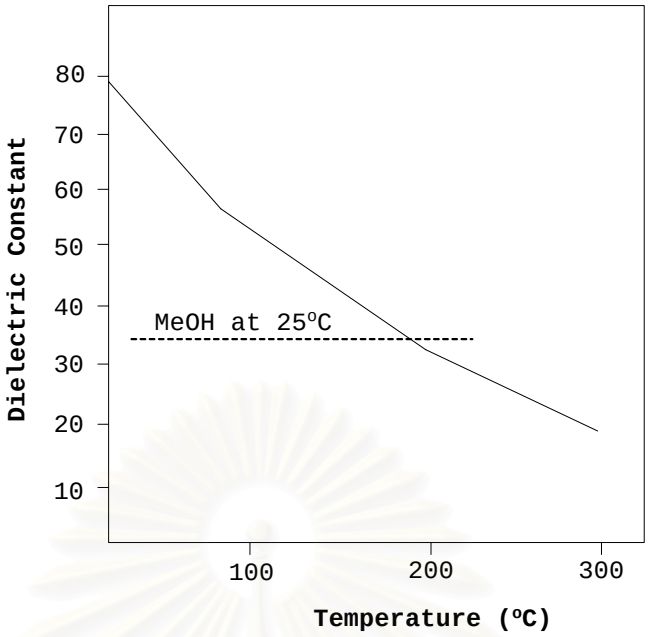


Figure 2.6 The dielectric constant of liquid water along the saturation line[1]

[1]Source: Association of Finnish Chemical Societies (2005), redrawn by the authors.

Ionization constant (Kw)

Naturally, the molecule of water endothermically ionizes or dissociates into hydrogen [H^+] and hydroxide [OH^-] ion. This is due to electric field fluctuations caused by nearby dipole libration (Geissler *et al.*, 2001). The reaction equation can be written as:

$$H_2O \rightleftharpoons H^+ + OH^-$$

As a result of the instability of hydrogen ion, it is hydrated with water itself which results in the formation of hydronium ion [H_3O^+]. The above equation is better written as:

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

The product of ionic concentrations (hydronium and hydroxide ion) is defined as ion product constant, K_W (also called ionization constant, dissociation constant). The relation is expressed as:

$$K_w = [H_3O^+][OH^-]$$

Although the extent of ionization is tiny ($[H^+]/[H_2O] = 2.8 \times 10^{-9}$ at 37°C), the ionization and changes in the tiny concentrations of hydrogen ions have absolute importance in several chemical processes.

Since a change in temperature causes a change in chemical equilibrium, therefore K_W is also temperature dependent; that is it increases with increasing temperature up to about 250^{o}C and then decreases onwards. Figure 2.7 illustrates the relationship of the K_W with water temperature.

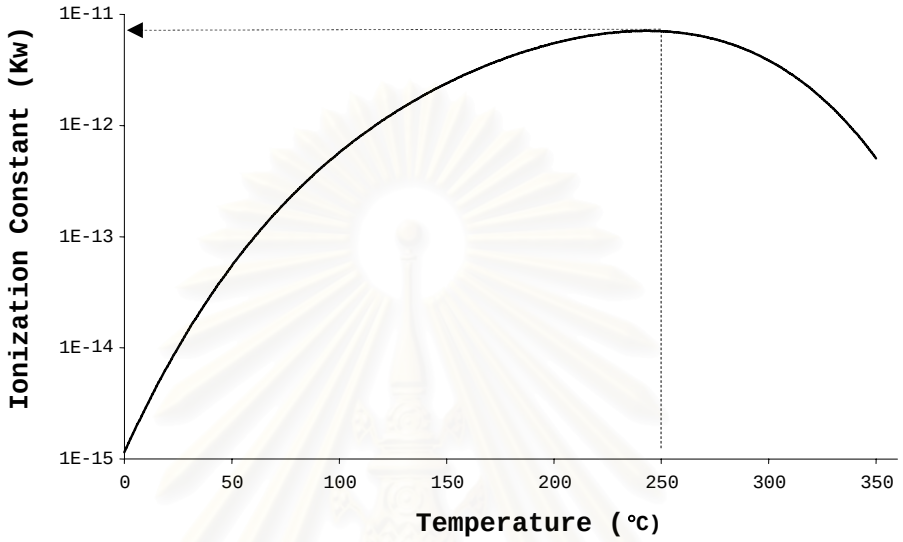


Figure 2.7 The ionization constant of water at various temperature[1]

[1]Source: Marczewski (2002), redrawn by the authors.

2.6 Hydrolysis

The hydrolysis reaction is defined as:

"A chemical reaction in which water reacts with another substance to form two or more new substances. This involves ionization of the water as well as splitting of the compound hydrolyzed."

(Source: Hawley's Condensed Chemical Dictionary, 11th Ed.)

According to the above definition, the hydrolysis of an organic molecule is a chemical transformation process in which an organic molecule (RX) reacts with water to form a new carbon-oxygen bond after cleaving a carbon-X bond in the original molecule, thus producing H^+X^-. The net reaction is therefore a *displacement* of X by a hydroxyl group (OH^-):

$$R\text{-}X + H_2O \rightarrow R\text{-}OH + X^- + H^+$$

For protein molecules, hydrolysis reaction breaks the peptide bonds through the addition of water, thus it forms two molecules: a carboxylic acid and an amine (two amino acid molecules). Figure 2.8 shows the hydrolysis reaction of a simple dipeptide bond.

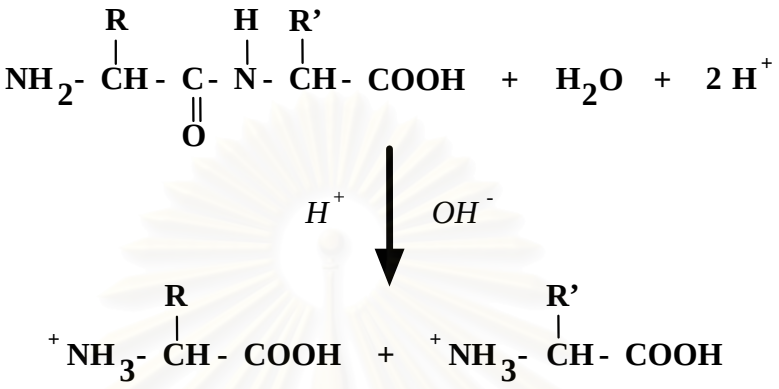


Figure 2.8 Hydrolysis reaction of dipeptide structure[1]

[1]Source: Clark (2004), redrawn by the authors.

Note that $2H^+$ ion is added to the reaction to complete the mechanism of amino acid solution. It is seen from the above figure that the peptide bond is hydrolyzed to two amino acid molecules. This concept can also be applied to a polypeptide (a protein chain); in which each of the peptide links is broken in exactly the same way. As a result, this reaction will end up with a mixture of amino acids that make up the protein.

It is clearly observed that the hydrogen and hydroxide ion play important role in hydrolysis reaction. It is therefore implied that the higher ionic concentration results in higher hydrolysis activity. As mentioned in the earlier topic, the increase of temperature in the selected subcritical region (100-250^{O}C) results in the increase of K_W which is related to the ionic concentration. The hydrolysis using subcritical water is therefore a promising technique.

2.7 Literature reviews

In recent years, research and development on the subject of subcritical water has been intensified. Researchers explore the usage of subcritical water for waste

treatment, polymer degradation, pharmaceutical manufacturing, chromatographic analysis, nuclear reactor cooling, to name a few. Other significant advances have also been made in material processing, ranging from fine particle manufacture to the creation of porous materials. The following reviews deal with past successful researches that employed subcritical water for processing of natural materials for production of value-added products.

Initially, subcritical water has been widely used in agricultural environmental analysis and treatment processes. The majority of usage was the removal of contaminants in soil and groundwater. The good reproducibility, safety, and low cost make subcritical water a promising processing medium. Other than extraction of environmental substances from solid matrix, subcritical water has been used for extraction of natural materials for useful metabolites such as in extraction of essential oils from *Rosmarinus officinalis* (Rosemary) (Basile *et al*., 1998), *Satureja gortensis* (Savory) (Kubatova *et al*., 2002), *Thymbra spicata* (Labiatae) (Ozel *et al*., 2003), and *Juniperus virginianna* (Eastern red cedar) (Eller and Taylor, 2004). Moreover, subcritical water has been used to extract some antioxidant compounds from *Rosmarinus officinalis* (Rosemary) (Ibanez *et al*., 2003), *Rhizoma coptidis* (Colden Thread), *Radix glycyrrhizae* (Liquorice), and *Radix scutellariae* (Baical Skullcap) (Ong and Len, 2003). Other recent examples of recent researches using subcritical water for extractions of medicinal active components include extraction of *Pimenta dioica* (Ginger) (Moreschi *et al*., 2004), extraction of anthraquinines from the roots of *Morinda citrifolia* (Noni) (Shotipruk *et al*., 2004), and extraction of anthocyanins and phenolics from dried red grape skin (Ju and Howard, 2005).

Moreover, hydrolysis in subcritical water has also been used to recover proteins, amino acids and organic acids. Recently, various applications of noncatalytic hydrolysis with subcritical water to natural materials such as cellulose (Sasaki *et al*., 1997), saccharides (Oomori *et al*., 2004), fish meat (Yoshida *et al*., 1999), baker's yeast (Lamoolphak *et al*., 2006), and silk fibroin (Kang *et al*., 2004) have been reported. Our previous study also demonstrated that subcritical water could be used for extraction of deoiled rice bran and the method yielded higher amount of protein and amino acids obtained by a conventional alkali hydrolysis (Sereewatthanawut and Prapintip *et al*., 2006).

CHAPTER III

EXPERIMENTAL METHODS

3.1 Materials and chemicals

Raw and deoiled rice bran was obtained from Thai Edible Oil Co., Ltd., Ayuthaya, Thailand and raw and deoiled soybean meal (whose seed coat was removed) was obtained from Thanakorn Vegetable Oil Products Co., Ltd., Samutprakarn, Thailand. Bovine serum albumin (BSA) was purchased from Acros organics, USA; Dinitrosalicylic acid (DNS) from Fluka, Germany and L-glutamic acid was purchased from Wako, Japan. D-glucose and all the other chemicals for Lowry's and and Ninhydrin's assay were purchased from APS Fine chem, NSW, Australia.

3.2 Subcritical water hydrolysis

The raw material was suspended in distilled water at the ratio of raw material-to-water of 1:5 or 2:5. Then, this suspension was charged into an 8.8-ml stainless steel (SUS-316) closed batch reactor (AKICO Co., Japan). The reactor was then heated with an electric heater to the desired temperature (200-220 ^{o}C). The pressure in the reactor was estimated based on saturated steam to be between 101.35 kPa and 3.97 MPa for the temperature range studied. After a desire reaction time (10-30 min), the reactor was immediately cooled to room temperature by immersion in a cool water bath (5 min). The liquid and solid contents in the reactor were collected and the remaining solid in the reactor was washed out with 5 ml water. The residue bran was separated from the soluble product with a vacuum filter using a filter paper (Whatman No. 1) and weighed after drying in a vacuum oven at 65^{o}C. The soluble portion was assayed for the amount of protein, amino acids and reducing sugars. All experiments were performed in duplicate.

3.3 Analytical methods

3.3.1 Analysis of protein

The protein content of the soluble portion was assayed using Lowry's method (Lowry *et al*., 1951), using bovine serum albumin (BSA) as a standard. The Lowry procedure is one of the most venerable and widely used protein assays. This method was selected for protein analysis in this present study due to its accuracy and simplicity. Under alkali conditions, copper reacts with protein. When folin phenol reagent (phospho-molybdic-phosphotungstic reagent) was added, the Folin-phenol reagent would bind to the protein. Bound reagent was slowly reduced and changes color from yellow to blue.

Lowry Stock Solutions

Lowry A: 1% (w/vol) copper sulphate ($CuSO_4$)

Lowry B: 2% (w/vol) sodium potassium tartrate ($NaKC_4H_4O_6 \bullet 4H_2O$)

Lowry C: 0.2 M sodium hydroxide (NaOH)

Lowry D: 4% (w/vol) sodium carbonate (Na_2CO_3)

Lowry Reagents

Lowry E: Solution of ABCD (1% A, 1% B, 49% C and 49% D by vol)

Lowry F: Folin's reagent dilute 1:1 in H_2O

Procedure

1) Add 0.5 ml of sample of the diluted soluble product (approximately 40-60 times) per tube.
2) Add 2.5 ml of Lowry E solution to each tube.
3) Incubate 10 min at room temperature.
4) Add 0.25 ml of Folin's reagent to each tube.
5) Incubate 10 min at room temperature.
6) Record the absorbance with a spectrophotometer at 750 nm.

3.3.2 Analysis of amino acids

Amino acids content was analyzed by Ninhydrin assays using L-Glutamic acid as a standard. Ninhydrin detection is one of the most common methods employed for quantitative amino acid analysis. As a rule, a Li-based cation-exchange system is

employed for the analysis of the more complex physiological samples, and the faster Na-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has characteristic of purple or yellow color. Amino acids, except imine acid, give a purple color, and show the maximum absorption at 570 nm.

Ninhydrin Stock Solutions

Reagent A: 1% (w/w) ninhydrin solution

Reagent B: 55% (vol/vol) glycerol

Reagent C: 0.5 M buffer citrate solution (pH = 5.5)

Reagent D: 100 mg/ml manganese chloride ($MnCl_2$)

Procedure

1) Add 0.2 ml of sample of diluted soluble product (approximately 2- 4 times) per tube.
2) Add 1 ml of Ninhydrin A solution to each tube.
3) Add 2.4 ml of Ninhydrin B solution to each tube.
4) Add 0.2 ml of Ninhydrin C solution to each tube.
5) Add 0.2 ml of Ninhydrin D solution to each tube.
6) Shake to mix the solution together.
7) Heat the solution in boiled water for 12 min.
8) Cool the solution down for 10 min.
6) Record the absorbance with a spectrophotometer at 570 nm.

