การแยกแกมมาออริซานอลจากไขสบู่ผลพลอยได้จากอุตสาหกรรมน้ำมันรำข้าว

นางสาววาสิณี แก้วบุญนำ

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SEPARATION OF GAMMA ORYZANOL FROM SOAPSTOCK BY PRODUCT FROM RICE BRAN OIL INDUSTRY

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
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งานวิจัยนี้เป็นการศึกษาแนวทางในการสร้างมูลค่าเพิ่มให้กับไขสบู่ ผลพลอยได้จากโรงงานกลั่นน้ำ
ข้าว ซึ่งเป็นแหล่งที่สำคัญของสารแกมมาออริซานอล โดยนำไขสบู่ซึ่งเป็นวัตถุดิบภายในประเทศมาทำกา
สภาวะที่เหมาะสมในการแยกแกมมาออริซานอล โดยพิจารณาจากผลได้และความบริสุทธิ์ของสารดั
การศึกษาจะเริ่มจากการนำไขสบู่มาทำปฏิกิริยาสปอนนิฟิเคชันด้วยโซเดียมไฮดรอกไซด์ 2.4 % โดยน้ำหนัก
สบู่ จากนั้นนำไขสบู่ที่ผ่านการทำปฏิกิริยาสปอนนิฟิเคชันแล้ว ไปอบแห้ง และหาปริมาณแกมมาออริซาน
พบว่ามีปริมาณ 5.83 % โดยน้ำหนัก ซึ่งปริมาณดังกล่าว จะถูกใช้เป็นปริมาณเริ่มต้นในการคำนวณผลได้แล
บริสุทธิ์ของแกมมาออริซานอล ไขสบู่ที่ผ่านการทำปฏิกิริยาสปอนนิฟิเคชันและอบแห้งแล้วจะถูกนำไปส
วิธี soxhlet โดยศึกษาอัตราส่วนของวัตถุดิบต่อตัวทำละลายเอทิลอะซิเตด ในช่วง 3 – 15 กรัมวัตถุดิบ ต่
ละลาย 200 มิลลิลิตร ซึ่งพบว่าอัตราส่วนที่เหมาะสม คือ15 กรัมต่อ 200 มิลลิลิตร จากนั้นสารสกัดจะถูกระ
ทำละลายออก และนำไปละลายในระบบตัวทำละลายผสม ก่อนนำไปตกผลึก 2 ขั้นตอน ซึ่งขั้นตอนแรกจะ
ตกผลึกที่อุณหภูมิห้องในช่วง 25 -30 องศาเซลเซียส เป็นเวลา 1- 2 ชั่วโมง เพื่อกำจัดสารยางเหนียวที่เป็นสิ่ง
สารละลายใสที่ได้จากขั้นตอนแรกจะนำไปตกผลึกต่อที่อุณหภูมิ 2-10 องศาเซลเซียส เป็นเวลา 8-16 ชั่วโ
แยกแกมมาออริซานอล ซึ่งในงานวิจัยนี้ ได้ศึกษาระบบตัวทำละลายผสม 2 ระบบ คือ อะซิโตนกับเมท
ในช่วง 15-35 % โดยปริมาตรของอะซิโตน และเอทิลอะซิเตดกับเมทานอล ในช่วง 10-30 % โดยปริมา
เอทิลอะซิเตด ซึ่งพบว่า ระบบตัวทำละลาย 20 % โดยปริมาตรของเอทิลอะซิเตดในเมทานอล มีความเห
ที่สุดซึ่งใกล้เคียงกับระบบตัวทำละลาย 25 % โดยปริมาตรของอะซิโตนในเมทานอล แต่เนื่องจากเอทิลอะซิ
ตัวทำละลายที่ใช้ในขั้นตอนการสกัด ซึ่งสามารถรีไซเคิล นำมากลับมาใช้ใหม่ได้ และเป็นที่ยอม
อุตสาหกรรมอาหารและยา จึงได้เลือกระบบตัวทำละลาย 20 % โดยปริมาตรของเอทิลอะซิเตดในเมทานอ
ในการศึกษาต่อไป นอกจากนั้นยังได้ศึกษาอุณหภูมิและเวลาที่เหมาะสมในการตกผลึกทั้ง 2 ขั้นตอน ซึ่
อุณหภูมิและเวลาที่เหมาะสมในการตกผลึกขั้นตอนแรก คือ 30 องศาเซลเซียส และ1 ชั่วโมง ส่วนการต
ขั้นตอนที่ 2 พบว่า สภาวะที่เหมาะสม คือ 5 องศาเซลเซียส และ 24 ชั่วโมง โดยความบริสุทธิ์และผลได้ของแ
ออริซานอลหลังการตกผลึกครั้งที่ 2 เท่ากับ 55.17 % และ 74.6 % ตามลำดับ เมื่อทำการวิเคราะห์ด้วยวิธี HPL
พบว่าใกล้เคียงกับงานวิจัยที่ผ่านมา นอกจากนั้นผลการวิเคราะห์แกมมาออริซานอลโดยใช้ UV-
spectrophotometer ยังให้ผลสอดคล้องและให้ข้อสรุปการเลือกสภาวะที่เหมาะสมเช่นดียวกับวิธี HPLC

ภาควิชา.....วิศวกรรมเคมี..... ลายมือชื่อนิสิต.....วาสิณี.....แก้วบุญนำ.....