3.3.3 Analysis of reducing sugar

Reducing sugars content was assayed by dinitrosalicylic colorimetric method, using D-Glucose as a standard using dinitrosalicylic reagemt. This method, developed by Sumner and co-worker (Sumner and *et al*., 1921), tests for the presence of free carbonyl group (C=O), the so-called reducing sugar. This involves the oxidation of the aldehyde functional group which presents, for example, glucose and the ketone

functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid is reduced to 3-amino, 5-nitrosalicylic acid (Figure 3.1).

aldehyde group $\xrightarrow{oxidation}$ carboxyl group

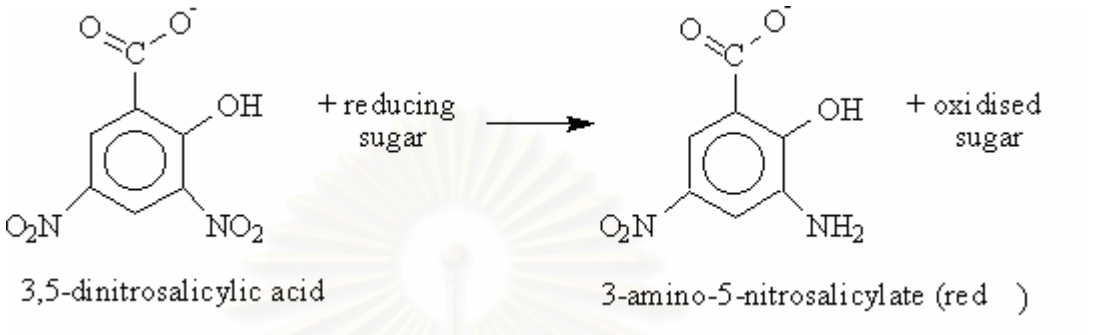


Figure 3.1 Reaction of 3,5-dinitrosalicylic acid in dinitrosalicylic colorimetric method.

The dinitrosalicylic acid reagent, for the determination of reducing sugar, is composed of dinitrosalicylic acid, Rochelle salt, phenol, sodium sulfite and sodium hydroxide. According to the authors of the test, Rochelle salt is introduced to prevent the reagent from dissolving oxygen and sulfite, to stabilize the color (given red-brown color) obtained in the presence of the phenol. The alkali is required for the reducing action of glucose on dinitrosalicylic acid.

Dinitrosalicylic Acid Reagent Solution, 1%

Dinitrosalicylic acid 10 g

Phenol 2 g

Sodium sulfite 0.5 g

Sodium hydroxide 10 g

Add water to 1 liter.

Rochelle salt

40% (w/vol) Potassium sodium tartrate solution

Procedure

1) Add 3 ml of DNS reagent to 3 ml of sample of diluted soluble product (approximately 10-80 times) in test tube.
2) Heat the mixture in boiled water for 5 min to develop the red-brown color.

3) Add 1 ml of a 40% Potassium sodium tartrate (Rochelle salt) solution to stabilize the color.
4) After cooling to room temperature in a cold water bath, record the absorbance with a spectrophotometer at 575 nm.

3.4 $ABTS^{\cdot+}$ scavenging assay

ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging assay was carried out following a modified method described by Re *et al*. (1999). The extract was diluted in series with water and each diluted solutions were added into $ABTS^{\bullet+}$ solution (aqueous solution of 7 mM ABTS and 2.45 mM potassium persulfate having absorbance of 0.70 ± 0.02 at 734 nm) with the volume ratio of 1:30 (sample solution: ABTS solution). The solutions were mixed using a vortex and the mixtures were incubated in the dark at room temperature for 10 min, after which the absorbance was measured at 734 nm.

For comparing the antioxidant activity of the extracts obtained at various conditions, concentration of sample producing 50% reduction of the radical absorbance (IC_{50}) was used as an index. The IC_{50} values for various extracts were found from the plots of percent inhibition (PI) versus the corresponding concentration of the sample. The values of PI were calculated using the following equation:

$$PI\ (\%) = [1-(A_t / A_r)] \times 100$$

Where A_t and A_r are absorbance of test sample and that of the reference, respectively.

3.5 Color measurement

For the purpose of quantifying the appearance of an object, the color of the product solution was measured using a Minolta Colorimeter CR-300 (Minolta Camera Co., Ltd. Japan) and the results are expressed in terms of 3-coordinate values (L, a*, and b* color space) which shows in Figure 3.2. L in the color space represents the luminance of the color (or value) on a numerical scale from 0 (black) to 100 (white). The color coordinates a* and b* represent the positions between red (+a*) and green (-a*), and between yellow (+b*) and blue (-b*). Prior to the color measurements, the instrument was calibrated against a standard white calibration plate to assure the

reliability of the instrument. Two readings were taken from randomly selected locations and the mean values of L, a* and b* were recorded.

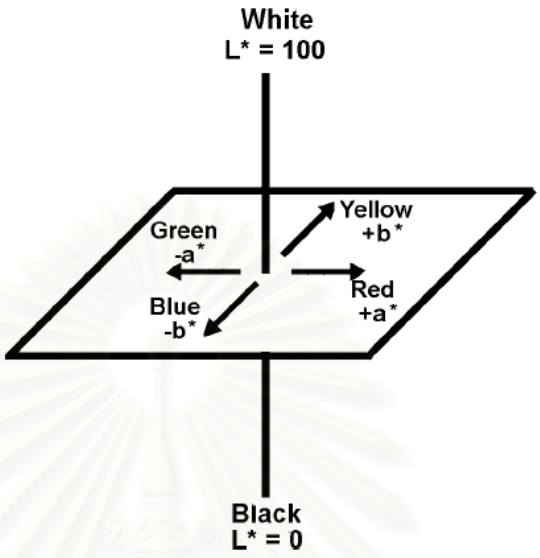


Figure 3.2 Color space stereoscopic image of L a*b* colorimetric system

3.6 Use of hydrolysis product for yeast growth

The product obtained after 30 min of SW deoiled rice bran hydrolysis at 200 ^{o}C was tested as a culture medium for yeast (*Saccharomyces cerevisiae*) growth. The yeast suspension was precultured overnight in a medium comprised of 2 w/v% of glucose, 2 w/v% of commercial yeast extract, and 2 w/v% SW deoiled rice bran hydrolysis in 60 ml distilled water. Under sterile condition, 5 ml of this culture was added to each of the culture flasks containing 100 ml of different culture medium that was autoclaved at 110 ^{o}C for 10 min and cooled prior to use. The different culture media tested were (1) control medium containing 2 w/v% glucose solution in distilled water, (2) the test medium containing 2 w/v% of glucose and 2 w/v% of subcritical water hydrolysis product (3) a culture medium containing 2 w/v% of glucose and 2 w/v% of commercial yeast extract granulated for microbiology. The test cultures were incubated in a controlled environment incubator shaker (New Brunswick Scientific Co., Inc., Edison, NJ, USA) at 30 ^{o}C and 150 rpm. The yeast growth was examined by

sampling the suspension every 2 h for the measurement of the optical density with spectrophotometer at 660 nm. All experiments were carried out in triplicates.

3.7 Paired preference test

Paired preference test is an initial consumer response test which two sets of samples (a pair) are required for the comparison of the taste preference. In this study, we conducted two sets of paired preference test. The first pair was to compare between milk control and the milk added with extraction product and the second pair was to compare between coffee control and the coffee added with extraction product. Before carrying out a paired preference test, the food samples being tested must be coded. The codes used can be three-digit numbers such as 457 for milk control, 425 for milk added with extraction, 946 for coffee control and 394 for coffee added with extraction. In this study, 40 tasters were used. For each pair of the samples, the samples were placed alternately into two columns. Each row is consisted of samples with and without the rice bran extract. The arrangement of the samples is illustrated in Figure 3.3. The number of place in configuration AB is equal to that of configuration BA, each consists of 20 set. Each taster was asked which of the pair of the products they prefered and the response was recorded. The results were then collected and analysed.

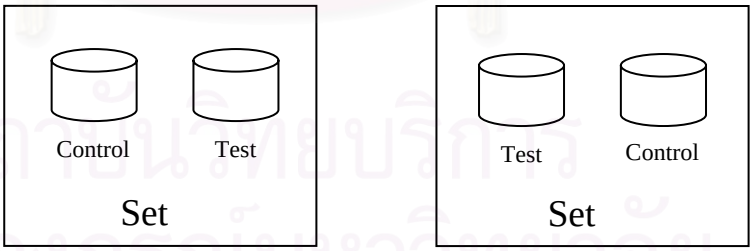


Figure 3.3 Sample arrangements for pair preference test

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Composition of raw materials

The composition of rice bran and soybean both as raw and deoiled materials are shown in Table 4.1. The information in the table was provided by the manufacturers of the raw materials, Thai Edible Oil CO., Ltd. and Thanakorn Vegetable Oil Products CO., Ltd for rice bran and soybean, respectively. The composition of the rice bran was analyzed by Near Infrared Spectroscopy (NIR), while that of the soybean was analyzed following the American Oil Chemists'Society (AOCS) procedure.

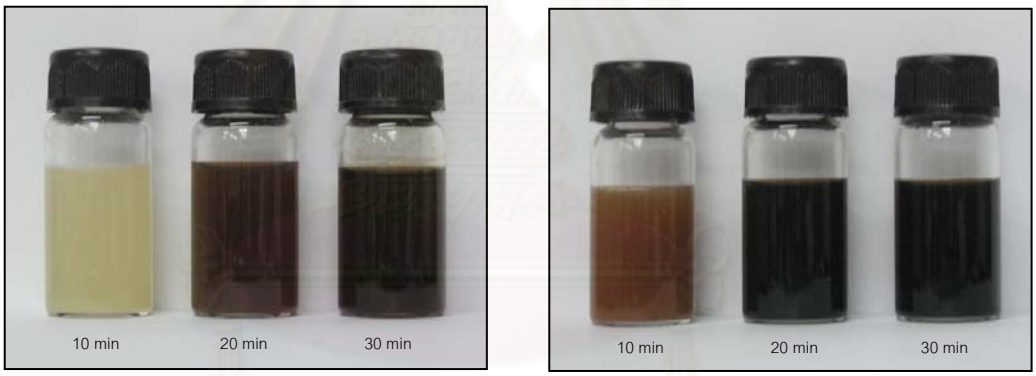
Table 4.1 Composition of raw materials.

Composition (%)	Raw Rice Bran	Deoiled Rice Bran	Raw Soybean Meal	Deoiled Soybean Meal
Moisture	8.80	11.00	11.09	11.25
Oil	24.04	0.70	20.19	0.87
Protein	14.06	15.53	33.87	43.55
Fiber	10.53	7.38	4.88	4.85
Non-Protein	42.57	65.39	24.50	33.34

The fat contents of the raw rice bran and raw soybean were high, and were approximately 24% and 20%, respectively. Protein content of the raw rice bran (14%) was much smaller than protein content of the soybean (34%), while the percentage of non-protein which includes ash and carbohydrates in the rice bran was higher than that of soybean (42.57 vs. 24.50%). After deoiling, protein content of both rice bran and soybean increased to 15.53 and 43.50%, respectively.

4.2 Characteristics of hydrolysis product

In this research, we conducted the hydrolysis experiment of rice bran and soybean meal at the temperatures range between 200–220 ^{o}C for various reaction times of 10, 20 and 30 min. Generally, the product obtained was a solid-liquid mixture. After allowing a period of precipitation, the product was settled into two layers. The upper layer was aqueous solution which was clearer and less viscous, whereas the lower layer was a wet solid rice bran and soybean meal residue. At reaction temperature lower than 200 or shorter reaction time, the mixture products were viscous and it was difficult to separate the unhydrolyzed solid from the soluble product. Hydrolysis product obtained at this condition for both rice bran and soybean had a mild toasty smell, while at the higher hydrolysis temperature and longer time, the strong coffee-like odor was developed. At the same conditions, the odor of soluble rice bran products was more pungent than that of the soybean meal.



(a) (b)

Figure 4.1 Soluble portions of raw rice bran and soybean obtained from hydrolysis at 220^{o}C at various times of exposure: (a) Soluble portions of raw rice bran; (b) Soluble portions of raw soybean

At the temperature above 220 ^{o}C, the color of both raw materials changed to dark brown and the product had strong and unfavorable pungent odor. The temperature of hydrolysis was therefore limited to the maximum of 220 ^{o} C.

4.3 Color parameter

The colors of the soluble products obtained at various hydrolysis conditions were measured using a Colorimeter (CR-300, Minolta Camera Co., Ltd., Japan). The color was expressed in terms of a 3-coordinate values (L, a*, and b* color space). The L value for each scale therefore indicates the level of light or dark, the a* value redness or greenness, and the b* value yellowness or blueness. All three values are required to completely describe an object's color. The results of the color measurements are shown in Figure 4.2 in which the solution products gave the highest lightness values (L) at the hydrolysis time 10 min. The L values then decreased when the reaction time increased. The results agree with the visual observation in which the color of the resulted solution products appeared darker when the reaction times were longer (Figure 4.1). Comapre with the L values, the blueness (-b*) values and redness values (+a*) are small, and are not significantly different for hydrolysis samples obtained at various conditions. The dark color of solution products were the result of Maillard reaction, a non-enzymatic browning process that depends on the reducing sugar content, amino acid content and the reaction temperature and reaction time. It is a chemical reaction between carbonyl group of the sugar and the nucleophilic amino group of the amino acid. This reaction usually requires the addition of heat and is highly time dependent. Thus, the higher the reaction time, the darker the solution.

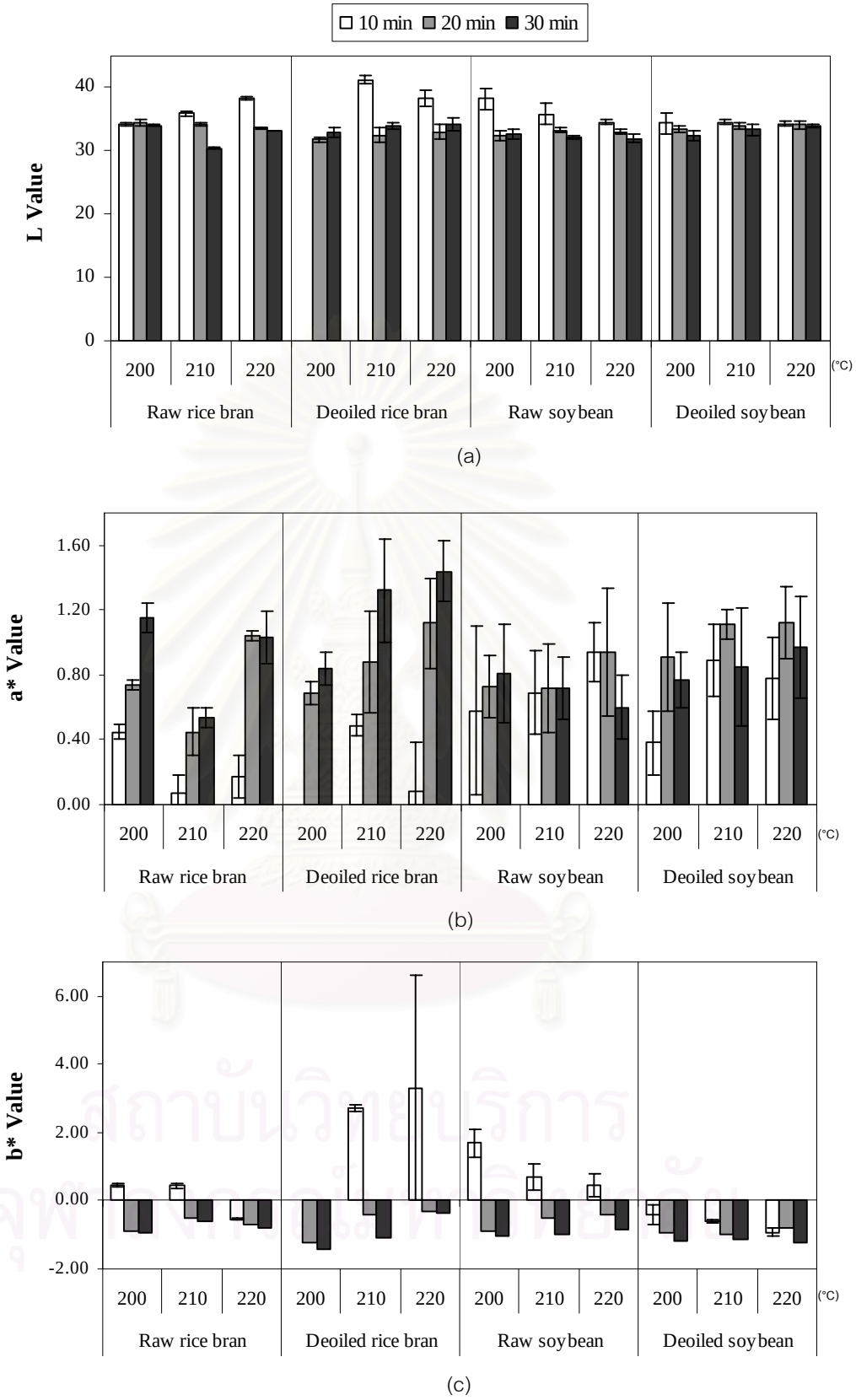
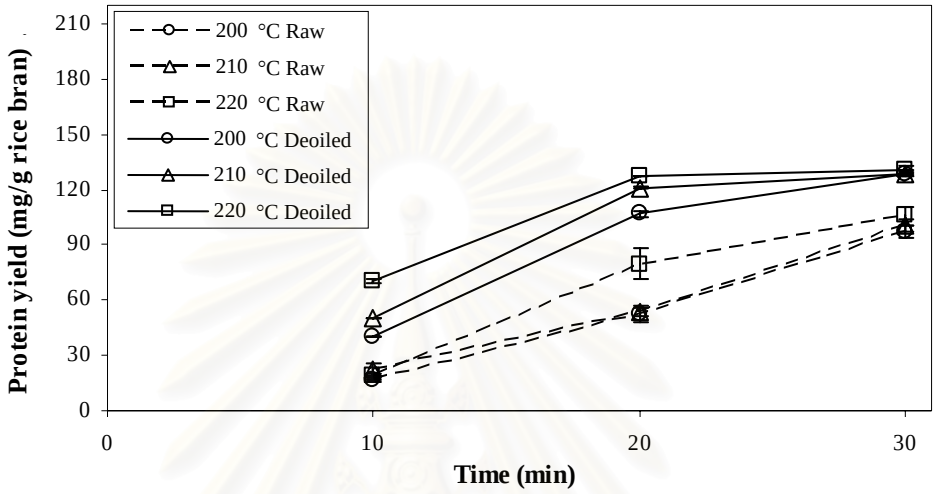


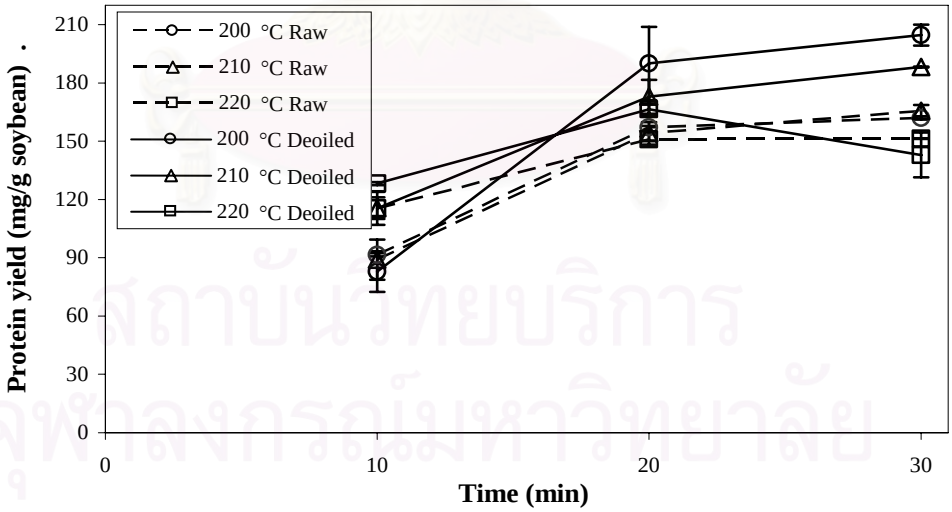
Figure 4.2 The color parameter of solution products was measured using Colorimeter: (a) L value, (b) a* value and (c) b* value

4.4 Protein yields of soluble products

The amounts of protein in the soluble products obtained by SW hydrolysis of raw and deoiled rice bran and raw and deoiled soybean meal at various conditions are shown in Figure 4.3.



(a)



(b)

Figure 4.3 Protein yield after hydrolysis of raw materials at different temperature and time: (a) raw and deoiled rice bran (b) raw and deoiled soybean

The protein yields of raw rice bran and soybean meal are lower than those of deoiled materials possibly because the hydrophobic oil content within the materials made them less accessable to water. For rice bran, the highest protein yield (106.02 ± 5.01 mg/g raw rice bran and 130.17 ± 2.48 mg/g deoiled rice bran) were obtained from the 220 ^{o}C hydrolysis for 30 min. Based on the amount in the original bran reported by NIR in Table 4.1, these account for the protein recovery of 75% and 84%, respectively. This suggested that the majority of protein in the original bran could be recovered in the soluble product.

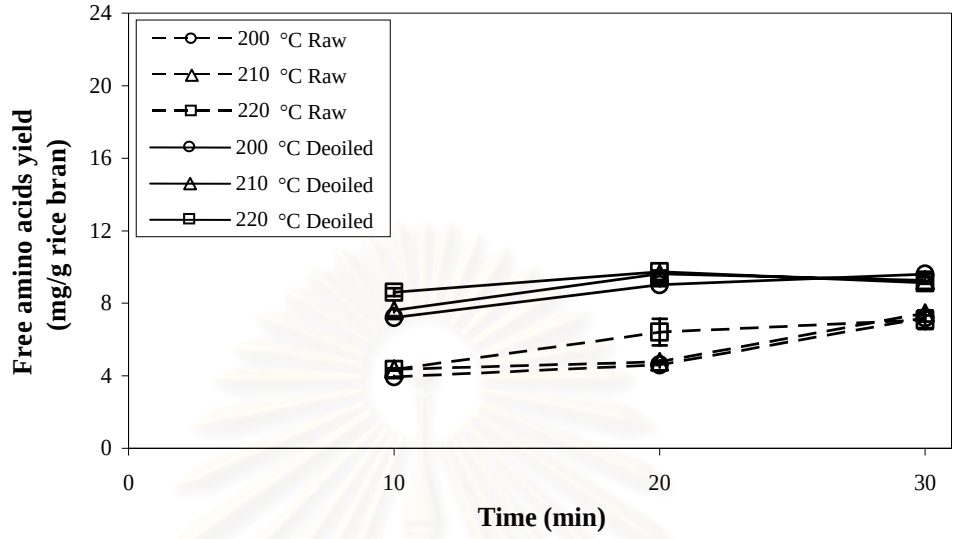
For soybean meal, the highest protein yield for raw soybean (165.72 ± 2.90 mg/g raw soybean) was obtained by hydrolysis at 210 ^{o}C for 30 min. The highest protein yield for deoiled soybean (204.64 ± 5.38 mg/g deoiled soybean) was obtained by hydrolysis at 200 ^{o}C for 30 min. The higher protein yield in soybean products compare with the rice bran products were due to the higher protein content in the raw materials as shown in Table 4.1. However, higher protein recovery was obtained for the rice bran product than the soybean products, in which only approximately 50% recovery was obtained. This could be a result of larger particle size of soybean compared to rice bran used in this experiment.

From the results shown in Figure 4.3, protein yields were generally found to increase with the reaction temperature. At ambient temperature, protein normally has low solubility in water due to strong aggregation through hydrophobic interactions but at higher temperatures the solubility in water is increased. When the temperature increases, protein solubility increases. Moreover, the increase in protein yield at elevated temperature is due to the increased hydrolysis activity caused by the increase in water ionization constant (also called dissociation constant or ion product constant or K_w). K_w increases from 1×10^{-14} at ambient temperature to 7×10^{-12} at 220 ^{o}C, and thus the concentration of hydronium and hydroxide ions, which is equal to the square-root of the K_w, increases. In the presence of hydronium and hydroxide ions, peptide bonds are broken down into smaller molecules of soluble protein or amino acids, which could further degrade to low molecular weight carboxylic acids such as formic acids, acetic acids, propionic acids, and etc (Quitain *et al*., 2002). Note however that for both raw and deoiled soybean meal, the protein yield was also found to decrease when the temperature increased from 210 to 220 ^{o}C, which is probably due to the decomposition of soluble protein into amino acids and other organic acids.

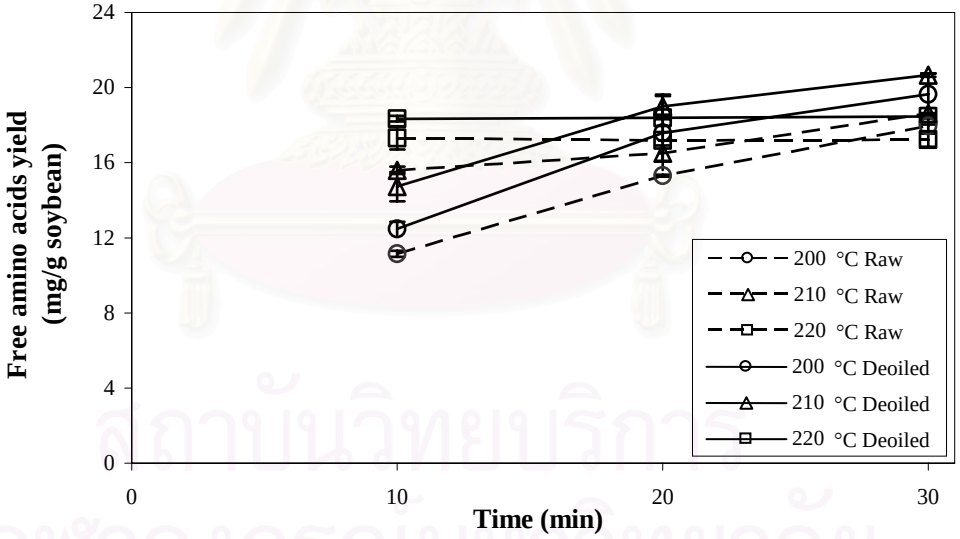
Higher protein yield could also be obtained by increasing the hydrolysis time. Nevertheless, longer time did not always give the positive results. The effect of reaction time is less important when it is increased from 20 to 30 min hydrolysis time for most of the samples tested. In particular, for the hydrolysis of deoiled soybean meal at hydrolysis temperature of 220 ^{o}C, increasing the hydrolysis times from 20 and 30 min decreased the protein yields. This is possibly because extent of protein decomposition to amino acids or organic acids was higher than the extent of protein production.

4.5 Total amino acid yields in soluble products

The amino acid yields of non-deoiled materials were lower than those from the deoiled materials which was in agreement with the results of protein yields. The highest amino acids yield for raw rice bran (7.47 ± 0.14 mg/g raw rice bran) were obtained at 210 °C for 30 min, while the highest yield for deoiled rice bran was 9.74 ± 0.08 mg/g raw rice bran, which was obtained at 220 °C for 20 min. This relatively low yield indicates that the extent of amino acids decomposition to smaller molecules of organic acids or other products was higher than the amino acid production. For rice bran, temperature and reaction time do not have significant effects on the amount of total amino acids yields. For soybean meals, the amino acid yields increased when the hydrolysis time increased for the hydrolysis at lower temperatures. At higher temperature for 220 ^{o}C, the amount of amino acids in solution was not affected greatly by time of reaction. The result indicated that at this condition, the extent of amino acid production was comparable to the extent of amino acid decomposition into smaller organic acids.