สาขาวิชา.....วิศวกรรมเคมี..... ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2550.....

4970566321 : MAJOR CHEMICAL ENGINEERING
KEY WORD : GAMMA ORYZANOL / SOAPSTOCK / RICE BRAN O
CRYSTALLIZATION

WASINEE KAEWBOONNUM : SEPARATION OF GAMMA ORYZA
FROM SOAPSTOCK BY PRODUCT FROM RICE BRAN OIL INDUS
THESIS PRINCIPLE ADVISOR: ASST.PROF. ARTIWAN SHOTIPRUK, P
91 pp.

This study aims to produce value added product, γ-oryzanol, by extraction c compound from rice bran oil soapstock, the by product from rice bran oil industry. suitable conditions for the separation of γ-oryzanol were determined. First, soapstock saponified with 2.4 % wt of sodium hydroxide. The saponified product was dehydrated and quantified for the content of γ-oryzanol. The γ-oryzanol content i dried saponified soapstock was found to be 5.83 % by weight. Since the dried sapor soapstock was used as a starting material for extraction, the amount of γ-oryzanol i dried saponified soapstock was taken as a basis for the calculation of the γ-oryzanol Extraction was carried out using ethyl acetate as a solvent in a soxhlet apparatus. The of raw material to solvent was varied in the range of 3-15 g raw material to 20 solvent. The results showed that the suitable ratio was 15 g/200 ml solvent. The e was then evaporated and the solution was crystallized twice. For the first crystallization, two suitable solvent mixture systems (15-35 % v/v of acetone in met and 10-30 % v/v of ethyl acetate in methanol), the temperature (25 and 30 °C), and ti and 2 hr) for crystallization were studied. The result showed that 20 % v/v of ethyl a in methanol was suitable and the yields are comparable to original solvent mixture at v/v of acetone. The suitable temperature and crystallization time was 30 °C and respectively. The supernatant from first step crystallization was then allowed to cryst at lower temperature in second step. The suitable temperatures (2, 5 and 10 °C extraction times (8, 16 and 24 hr.) were studied. The suitable temperature and time found to be 5 °C and 24 hr., respectively. After the second crystallization step, the and yield of γ-oryzanol as analyzed by HPLC were found to be 55.17 % wt and 7 wt, respectively. Moreover, quantification of γ-oryzanol by using UV-v spectrophotometric analysis was consistent and gave the same conclusion with HPLC

Department: .....Chemical Engineering... Student's signature: ...วาสินี....แก้วบุญน้อ...
Field of study:...Chemical Engineering... Principle Advisor's signature: .....
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CHAPTER I

INTRODUCTION

1.1 Motivation

Due to suitable topography and climate, rice is the major agricultural product of Thailand. With the annual productivity of approximately 29 million tons, it is the sixth of the world rice producer [www.oae.go.th]. About 55 % of the rice produced is consumed domestically and the rest (45 %) is exported to other counties [www.nfi.or.th]. Since consumers prefer white rice over brown rice, the rice bran is removed and is therefore an important by product of the milling process. Rice bran is an enriched source of fibre, proteins, oil, and important antioxidants mainly, and is mainly utilized as animal feed. A small amount of rice bran extracted for oil used for cooking, and when compared with other vegetable oils, rice bran oil contains higher content nutraceutical substances such as vitamin E and γ-oryzanol. In particular, γ-oryzanol is a characteristic compound found only in rice bran oil and not in other vegetable oils. It exhibits several health benefits such as being an anti-aging agent and reducing blood cholesterol with the antioxidant activity that is six times of that of vitamin E [Haung et al. (2002)]. Moreover, γ-oryzanol is non-genotoxic and non-inhibitory of cellular communication, therefore it is safe for use as supplement, food additive, or cosmetics [Tsushimoto et al. (1991)].