(a)

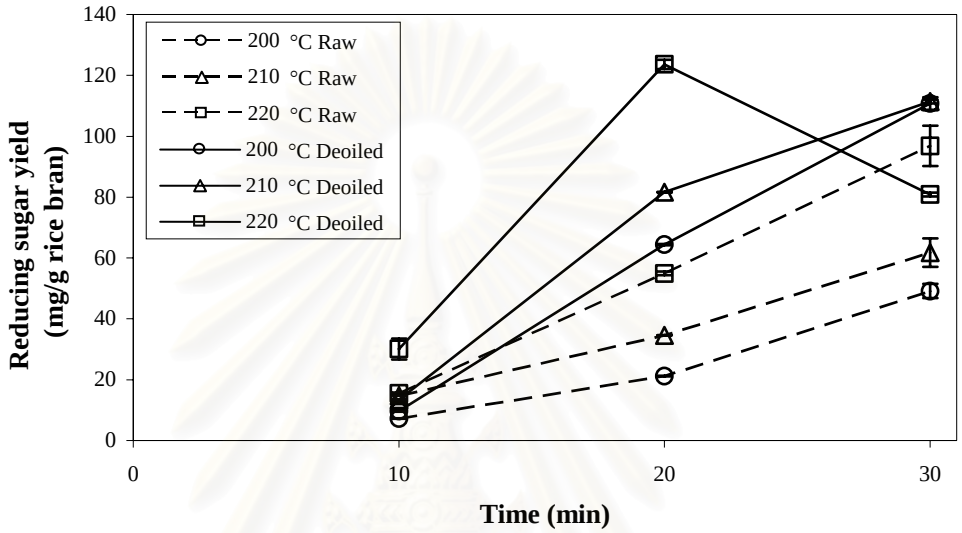


(b)

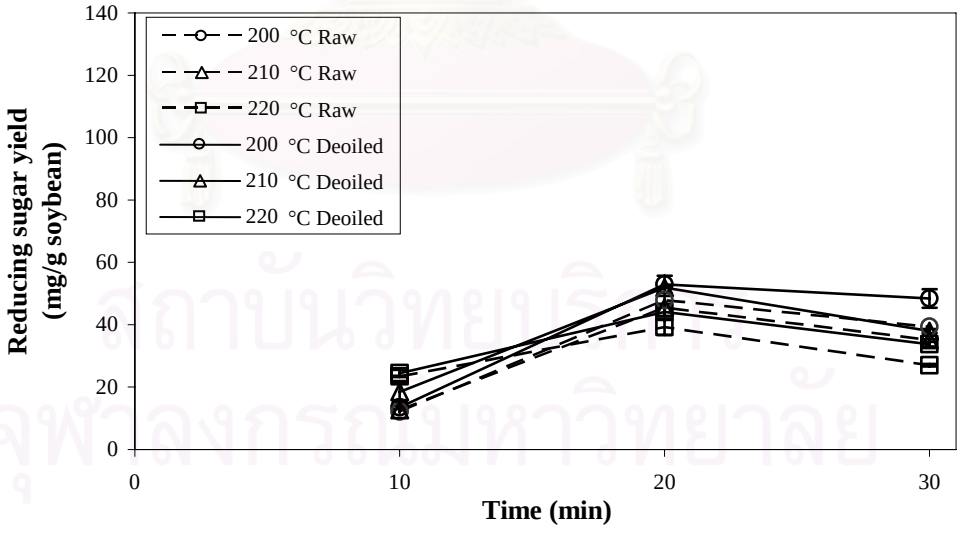
Figure 4.4 Free amino acid yield after hydrolysis of raw materials at different temperature and time: (a) raw and deoiled rice bran (b) raw and deoiled soybean

4.6 Reducing sugar yields in soluble products

When carbohydrate reacts with hydronium and hydroxide ions, reducing sugars are produced. The reducing sugar yields of the soluble hydrolysis products at different temperature and time are shown in Figure 4.5.



(a)



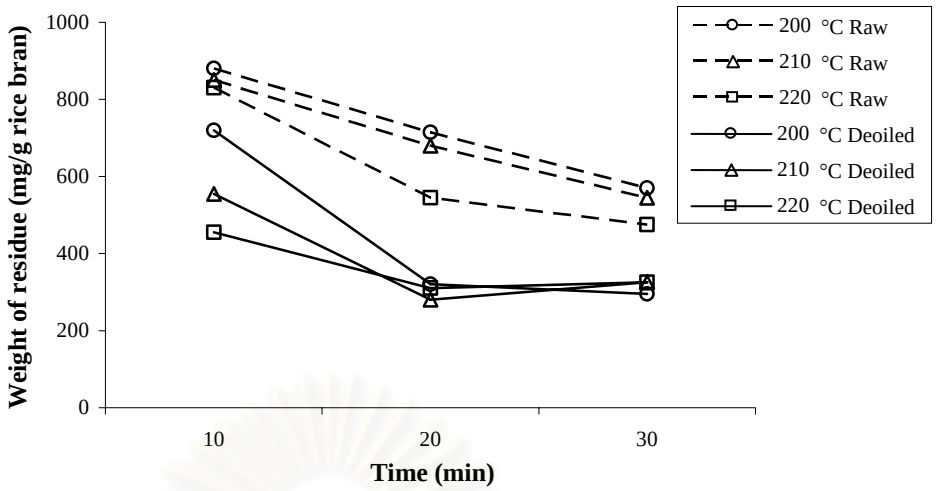
(b)

Figure 4.5 Reducing sugar yields after hydrolysis of raw materials at different temperature and time: (a) raw and deoiled rice bran (b) raw and deoiled soybean

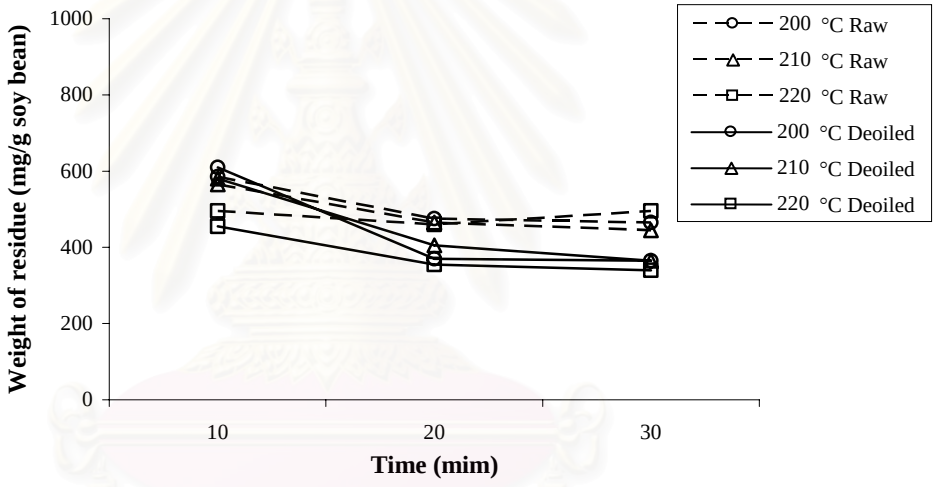
For raw and deoiled rice bran, the reducing sugar content in the soluble products increased with increasing temperatures and times of reaction, except for the hydrolysis product obtained at 220^{o}C and 30 min, whose reduce sugar content decreased. This result again indicated that at this condition, the extent of reducing sugar decomposition into smaller molecules of organic carbon was high, and that the decomposition of reducing sugar to other products was favored over the production of reducing sugar. Similar decrease in reducing sugar as a result of increasing hydrolysis time was also observed for soybean meal. The reducing sugar contents of the soybean products were lower than those of the rice bran products, which agreed with the results shown in Table 4.1, which indicated lower non-protein (mostly carbohydrate) content of soybean meal compared with rice bran.

4.7 Weight of solid residue

Consideration of the weight of the residue remaining after hydrolysis allows one to determine the amount of the original materials that was converted into the extraction product. For all experiments, the residue was separated from the soluble product with a vacuum filter using a filter paper and weighed after drying in a vacuum oven at 65 ^{o}C, and the weight of the dried residue was measured. The weight of the residue of all experimental generally decreased with time and temperature as shown in Figure 4.6. As the reaction proceeded over time, the residue weight decreased. This trend agreed with the amount of protein, amino acid, and reducing sugar yields obtained in the soluble product which increased with reaction time. Note that the weight of the raw rice bran and soybean residue were higher than those of the residue of deoiled rice bran and soybean, indicating that smaller amount of raw rice bran and soybean could be converted into the soluble products. This also agreed with that observed for the extraction yield as shown in the previous sections.



(a)



(b)

Figure 4.6 Weight of residue after subcritical water hydrolysis at different temperature and time: (a) raw and deoiled rice bran (b) raw and deoiled soybean

However, the original materials were not all converted into protein, amino, and reducing sugars as the amount of the raw materials hydrolyzed (difference between the weight of original material and the weight of residue) was higher than the weight of protein, amino acids, and reduced sugar contents measured in the soluble products. Taking deoiled rice bran as an example, Figure 4.7 shows the amount of rice bran hydrolyzed (the total height) are significantly higher than the sum of the amount

of protein, amino acids, and reduced sugars in the soluble products (the bottom white section of the bars of the chart in Figure 4.7).

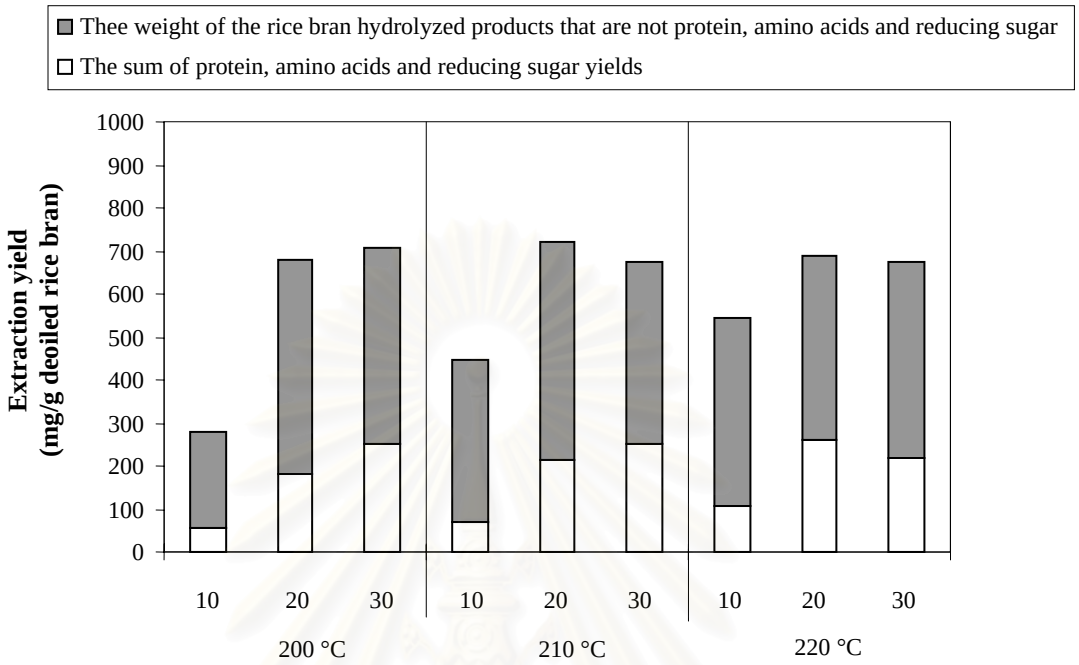


Figure 4.7 Extraction yield after subcritical water hydrolysis deoiled bran at different temperature and time

Consider for example the experiment at 220 ^{o}C and 30 min, in which from Figure 4.6 (a), 33% of the starting rice bran remained (330 mg rice bran residue/g deoiled rice bran), this suggested that approximately 67% of deoiled rice bran (670 mg/g rice bran) was hydrolyzed as shown in Figure 4.7. Since the measured contents (protein, amino acids, and reducing sugars) of deoiled rice bran soluble product was approximately 22% (220.125 mg/g deoiled bran), thus the remaining fraction of 45 % (the difference between 67% and 22% or the top grey section of the bars in Figure 4.7) was the unmeasured product, which could be the hydrolysis product of the non-protein content such as smaller organic acids.

4.8 Suitable conditions

The objective of this research is to produce value-added products from rice bran and soybean meal. The yields of protein and amino acids were therefore important factors for selecting suitable hydrolysis condition. Since the highest protein

and amino acids yields were obtained mostly after 30 min hydrolysis time, the yields of protein and amino acids in the soluble products obtained from 30 min hydrolysis at various temperatures are summarized in Table 4.2. Note that if comparable yields are resulted from two different conditions, the lower temperature condition could be more desirable due to lower energy requirement.

Table 4.2 Protein and amino acids content after hydrolysis of raw materials at different temperature for 30 min

Temperature	Raw rice bran		Deoiled rice bran		Raw soybean		Deoiled soybean	
(°C)	Protein (mg)	Amino acid (mg)	Protein (mg)	Amino acid (mg)	Protein (mg)	Amino acid (mg)	Protein (mg)	Amino acid (mg)
200	97.14	7.20	128.60	9.59	162.00	17.96	**204.64**	19.65
210	100.50	**7.47**	128.79	9.27	**165.72**	**18.62**	188.22	**20.67**
220	**106.02**	7.09	**130.17**	9.14	151.37	17.23	142.96	18.47

The bold-type numbers in the table above represents the highest protein and amino acid yields for each raw material. Since the temperatures have much smaller effect on the yield of amino acids, the most suitable temperature for the production of each product could be drawn from that giving the highest protein yields. From these results, the most suitable conditions are summarized in Table 4.3.

Table 4.3 Suitable hydrolysis conditions (the time was 30 min)

Raw materials	Temperature (°C)	Protein yield (mg/g raw material)	Amino acid yield (mg/g raw material)
Raw rice bran	220	106.02	7.09
Deoiled rice bran	220/210	130.17/128.79	9.14/9.27
Raw soybean	210/200	165.72/162.00	18.62/17.96
Deoiled soybean	200	204.64	19.65

4.9 Effect of ratio raw material-to-water on extraction yield

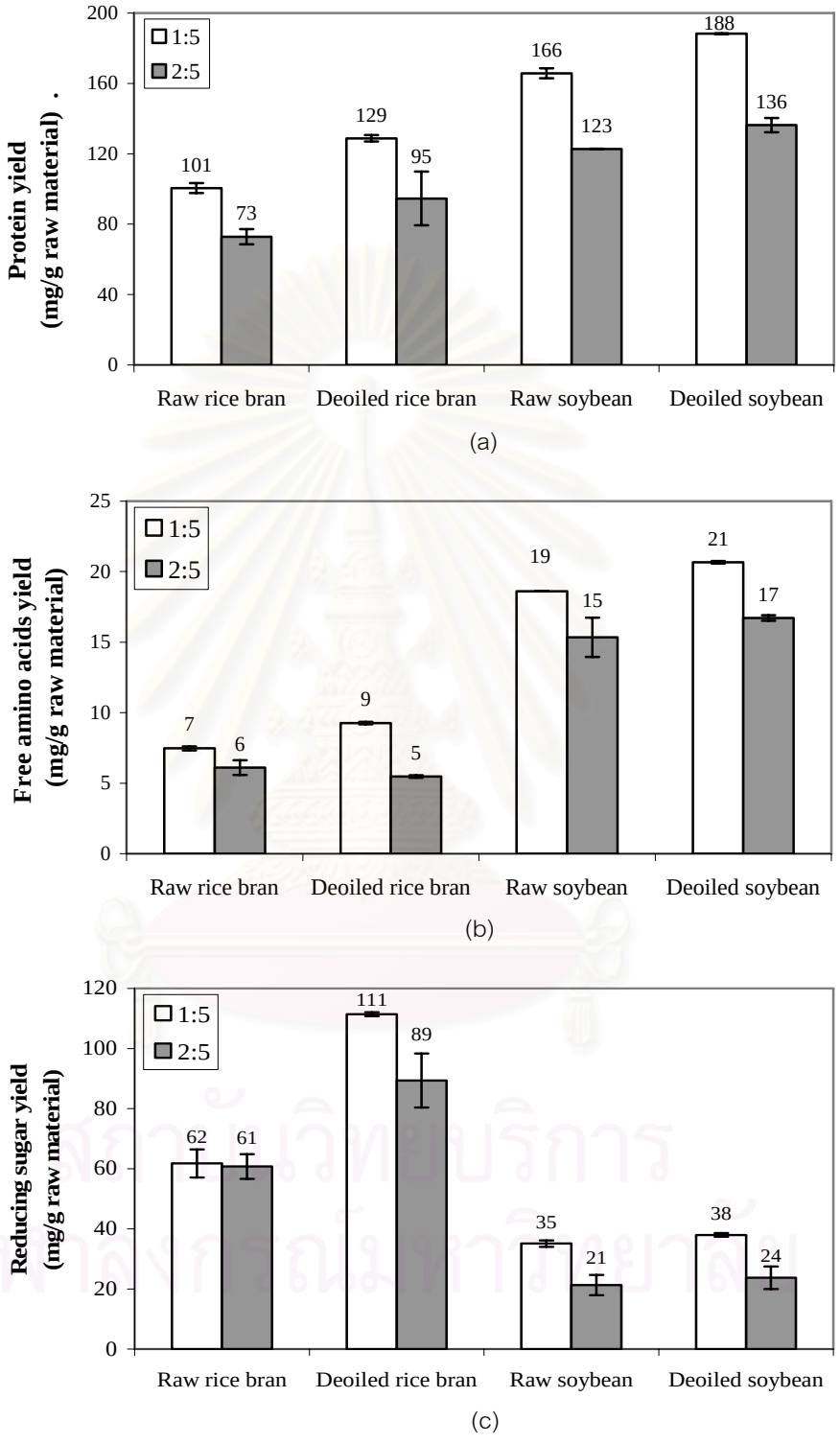


Figure 4.8 Effect of ratio of raw material-to-water for 210 ^{o}C and 30 min; (a) the protein yield (b) the amino acid yield and (c) the reducing sugar yield.

The effect of ratio raw material-to-water on extraction yield was studied by comparing the product yields obtained from two different ratios of raw material-to-water, 1:5 and 2:5 for all reaction conditions. As an example, only the results from the hydrolysis experiment at 210 ^{o}C and 30 min are shown here in Figure 4.8. Those for all the other conditions are found to be similar and are shown in Appendix B. From Figure 4.8, it was found that the yields of protein, amino acids and reducing sugar decreased when the ratio raw material-to-water was increased from 1:5 to 2:5. This is mainly because with too high amount of raw material, the increase in the density and viscosity caused poor mixing of raw material and water, thus mass transfer decreased and the accessibility of water to particles of raw materials was difficult. The ratio raw material-to-water of 1:5 was therefore more suitable.

4.10 Antioxidant activity

In recent years, research related to active compounds of bioresources that exhibit antioxidant activity has received much interest from scientific professionals. It is well known that rice bran and soybean are highly nutritious foods which contain various antioxidants that impart beneficial effects on human health. Those contained in rice bran include vitamin E (Tocopherols and tocotrienols), vitamin C, anthocyanidins, isoflavones, beta-carotene, polyphenols and oryzanol. Among these, the most powerful antioxidative compound in rice bran is oryzanol (Godber and Wells, 1994), which has been reported to be used in cure of nerve imbalance and disorders of menopause (Roger *et al*., 1993). For soybean, the powerful antioxidants are isoflavones, particularly daidzein and genistein which have been reported for their ability to fight against breast cancer, prostate cancer, menopausal symptoms, and heart disease (Ruiz-Larrea *et al*., 1997). Several protein hydrolysates derived from rice bran and soybean meal which contain amino acids such as tyrosine, methionine, histidine, tryptophan and proline have also been found to exhibit antioxidant activity (Saiga, Tanabe and Nishimura, 2003, Jung and Kim, 1995 and Marcuse, 1960). At ambient temperature, some of the antioxidants such as vitamin C and certain amino acids are water soluble. However, most of other antioxdants are insoluble in water.

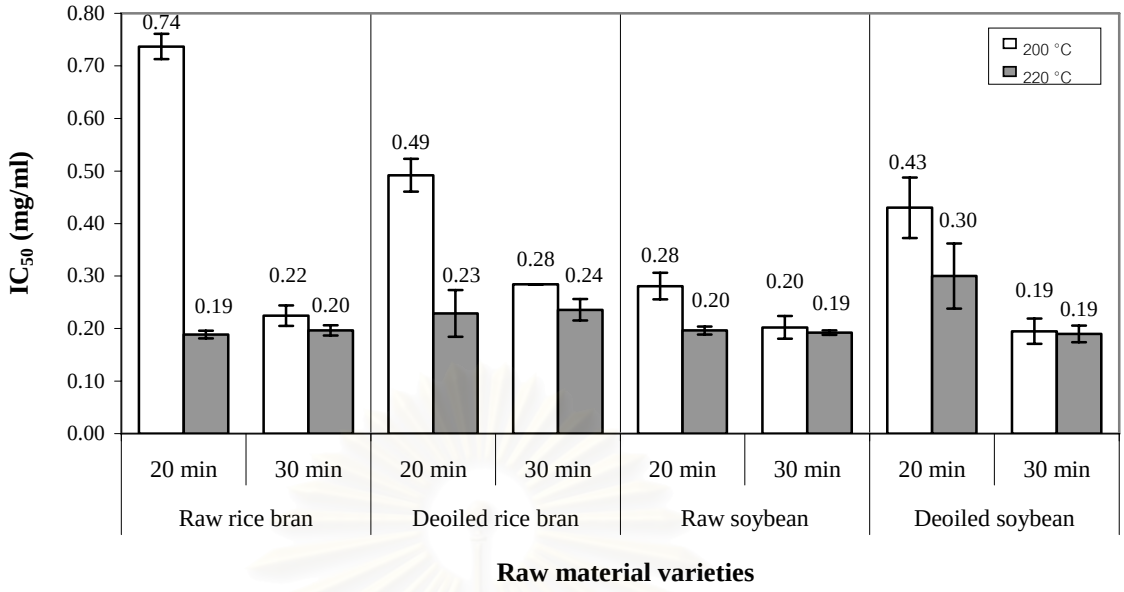


Figure 4.9 Antioxidant activity (IC_{50}) of the soluble products at hydrolysis times of 20 and 30 min and temperatures of 200 (C and 220 (C.

In this study, the antioxidant activity of soluble products obtained by SW hydrolysis was evaluated with $ABTS^{\bullet+}$ scavenging assay. This method is popular because it is operable over a wide range of pH, inexpensive, rapid and reproducible and requires simple conventional laboratory equipment (Awika *et al.*, 2003). Antioxidant activity was represented by IC_{50} index which is the concentration of sample producing 50% reduction of the radical absorbance. This means that the higher IC_{50} of the product, the lower antioxidant activity. The antioxidant activity was measured for hydrolysis temperature of 200 °C and 220 °C and hydrolysis time at 20 min and 30 min. These conditions were found to be the most suitable hydrolysis conditions and were therefore selected for the antioxidant test.

The result in Figure 4.9 indicated that with the hydrolysis time of 20 min, antioxidant activity increased as temperature increased from 200 (C to 220 (C for all materials. For the reaction time of 30 min; however, the antioxidant activity only increased slightly or stayed constant with the increase in temperature. The increase in water temperature not only increases the ion product which causes hydrolysis reaction as discussed previously, but it also causes the breakdown of hydrogen bonds. Hydrogen bonds of water keep the water molecules together, thus separating themselves from other organic compounds. When the H-bonds breakdown, several

antioxidative organic compounds within the rice bran and soybean samples were able to better dissolve in water, thus the soluble products exhibited higher antioxidant activity than those obtained at lower temperature. At long exposure time with high temperature however, some antioxidantive compounds might be degraded, thus the activity might decrease (Clifford, 2000). It is recommended that in the future study, analysis should be carried out to identify the antioxidant compounds obtained in the soluble products.

4.11 Possible application of products

4.11.1 Use of product of SW deoiled rice bran hydrolysis for yeast growth

As a preliminary examination of the possible use of the hydrolysis products, the SW hydrolysis product of deoiled rice bran was tested as nutrient source for yeast growth. This is the initial test for possible product use. Figure 4.10 shows the value of optical density at 660 nm of the yeast cell suspension grown in the deoiled rice bran product, compared with growth in commercial yeast extract, and the glucose solution control.

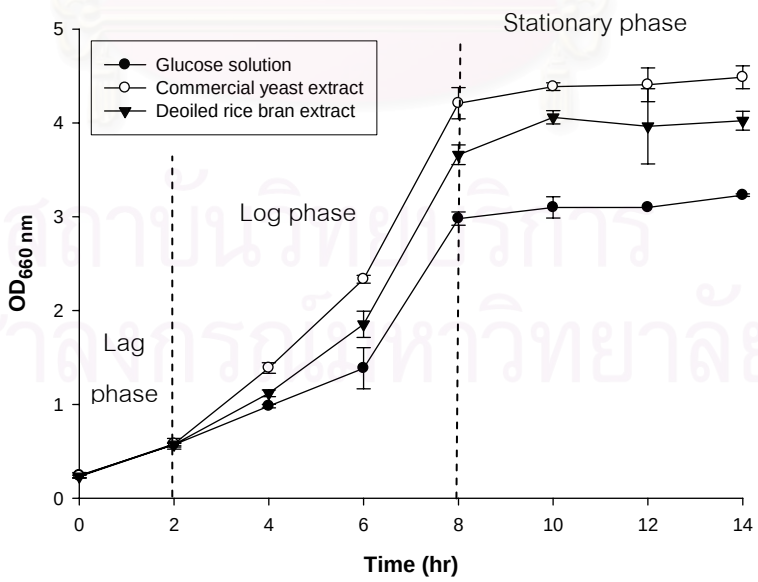


Figure 4.10 Optical densities of yeast cultures incubated in SW deoiled rice bran hydrolysis product.

All three growth curves in Figure 4.10 exhibited similar typical growth behavior which consisted of three phases: lag, log, and stationary phases. From the figure 4.10, it can be observed the yeast cells cutivated with various growth media enter the different phases approximately at the same time. Furthermore, the results showed that the yeast growth in subcritical water hydrolysis product of deoiled rice bran lie between the suspension of yeast cells grown in glucose solution (the negative control) and the glucose solution containing the same 2w/v% commercial yeast extract (positive control). This result here confirms that the deoiled bran product from SW could positively be applied as nutrients for the culture of yeast cells. Further toxicity tests of this product and other SW hydrolysis, i.e. toxic dose and/or acceptable daily intake (ADI) for food additive, are recommended that in the future study.

4.11.2 Paired preference test

Paired preference test is initial consumer response to the taste of the product in which a pair of samples is required in order to compare the taste preference. Here, only the product of deoiled rice bran was tested. In the samples in which the rice bran products were added, 2 g of dried products were added to 600 ml of the control samples. This is equivalent to 1.62 mg/ml of protein and 0.12 mg/ml of amino acids from the added products. In this study, two pairs of samples were compared; the first pair was that of milk control and milk added with deoiled rice bran hydrolysis product and the second pair was that of coffee control and the coffee added with extraction product. The test was conducted on 40 testers. The results of paired preference test are shown in Table 4.4.

Table 4.4 Paired preference test

Set	Preference		
	Control	Added with extraction product	Total
Milk	14	26	40
Coffee	25	15	40

From the statistical analysis based on the 40 testers, the minimum correct answers necessary to establish significant differentiation at a 5% probability level was 26 (Appendix E). From these results, it can be concluded that the milk added with extraction product exhibited a strong preference ($P \leq 0.05$) compared with the milk control. The coffee added with extraction product and the coffee control exhibited a similar preference ($P > 0.05$). It should be noted that the pair preference test is only a basic test conducted on testers with minimal training whose age range between 22 and 36 who were asked to select one of the two choices they preferred. The descriptive evaluation of the product was not the main results used in the analysis as these testers are inexperienced. Further detailed sensory evaluation such as descriptive analysis which is conducted on trained testers would be required. Furthermore, the product preference could be dependent on the age or gender of the testers, as well as on the serving size of the product, thus further study should be conducted on testers of different age or gender groups, and with different serving sizes. Nevertheless, the results of the pair preference test conducted in this study proved the potential application of the SW hydrolysis products as functional food for human.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 This study demonstrates that subcritical water could be used to potentially hydrolyze raw and deoiled rice bran and raw and deoiled soybean meal into more valuable proteins, amino acids and reducing sugar.

5.1.2 The suitable conditions for protein and amino acid production from rice bran (raw and deoiled) and soybean meal (raw and deoiled) by subcritical water hydrolysis were 1:5 raw material-to-water weight ratio at 30 min and hydrolysis temperature of 220 ^{o}C for raw and deoiled rice bran and 210 ^{o}C and 200 ^{o}C for raw and deoiled soybean meal, respectively.

5.1.3 Apart from protein, amino acids and reducing sugar, active compounds that exhibit antioxidant activity were also extracted by subcritical water.