Normally, there are two alternative processes for oil refining: chemical refining and physical refining. These processes are distinct in the initial step for removing free fatty acids contained in crude oil. In chemical process, sodium hydroxide is used to remove free fatty acids and other impurities such as phosphatides, proteinaceous, and mucilaginous substances, while in physical refining, the separation of these comounds is achieved by steam distillation. Although the physical process results in a smaller loss of nutraceutical components, nowadays rice bran oil industries still use chemical refining due to the simplicity of the process design. In the chemical refining process, the deacidification of the crude oil by alkali treatment gives rise to *soapstock* that is then separated from the oil (triglycerides). At this alkali treatment step that a large amount of γ-oryzanol (83-95 % of the amount

contained in crude oil) is lost from the oil into the soapstock by product. For this reason, soapstock is therefore considered one of the most interesting by products from rice bran oil refining process due to its high content of γ-oryzanol. Due to the beneficial effects of γ-oryzanol on human health mentioned above, the global interest has increased in developing a process for separation γ-oryzanol from rice bran oil soapstock into commercial scale. Unfortunately, the soapstock produced in Thailand is currently used as animal feed or sold to other countries at low price (13-15 baht/kg). It is therefore the aim of this study to examine the process by which the value added γ-oryzanol could be separated from the soapstock.

In the development of such process into commercial scale, several factors such as productivity, environment and health problems, process investment, and separation efficiency (purity and yield) must be considered. Furthermore, isolation procedure developed for one soapstock does not necessarily work well with another soapstock [Narayan et al. (2006)]. Indira et al. (2005) proposed a process to obtain γ-oryzanol enriched fraction following simple procedures starting with saponification of the soapstock, dehydration of the saponified product, and leaching of γ-oryzanol from the dried matter with organic solvent. The extract (γ-oryzanol enriched fraction) could then be refined further to achieve higher purity following the process developed by Narayan et al. (2004) Their procedure involved nonselective temperature assisted dissolution of γ-oryzanol enriched fraction in a suitable mixture, followed by fractional precipitation of mucilaginous impurities, and crystallization of γ-oryzanol from supernatant.

In this study, the above processes for separation of γ-oryzanol from rice bran oil soapstock were adapted and employed. The suitable separation conditions such as the amount of sodium hydroxide used, the ratio of the leaching solvent to sample, the types and temperature of the solvent mixtures used for crystallization, were determined in order to enhance the purity and yield of γ-oryzanol obtained from local rice bran oil soapstock.

1.2 Objectives

1. To study the effect of ratio rice bran oil soapstock to ethyl acetate in the leaching step on yield and purity of γ-oryzanol extracted.

2. To study the effects of crystallization condition (binary solvent mixtures, temperature and time) on yield and purity of γ-oryzanol.

1.3 Working scopes

1. Determine the suitable amount of sodium hydroxide required for the saponification of the local soap stock.

2. Determine the suitable ratio of rice bran oil soapstock to ethyl acetate (3g/ 200 ml, 6g/ 200 ml, 10 g/ 200 ml and 15 g/ 200 ml) for leaching process.

3. Determine the suitable binary solvent mixtures for crystallization such as acetone: methanol (15 %, 25 % and 35 % v/v of acetone) and ethyl acetate: methanol (10 %, 20 % and 30 % v/v of ethyl acetate) on yield and purity of γ-oryzanol.

4. Determine the suitable temperature (at 25 and 30 °C) and time (1 and 2 hour) for the first crystallization step based on yield and purity of γ-oryzanol.

5. Determine the suitable temperature (at 2, 5 and 10 °C) and time (at 8, 16 and 24 hours) for the second crystallization step based on yield and purity of γ-oryzanol.

1.4 Expected benefits

1. To provide suitable conditions for separation of γ-oryzanol from local rice bran oil soapstock.

2. To propose the procedure for the production of value added by product from rice bran oil refining process.

CHAPTER II

BACKGROUND & LITERATURE REVIEWS

2.1 Rice bran and rice bran oil

Rice bran is one of the valuable by product from rice processing industry. During the milling process to obtain white rice, rice bran (8-9 wt % of the rice paddy) and rice germ (1-2 %) are usually removed, and the removed by-products are generally called rice bran. Rice bran is a rich source of fiber, natural oil, and nutrients such as vitamin E and vitamin B [Peretti et al. (2002)]. It's usually used as animal feed, food supplements and for oil production. Rice bran contains about 16-32 % oil [Marshall et al. (1994)] and rice bran oil is considered the most nutritious oil due to a unique complex of naturally occurring biological active and antioxidative compounds such as tocopheral, tocotrienol and γ-oryzanol [Cicero and Gaddi (2001)].

2.2 Rice bran oil processing

Among the production all vegetable edible oils, processing of rice bran oil is considered the most challenging due to rapid deterioration of the crude fat by lipase enzymes in rice bran. The 1, 3–site of triacylglycerol is cleaved by several types of lipase in the bran, resulting higher free fatty acid level. To solve this problem, heating of the bran immediately after milling inactivates the lipase and prohibit the formation of free fatty acid (stabilization) before oil extraction [Zullaikah et al. (2005)].