5.1.4 The product of subcritical water of the deoiled rice bran was proven to potentially be used as nutrient source for yeast growth.

5.1.5 The product of subcritical water of the deoiled rice bran was proven to have the potential for the application of the product for human foods.

5.2 Recommendations

5.2.1 Due to the different sizes of various raw materials obtained from the suppliers, it is recommended to control their size range before conducting experiment.

5.2.2 For future studies, more detailed analysis of the products should be conducted using high performance liquid chromatography (HPLC) in order to determine the amount and types of active compounds in the extract.

5.2.3 Although the method involves no addition of toxic chemicals and the product was tested and found to be used as nutrient source for yeast growth, toxic compounds could potentially be produced in a reaction at such high

temperature. Therefore, the composition of the product should be analyzed to determine potential existence of other toxic chemicals produced.

5.2.4 Other sensory evaluation techniques, i.e. descriptive sensory analysis should be conducted on the products, which give more precise evaluation of the potential use of the product.

5.2.5 Kinetics of the hydrolysis could be determined, which would be useful for the process scale-up.

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APPENDICES

APPENDIX A

EXPERIMENTAL AND DATA ANALYSIS

A-1 Standard calibration curve of protein

Table A-1 Standard calibration curve data

Concentration of BSA (mg/ml)	Absorbance at 750 nm				
	Exp.1	Exp.2	Exp.3	Exp.4	Average
0.00	0.000	0.000	0.000	0.000	0.000
0.05	0.120	0.137	0.147	0.137	0.135
0.10	0.237	0.235	0.253	0.245	0.243
0.15	0.322	0.324	0.384	0.376	0.352
0.20	0.437	0.435	0.456	0.449	0.444
0.25	0.523	0.520	0.544	0.527	0.529
0.30	0.635	0.622	0.615	0.660	0.633

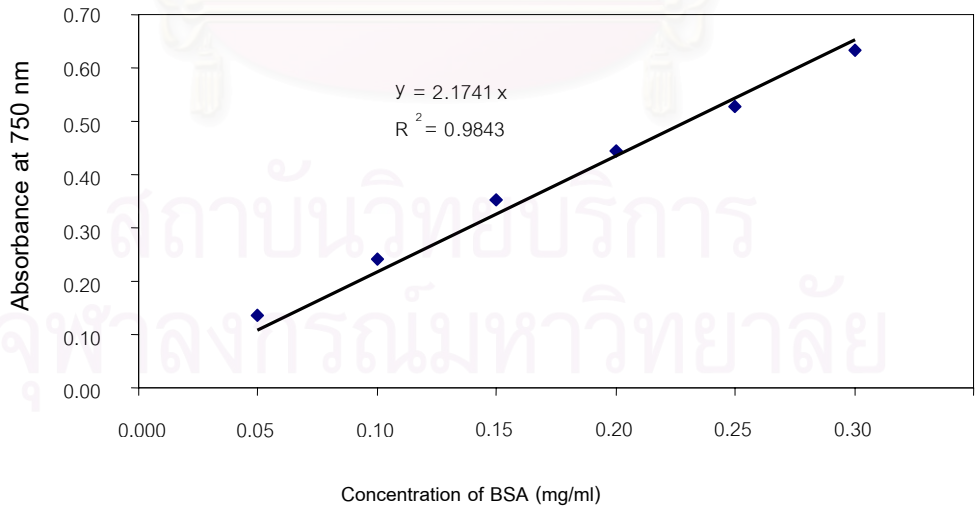

Figure A-1 Standard calibration curve of protein

A-2 Standard calibration curve of amino acids

Table A-2 Standard calibration curve data

Concentration of Glutamic Acid (mg/ml)	Absorptivity at 570 nm				
	Exp.1	Exp.2	Exp.3	Exp.4	Average
0.40	0.072	0.058	0.060	0.074	0.066
0.50	0.092	0.123	0.110	0.114	0.110
0.60	0.126	0.163	0.144	0.151	0.146
0.70	0.159	0.262	0.214	0.231	0.217
0.80	0.223	0.321	0.216	0.345	0.276
0.90	0.247	0.375	0.302	0.401	0.331
1.00	0.325	0.421	0.326	0.545	0.404

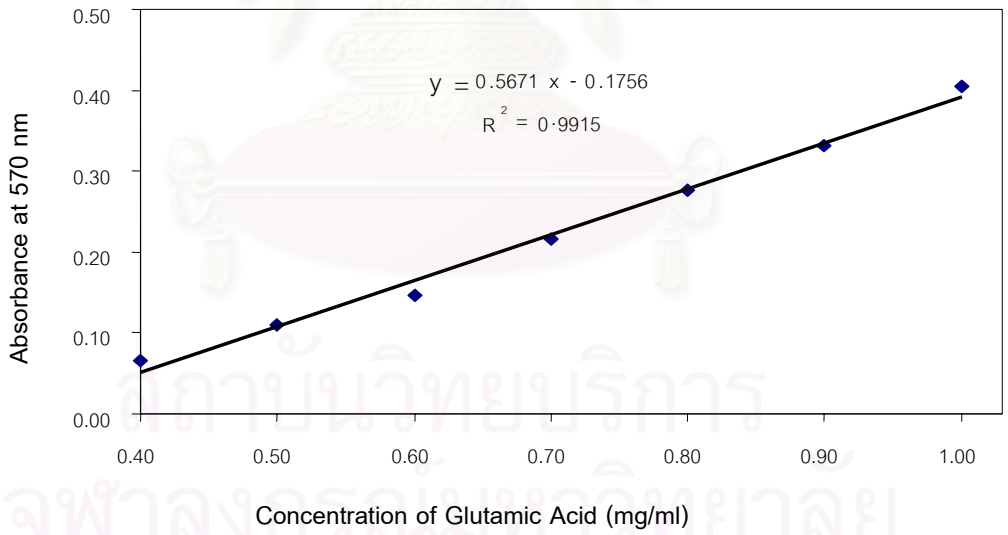

Figure A-2 Standard calibration curve of amino acids

A-3 Standard calibration curve of reducing sugar

Table A-3 Standard calibration curve data

Concentration of Glucose (mg/ml)	Absorptivity at 575 nm				
	Exp.1	Exp.2	Exp.3	Exp.4	Average
0.10	0.160	0.147	0.159	0.155	0.155
0.14	0.366	0.329	0.352	0.350	0.349
0.18	0.544	0.513	0.536	0.538	0.533
0.22	0.763	0.711	0.737	0.747	0.740
0.26	0.966	0.904	0.918	0.909	0.924

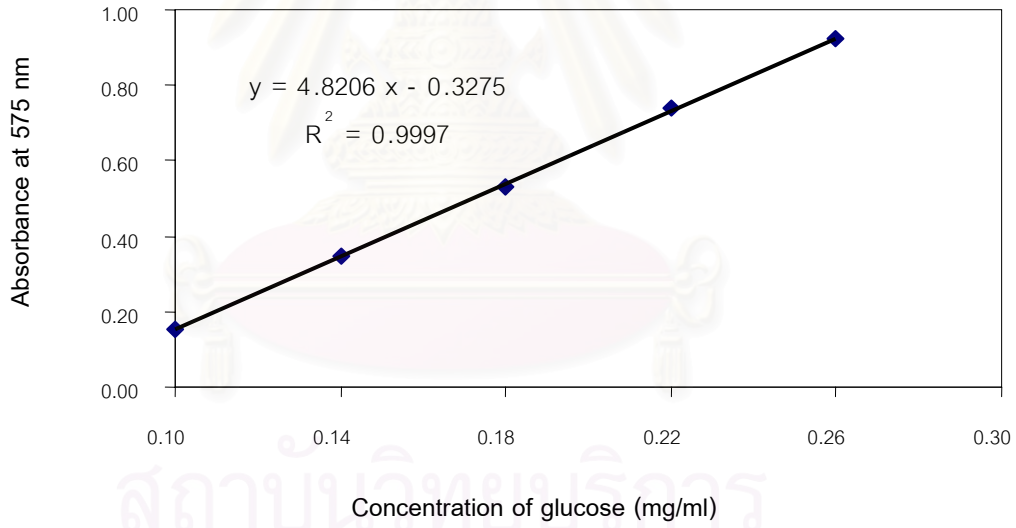

Figure A-3 Standard calibration curve of reducing sugar**.**

APPENDIX B

EXPERIMENTAL DATA

B-1 Experimental data of deoiled rice bran hydrolysis with subcritical water

B-1.1 Experimental data of subcritical water hydrolysis of 1:5 deoiled rice bran to water ratio

Table B-1.1.1 Protein yield of soluble product of subcritical water hydrolysis at 1:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	Protein Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	39.833	40.661	40.247	0.414
	20	105.055	108.735	106.895	1.840
	30	129.157	128.053	128.605	0.552
210 °C	10	50.044	50.228	50.136	0.092
	20	120.510	121.614	121.062	0.552
	30	126.949	130.629	128.789	1.840
220 °C	10	71.202	69.086	70.144	1.058
	20	126.949	127.317	127.133	0.184
	30	127.685	132.653	130.169	2.484

Table B-1.1.2 Amino acids yield of soluble product of subcritical water hydrolysis at 1:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	Amino Acids Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	7.293	7.135	7.214	0.079
	20	8.951	9.074	9.013	0.062
	30	9.727	9.462	9.594	0.132
210 °C	10	7.505	7.734	7.619	0.115
	20	9.656	9.603	9.630	0.026
	30	9.356	9.180	9.268	0.088
220 °C	10	8.827	8.404	8.616	0.212
	20	9.815	9.656	9.735	0.079
	30	9.074	9.198	9.136	0.062

Table B-1.1.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 1:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	Reducing Sugar Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	9.553	9.968	9.760	0.293
	20	64.183	64.515	64.349	0.235
	30	112.227	109.240	110.733	2.112
210 °C	10	13.349	13.515	13.432	0.117
	20	81.691	81.525	81.608	0.117
	30	110.982	111.895	111.438	0.645
220 °C	10	32.517	27.663	30.090	3.432
	20	124.756	122.599	123.678	1.526
	30	81.276	80.363	80.820	0.645

Table B-1.1.4 Antioxidant activity (IC_{50}) of soluble product of subcritical water hydrolysis at 1:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	IC_{50}			
		Exp.1	Exp.2	AVG	SD
200 °C	20	0.74	0.73	0.74	0.004
	30	0.24	0.21	0.22	0.020
220 °C	20	0.19	0.19	0.19	0.002
	30	0.20	0.19	0.20	0.010

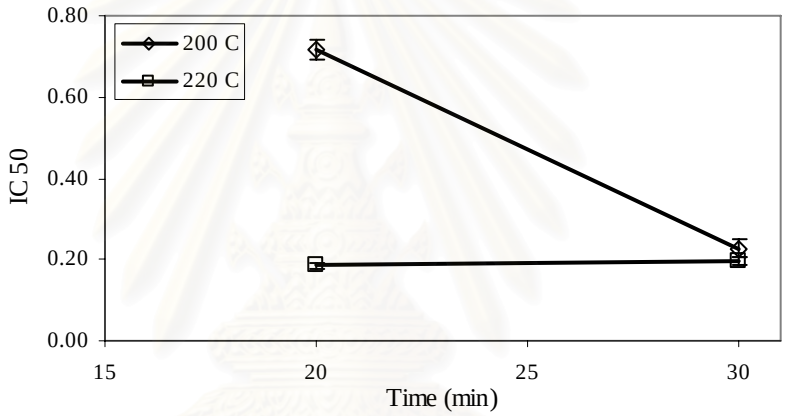

Figure B-1.1.4 Antioxidant activity (IC_{50}) of soluble product of subcritical water hydrolysis at 1:5 deoiled rice bran to water ratio

B-1.2 Experimental data of subcritical water hydrolysis of 2:5 deoiled rice bran to water ratio

Table B-1.2.1 Protein yield of soluble product of subcritical water hydrolysis at 2:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	Protein (mg/g deoiled rice bran)			SD
		Exp.1	Exp.2	Avg	
200	10	7.267	9.613	8.440	1.173
	20	44.892	64.026	54.459	9.567
	30	84.173	82.885	83.529	0.644
210	10	9.337	9.153	9.245	0.092
	20	52.895	56.023	54.459	1.564
	30	79.297	109.931	94.614	15.317
220	10	22.492	18.444	20.468	2.024
	20	86.933	82.977	84.955	1.978
	30	94.936	108.275	101.605	6.669

Table B-1.2.2 Amino acids yield of soluble product of subcritical water hydrolysis at 2:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	Amino acids (mg/g deoiled rice bran)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	2.483	2.642	2.562	0.079
	20	4.846	4.414	4.630	0.216
	30	7.658	7.517	7.588	0.071
210 °C	10	3.029	3.373	3.201	0.172
	20	5.145	6.256	5.701	0.555
	30	5.569	5.384	5.476	0.093
220 °C	10	4.167	3.876	4.021	0.145
	20	6.327	5.965	6.146	0.181
	30	4.863	4.960	4.912	0.048

Table B-1.2.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 2:5 deoiled rice bran to water ratio

Temperature (°C)	Time (min)	Reducing sugar (mg/g deoiled rice bran)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	3.947	3.708	3.827	0.169
	20	20.397	28.394	24.395	5.655
	30	54.532	54.895	54.713	0.257
210 °C	10	4.061	3.926	3.993	0.095
	20	24.131	19.370	21.750	3.366
	30	83.003	95.709	89.356	8.984
220 °C	10	12.400	11.819	12.109	0.411
	20	37.677	40.374	39.025	1.907
	30	90.730	117.231	103.981	18.739

B-2 Experimental data of raw rice bran hydrolysis with subcritical water

B-2.1 Experimental data of subcritical water hydrolysis of 1:5 raw rice bran to water ratio

Table B-2.1.1 Protein yield of soluble product of subcritical water hydrolysis at 1:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	Protein Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	18.766	15.731	17.249	1.518
	20	57.311	48.388	52.849	4.462
	30	93.648	100.639	97.144	3.496
210 °C	10	25.666	18.950	22.308	3.358
	20	50.872	56.023	53.447	2.576
	30	97.696	103.307	100.501	2.806
220 °C	10	19.870	17.203	18.536	1.334
	20	87.944	71.110	79.527	8.417
	30	101.007	111.034	106.021	5.014

Table B-2.1.2 Amino acids yield of soluble product of subcritical water hydrolysis at 1:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	Amino acids Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	3.996	3.890	3.943	0.053
	20	4.754	4.454	4.604	0.150
	30	7.135	7.258	7.196	0.062
210 °C	10	4.313	4.401	4.357	0.044
	20	4.825	4.719	4.772	0.053
	30	7.329	7.611	7.470	0.141
220 °C	10	4.278	4.419	4.348	0.071
	20	7.135	5.671	6.403	0.732
	30	6.570	7.611	7.090	0.520

Table B-2.1.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 1:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	Reducing Sugar Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	7.312	7.188	7.250	0.088
	20	21.253	21.190	21.221	0.044
	30	50.772	47.453	49.112	2.347
210 °C	10	14.749	14.708	14.728	0.029
	20	34.695	34.591	34.643	0.073
	30	58.447	65.085	61.766	4.694
220 °C	10	15.703	15.289	15.496	0.293
	20	55.335	54.506	54.921	0.587
	30	92.157	101.492	96.824	6.601

Table B-2.1.4 Antioxidant activity (IC_{50}) of the soluble products of subcritical water hydrolysis at 1:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	IC_{50}			
		Exp.1	Exp.2	AVG	SD
200 °C	20	0.47	0.51	0.49	0.031
	30	0.28	0.28	0.28	0.000
220 °C	20	0.26	0.20	0.23	0.045
	30	0.25	0.22	0.24	0.020

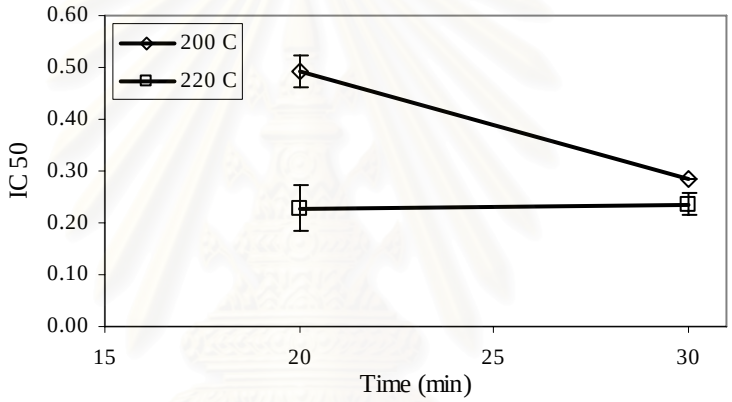

Figure B-2.1.4 Antioxidant activity (IC_{50}) of the soluble products of subcritical water hydrolysis at 1:5 raw rice bran to water ratio

B-2.2 Experimental data of subcritical water hydrolysis of 2:5 raw rice bran to water ratio

Table B-2.2.1 Protein yield of soluble product of subcritical water hydrolysis at 2:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	Protein (mg/g raw rice bran)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	14.489	13.937	14.213	0.276
	20	31.599	32.749	32.174	0.575
	30	56.897	67.568	62.233	5.336
210 °C	10	14.673	14.075	14.374	0.299
	20	50.136	49.032	49.584	0.552
	30	68.580	77.135	72.858	4.278
220 °C	10	14.811	13.799	14.305	0.506
	20	55.149	56.115	55.632	0.483
	30	73.548	78.837	76.192	2.645

Table B-2.2.2 Amino acids yield of soluble product of subcritical water hydrolysis at 2:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	Amino acids (mg/g raw rice bran)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	2.227	2.306	2.267	0.040
	20	3.109	3.638	3.373	0.265
	30	4.669	6.124	5.397	0.727
210 °C	10	2.271	2.342	2.306	0.035
	20	5.004	4.537	4.771	0.234
	30	5.586	6.636	6.111	0.525
220 °C	10	2.192	2.016	2.104	0.088
	20	4.440	4.326	4.383	0.057
	30	5.357	5.154	5.256	0.101

Table B-2.2.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 2:5 raw rice bran to water ratio

Temperature (°C)	Time (min)	Reducing sugar (mg/g raw rice bran)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	6.851	5.617	6.234	0.873
	20	14.034	16.419	15.226	1.687
	30	20.506	32.952	26.729	8.801
210 °C	10	4.673	5.181	4.927	0.359
	20	26.122	24.100	25.111	1.430
	30	58.800	62.619	60.709	4.114
220 °C	10	15.968	9.921	12.944	4.276
	20	38.227	26.122	32.174	8.559
	30	56.461	67.507	61.984	7.811

B-3 Experimental data of deoiled soy bean hydrolysis with subcritical water

B-3.1 Experimental data of subcritical water hydrolysis of 1:5 deoiled soy bean to water ratio

Table B-3.1.1 Protein yield of soluble product of subcritical water hydrolysis at 1:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	Protein Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	93.464	72.490	82.977	10.487
	20	171.105	208.914	190.010	18.904
	30	199.255	210.018	204.636	5.382
210 °C	10	124.005	107.079	115.542	8.463
	20	164.206	181.592	172.899	8.693
	30	188.492	187.940	188.216	0.276
220 °C	10	128.973	127.501	128.237	0.736
	20	169.449	163.654	166.552	2.898
	30	131.365	154.547	142.956	11.591

Table B-3.1.2 Amino acids yield of soluble product of subcritical water hydrolysis at 1:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	Amino Acids Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	12.083	12.858	12.470	0.388
	20	15.609	19.559	17.584	1.975
	30	18.536	20.758	19.647	1.111
210 °C	10	15.503	13.952	14.728	0.776
	20	19.630	18.360	18.995	0.635
	30	20.758	20.582	20.670	0.088
220 °C	10	18.501	18.184	18.342	0.159
	20	18.360	18.431	18.395	0.035
	30	18.431	18.501	18.466	0.035

Table B-3.1.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 1:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	Reducing Sugar Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	13.142	13.826	13.484	0.484
	20	51.010	54.910	52.960	2.758
	30	50.512	46.322	48.417	2.963
210 °C	10	20.672	16.170	18.421	3.183
	20	52.006	51.799	51.902	0.147
	30	37.568	38.398	37.983	0.587
220 °C	10	23.700	25.235	24.468	1.085
	20	45.036	43.086	44.061	1.379
	30	34.249	33.253	33.751	0.704

Table B-3.1.4 Antioxidant activity (IC_{50}) of the soluble product of subcritical water hydrolysis at 1:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	IC_{50}			
		Exp.1	Exp.2	AVG	SD
200 °C	20	0.30	0.26	0.28	0.025
	30	0.21	0.18	0.19	0.022
220 °C	20	0.19	0.20	0.20	0.008
	30	0.21	0.20	0.20	0.004

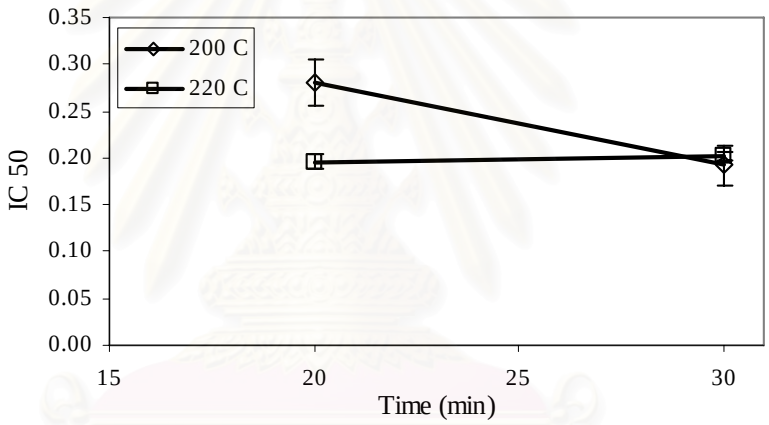

Figure B-3.1.4 Antioxidant activity (IC_{50}) of the soluble product of subcritical water hydrolysis at 1:5 deoiled soy bean to water ratio

B-3.2 Experimental data of subcritical water hydrolysis of 2:5 deoiled soy bean to water ratio

Table B-3.2.1 Protein yield of soluble product of subcritical water hydrolysis at 2:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	Protein (mg/g deoiled soy bean)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	45.536	51.608	48.572	3.036
	20	120.602	139.506	130.054	9.452
	30	131.779	141.990	136.884	5.106
210 °C	10	63.935	64.854	64.394	0.460
	20	144.060	131.917	137.988	6.071
	30	140.334	132.193	136.263	4.071
220 °C	10	73.410	83.713	78.561	5.152
	20	118.946	129.295	124.120	5.175
	30	108.183	108.321	108.252	0.069

Table B-3.2.2 Amino acids yield of soluble product of subcritical water hydrolysis at 2:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	Amino acids (mg/g deoiled soy bean)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	5.812	7.011	6.412	0.600
	20	12.601	15.246	13.923	1.323
	30	20.342	20.360	20.351	0.009
210 °C	10	8.898	7.858	8.378	0.520
	20	17.785	18.120	17.953	0.168
	30	16.904	16.516	16.710	0.194
220 °C	10	10.414	12.301	11.358	0.943
	20	12.901	15.740	14.320	1.420
	30	15.810	14.611	15.211	0.600

Table B-3.2.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 1:5 deoiled soy bean to water ratio

Temperature (°C)	Time (min)	Reducing sugar (mg/g deoiled soy bean)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	13.499	9.838	11.669	2.589
	20	39.316	41.400	40.358	1.474
	30	31.661	32.781	32.221	0.792
210 °C	10	11.176	11.000	11.088	0.125
	20	31.350	33.372	32.361	1.430
	30	26.371	21.113	23.742	3.718
220 °C	10	19.183	20.532	19.857	0.953
	20	23.042	27.927	25.484	3.454
	30	22.077	22.699	22.388	0.440

B-4 Experimental data of raw soy bean hydrolysis with subcritical water

B-4.1 Experimental data of subcritical water hydrolysis of 1:5 raw soy bean to water ratio

Table B-4.1.1 Protein yield of soluble product of subcritical water hydrolysis at 1:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	Protein Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	90.704	92.360	91.532	0.828
	20	159.790	154.547	157.168	2.622
	30	161.170	162.826	161.998	0.828
210 °C	10	78.745	99.351	89.048	10.303
	20	150.407	157.858	154.133	3.726
	30	162.826	168.621	165.724	2.898
220 °C	10	110.023	121.062	115.542	5.520
	20	148.199	153.995	151.097	2.898
	30	151.235	151.511	151.373	0.138

Table B-4.1.2 Amino acids yield of soluble product of subcritical water hydrolysis at 1:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	Amino Acids Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	10.989	11.307	11.148	0.159
	20	15.362	15.257	15.309	0.053
	30	18.360	17.549	17.955	0.406
210 °C	10	15.398	15.786	15.592	0.194
	20	16.103	16.914	16.509	0.406
	30	18.607	18.642	18.625	0.018
220 °C	10	16.703	17.937	17.320	0.617
	20	18.325	16.068	17.196	1.129
	30	17.619	16.844	17.232	0.388

Table B-4.1.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 1:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	Reducing Sugar Content (mg)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	11.835	12.498	12.167	0.469
	20	46.270	49.444	47.857	2.244
	30	38.802	39.798	39.300	0.704
210 °C	10	12.104	13.162	12.633	0.748
	20	44.030	47.017	45.523	2.112
	30	35.877	34.321	35.099	1.100
220 °C	10	23.306	23.555	23.431	0.176
	20	37.433	40.980	39.207	2.508
	30	27.351	26.729	27.040	0.440

Table B-4.1.4 Antioxidant activity (IC_{50}) of the soluble product of subcritical water hydrolysis at 1:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	IC_{50}			
		Exp.1	Exp.2	AVG	SD
200 °C	20	0.34	0.42	0.38	0.058
	30	0.18	0.21	0.19	0.024
220 °C	20	0.47	0.38	0.43	0.062
	30	0.20	0.18	0.19	0.016

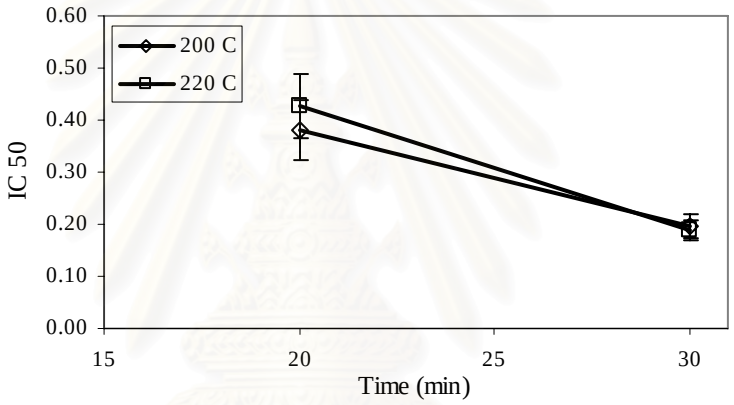

Figure B-4.1.4 Antioxidant activity (IC_{50}) of the soluble product of subcritical water hydrolysis at 1:5 raw soy bean to water ratio

B-4.2 Experimental data of subcritical water hydrolysis of 2:5 raw soy bean to water ratio

Table B-4.2.1 Protein yield of soluble product of subcritical water hydrolysis at 2:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	Protein (mg/g raw soy bean)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	54.367	45.812	50.090	4.278
	20	71.202	71.340	71.271	0.069
	30	85.691	93.280	89.485	3.795
210 °C	10	42.040	38.913	40.477	1.564
	20	77.825	84.311	81.068	3.243
	30	122.533	122.947	122.740	0.207
220 °C	10	40.017	35.785	37.901	2.116
	20	83.759	103.353	93.556	9.797
	30	97.282	99.213	98.248	0.966

Table B-4.2.2 Amino acids yield of soluble product of subcritical water hydrolysis at 2:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	Amino acids (mg/g raw soy bean)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	5.657	5.525	5.591	0.066
	20	9.580	10.206	9.893	0.313
	30	16.061	15.117	15.589	0.472
210 °C	10	5.516	5.093	5.304	0.212
	20	9.862	12.904	11.383	1.521
	30	13.945	16.731	15.338	1.393
220 °C	10	4.846	5.771	5.309	0.463
	20	17.119	13.460	15.289	1.829
	30	17.339	18.388	17.864	0.525

Table B-4.2.3 Reducing sugar yield of soluble product of subcritical water hydrolysis at 2:5 raw soy bean to water ratio

Temperature (°C)	Time (min)	Reducing sugar (mg/g raw soy bean)			SD
		Exp.1	Exp.2	Avg	
200 °C	10	9.278	10.201	9.739	0.653
	20	21.087	19.759	20.423	0.939
	30	25.671	29.135	27.403	2.450
210 °C	10	8.054	7.660	7.857	0.279
	20	18.348	19.966	19.157	1.144
	30	18.908	23.680	21.294	3.374
220 °C	10	9.662	7.712	8.687	1.379
	20	30.961	23.659	27.310	5.163
	30	27.725	24.095	25.910	2.567

B-5 Weight of residue

B-5.1 Residue weight of 1:5 raw materials to water ratio

Table B-5.1 Residue weight of 1:5 raw materials to water ratio

Raw materials	Temperature (^{o}C)	Reaction Time (min)	Residue Weight (g)		
			Exp.1	Exp.2	Avg
Deoiled rice bran	200 °C	10	0.73	0.71	0.72
		20	0.33	0.31	0.32
		30	0.30	0.29	0.30
	210 °C	10	0.56	0.55	0.56
		20	0.29	0.27	0.28
		30	0.32	0.33	0.33
	220 °C	10	0.45	0.46	0.46
		20	0.30	0.32	0.31
		30	0.34	0.31	0.33
Raw rice bran	200 °C	10	0.89	0.87	0.88
		20	0.71	0.72	0.72
		30	0.61	0.53	0.57
	210 °C	10	0.83	0.87	0.85
		20	0.68	0.68	0.68
		30	0.52	0.57	0.55

Table B-5.1 (continuous) Residue weight of 1:5 raw materials to water ratio

Raw materials	Temperature (^{o}C)	Reaction Time (min)	Residue Weight (g)		
			Exp.1	Exp.2	Avg
	220 °C	10	0.82	0.84	0.83
		20	0.53	0.56	0.55
		30	0.46	0.49	0.48
Deoiled soy bean	200 °C	10	0.60	0.62	0.61
		20	0.37	0.37	0.37
		30	0.37	0.36	0.37
	210 °C	10	0.59	0.54	0.57
		20	0.41	0.4	0.41
		30	0.37	0.36	0.37
	220 °C	10	0.52	0.39	0.46
		20	0.36	0.35	0.36
		30	0.34	0.34	0.34
Raw soy bean	200 °C	10	0.60	0.57	0.59
		20	0.48	0.47	0.48
		30	0.47	0.46	0.47
	210 °C	10	0.61	0.55	0.58
		20	0.47	0.46	0.47
		30	0.44	0.45	0.45
	220 °C	10	0.49	0.5	0.50
		20	0.46	0.46	0.46
		30	0.49	0.5	0.50

B-5.2 Residue weight of 2:5 raw materials to water ratio

Table B-5.2 Residue weight of 2:5 raw materials to water ratio

Raw materials	Temperature (^{o}C)	Reaction Time (min)	Residue Weight (g)		
			Exp.1	Exp.2	Avg
Deoiled rice bran	200 °C	10	1.69	1.65	1.67
		20	1.35	1.23	1.29
		30	0.88	0.87	0.88
	210 °C	10	1.72	1.73	1.73
		20	1.11	1.12	1.12
		30	0.66	0.68	0.67
	220 °C	10	1.6	1.59	1.60
		20	0.80	0.74	0.77
		30	0.78	0.76	0.77
Raw rice bran	200 °C	10	1.78	1.82	1.80
		20	1.77	1.70	1.74
		30	1.42	1.32	1.37
	210 °C	10	1.79	1.82	1.81
		20	1.35	1.47	1.41
		30	1.16	1.06	1.11
	220 °C	10	1.77	1.72	1.75
		20	1.43	1.39	1.41
		30	1.16	1.14	1.15
Deoiled soy bean	200 °C	10	1.54	1.37	1.46
		20	0.87	0.95	0.91
		30	0.79	0.81	0.80
	210 °C	10	1.37	1.52	1.45
		20	0.84	0.81	0.83
		30	0.80	0.78	0.79
	220 °C	10	1.33	1.27	1.30
		20	0.77	0.74	0.76
		30	0.74	0.79	0.77
Raw soy bean	200 °C	10	1.71	1.71	1.71
		20	1.34	1.29	1.32
		30	1.05	1.1	1.08
	210 °C	10	1.75	1.77	1.76
		20	1.44	1.46	1.45
		30	0.99	1.03	1.01
	220 °C	10	1.63	1.69	1.66
		20	1.15	1.31	1.23
		30	1.06	0.93	1.00

APPENDIX C

EXPERIMENTAL DATA OF COLOR MEASUREMENT

C-1 Color measurement

Table C-1 Color parameter of Deoiled and raw rice bran

Temperature \ Time		Deoiled rice bran			Raw rice bran		
		10 min	20 min	30 min	10 min	20 min	30 min
200 °C	L	n/a	31.76 ± 0.34	32.93 ± 0.69	34.20 ± 0.26	34.46 ± 0.41	33.99 ± 0.15
	a*	n/a	+0.69 ± 0.07	+0.84 ± 0.10	+0.45 ± 0.05	+0.74 ± 0.03	+1.15 ± 0.09
	b*	n/a	-1.23 ± 0.08	-1.40 ± 0.07	+0.45 ± 0.05	-0.89 ± 0.05	-0.94 ± 0.08
210 °C	L	41.24 ± 0.65	32.52 ± 1.15	33.99 ± 0.53	35.91 ± 0.31	34.18 ± 0.17	30.45 ± 0.23
	a*	+0.49 ± 0.07	+0.88 ± 0.31	+1.32 ± 0.32	+0.07 ± 0.11	+0.45 ± 0.15	+0.54 ± 0.06
	b*	+2.71 ± 0.09	-0.42 ± 0.03	-1.09 ± 0.15	+0.45 ± 0.07	-0.51 ± 0.03	-0.62 ± 0.09
220 °C	L	38.37 ± 1.22	33.02 ± 1.10	34.10 ± 1.02	38.32 ± 0.15	33.57 ± 0.10	33.09 ± 0.03
	a*	+0.76 + 0.31	+1.12 ± 0.28	+1.44 ± 0.19	+0.17 ± 0.13	+1.04 ± 0.03	+1.03 ± 0.16
	b*	+3.30 ± 3.30	-0.36 ± 0.13	-0.33 ± 0.27	-0.55 ± 0.02	-0.69 ± 0.04	-0.81 ± 0.06

Table C-2 Color parameter of Deoiled and raw soybean meal

Temperature \ Time		Deoiled soybean meal			Raw soybean meal		
		10 min	20 min	30 min	10 min	20 min	30 min
200 °C	L	34.33 ± 1.64	33.39 ± 0.47	32.39 ± 0.75	38.19 ± 1.63	32.42 ± 0.76	32.63 ± 0.68
	a*	+0.38 ± 0.20	+0.91 ± 0.33	+0.77 ± 0.17	+0.58 ± 0.52	+0.73 ± 0.19	+0.81 ± 0.3
	b*	-0.42 ± 0.30	-0.94 ± 0.11	-1.18 ± 0.05	+1.69 ± 0.40	-0.90 ± 0.20	-1.05 ± 0.11
210 °C	L	34.55 ± 0.47	33.95 ± 0.57	33.31 ± 0.94	35.85 ± 1.62	33.26 ± 0.43	32.04 ± 0.25
	a*	+0.89 ± 0.22	+1.11 ± 0.09	+0.85 ± 0.36	+0.69 ± 0.26	+0.72 ± 0.27	+0.72 ± 0.19
	b*	-0.59 ± 0.04	-1.00 ± 0.3	-1.13 ± 0.15	+0.68 ± 0.38	-0.52 ± 0.39	-0.97 ± 0.07
220 °C	L	34.28 ± 0.34	34.12 ± 0.68	33.87 ± 0.24	34.52 ± 0.34	32.99 ± 0.41	32.00 ± 0.62
	a*	+0.78 ± 0.25	+1.12 ± 0.44	+0.97 ± 0.26	+0.94 ± 0.18	+0.94 ± 0.39	+0.60 ± 0.20
	b*	-0.93 ± 0.11	-0.79 ± 0.38	-1.25 ± 0.07	+0.46 ± 0.33	-0.41 ± 0.46	-0.84 ± 0.14

APPENDIX D

EXPERIMENTAL DATA OF YEAST GROWTH

D-1 Use of hydrolysis product for yeast growth

Table D-1.1 Control medium containing 2 w/v% glucose solution in distilled water

Time (hr)	OD 660nm				
	Exp.1	Exp.2	Exp.3	Avg	SD
0	0.130	0.223	0.265	0.244	0.030
2	0.356	0.552	0.589	0.571	0.026
4	0.665	0.995	0.968	0.982	0.019
6	0.880	1.230	1.540	1.385	0.219
8	1.780	2.930	3.030	2.980	0.071
10	1.960	3.180	3.020	3.100	0.113
12	2.150	3.100	3.100	3.100	0.000
14	2.510	3.220	3.240	3.230	0.014

Table D-1.2 A culture medium containing 2 w/v% of glucose and 2 w/v% of commercial yeast extract

Time (hr)	OD 660nm				
	Exp.1	Exp.2	Exp.3	Avg	SD
0	0.257	0.213	0.238	0.236	0.022
2	0.634	0.520	0.583	0.579	0.057
4	1.444	1.333	1.386	1.388	0.056
6	2.380	2.300	2.320	2.333	0.042
8	4.240	4.030	4.360	4.210	0.167
10	4.400	4.420	4.340	4.387	0.042
12	4.420	4.580	4.220	4.407	0.180
14	4.530	4.350	7.627	5.520	4.010

Table D-1.3 The test medium containing 2 w/v% of glucose and 2 w/v% of subcritical water hydrolysis product

Time (hr)	OD 660nm				
	Exp.1	Exp.2	Exp.3	Avg	SD
0	0.243	0.233	0.218	0.231	0.013
2	0.559	0.571	0.585	0.572	0.013
4	1.080	1.123	1.150	1.118	0.035
6	1.860	1.710	1.990	1.853	0.140
8	3.670	3.550	3.760	3.660	0.105
10	4.010	4.140	4.030	4.060	0.070
12	3.500	4.180	4.210	3.963	0.402
14	4.010	3.930	4.130	4.023	0.101

APPENDIX E

DATA ANALYSIS OF PAIRED PREFERENCE TEST

E-1 Static analysis

Table E-1.1 Two sample analysis

Number of judgments	Two-tail tests Minimum agreeing judgments necessary to establish significant differentiation			One-tail tests Minimum correct answers necessary to establish significant differentiation		
	Probability level			Probability level		
	5%	1%	0.1%	5%	1%	0.1%
5	….	….	….	5	….	….
6	….	….	….	6	….	….
7	7	….	….	7	7	….
8	8	8	….	7	8	….
9	8	9	….	8	9	….
10	9	10	….	9	10	10
11	10	11	11	9	10	11
12	10	11	12	10	11	12
13	11	12	13	10	12	13
14	12	13	14	11	12	13
15	12	13	14	12	13	14
16	13	14	15	12	14	15
17	13	15	16	13	14	16
18	14	15	17	13	15	16
19	15	16	17	14	15	17
20	15	17	18	15	16	18
21	16	17	19	15	17	18
22	17	18	19	16	17	19
23	17	19	20	16	18	20
24	18	19	21	17	19	20
25	18	20	21	18	19	21
26	19	20	22	18	20	22
27	20	21	23	19	20	22
28	20	22	23	19	21	23
29	21	22	24	20	22	24
30	21	23	25	20	22	24
31	22	24	25	21	23	25
32	23	24	26	22	24	26
33	23	25	27	22	24	26
34	24	25	27	23	25	27
35	24	26	28	23	25	27
36	25	27	29	24	26	28
37	25	27	29	24	27	29
38	26	28	30	25	27	29
39	27	28	31	26	28	30
40	27	29	31	26	28	31
41	28	30	32	27	29	31
42	28	30	32	27	29	32
43	29	31	33	28	30	32
44	29	31	34	28	31	33
45	30	32	34	29	31	34
46	31	33	35	30	32	34
47	31	33	36	30	32	35
48	32	34	36	31	33	36
49	32	34	37	31	34	36
50	33	35	37	32	34	37
60	39	41	44	37	40	43
70	44	47	50	43	46	49
80	50	52	56	48	50	55
90	55	58	61	54	57	61
100	61	64	67	59	63	66

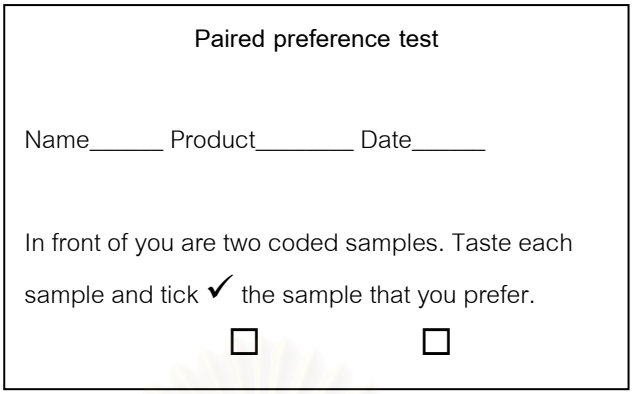

Figure E-1 Instructions on score-card

Table E-1.2 Result from answer of tester

Set	Preference		
	Control	Added with extraction product	Total
Milk	14	26	40
Coffee	25	15	40

Assumption H_o = Testers preference in product test < 50% ($p < ½$)

H_a = Testers preference in product test ≥ 50% ($p \geq ½$)

From Table E-1.1 (df=1 and one tailed) was found to minimum correct answers necessary to establish significant differentiation at probability level 5% was 26. Therefore the results we concluded that milk added with extraction product exhibited a strong preference for the flavor that had been paired with control (not less than 26 persons) at $P \leq 0.05$, which acceptance H_a and not acceptance H_o at probability level of 5% and coffee added with extraction product exhibited a similar preference for the flavor that had been paired with control (less than 26 persons) at $P > 0.05$, which acceptance H_o and not acceptance H_a.

VITA

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