2.2.1 Oil extraction

The oil may be removed from the stabilized bran by using hydraulic pressing or solvent extraction. Hydraulic pressing results in only 50 % oil recovery thus solvent extraction is preferred. Hexane is commonly used as the solvent in the commercial extraction of oil from stabilized bran. After extraction, solvent is removed to obtain crude rice bran oil and is further purified. The composition of crude rice bran oil is shown in Table 2.1.

Table 2.1 Composition of crude rice bran oil

Composition of crude rice bran oil	
Saponifiable lipid	
Triacylglycerols	81-84 %
Diacylglycerol	2-3 %
Monoacylglycerol	1-2 %
Free fatty acids	2-6 %
Wax	3-4 %
Glycolipids	0.8 %
Phospholipids	1-2 %
Unsaponifiable matter	4 %

Source: Ghosh (2007)

2.2.2 Oil refining

Refining process produces edible oil with characteristics that consumers desire such as bland flavor and odor, clear appearance, light color, stability to oxidation, and suitability for frying. There are two alternative processes for edible oil refining: chemical refining or alkali refining and physical refining.

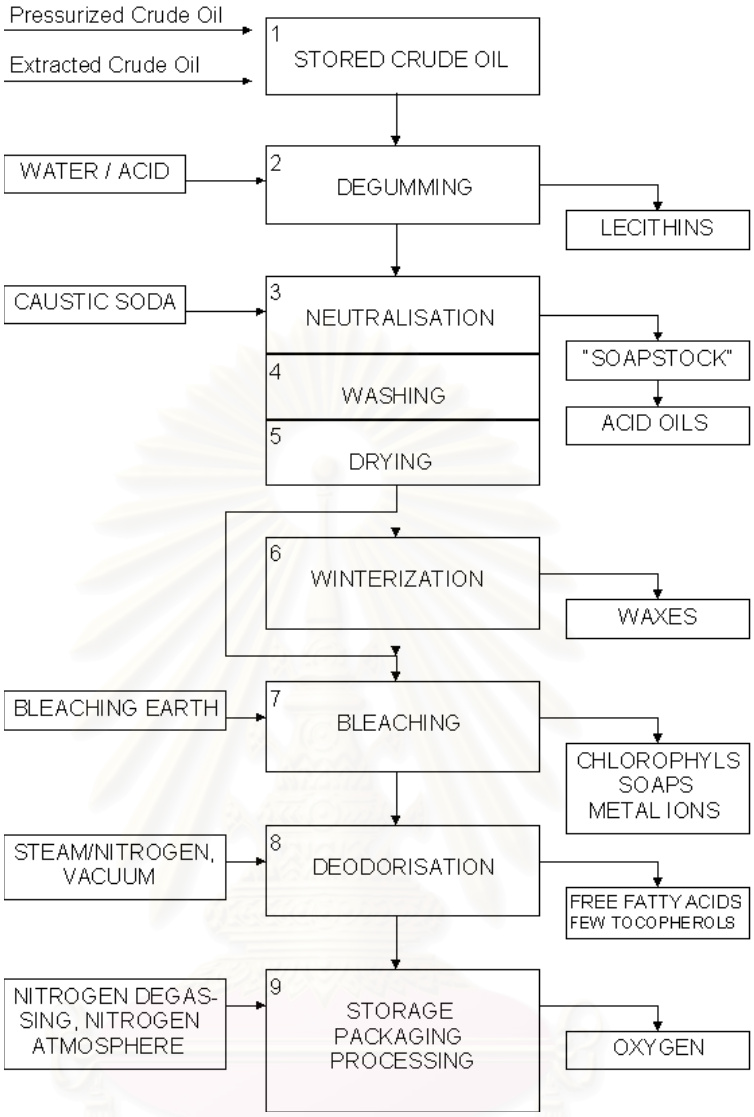


Figure 2.1 Rice bran oil production by chemical refining
Source: Belitz et al. (1987)

Chemical refining process usually comprises different steps as shown in Figure 2.1 and the details are described as follows.

1). Degumming

This step is designed to remove phospholipids (gums), both hydratable and nonhydratable gums in crude oil. Hydratable gums can be removed by adding water or acid into the oil. This process is called water degumming or acid degumming.

Another way to remove hydratable gums is by using surface active agents such as lauryl sulfate, sodium oleate, alkylated phenol ethylene oxides, or alkyl aryl sulfonate. This method was claimed to achieve a lower phosphorus content compared with water or acid degumming [Bhattacharyya et al. (1985)]. Methods for the removal of the nonhydratable gums from rice bran oil include superdegumming and enzymatic degumming. A superdegumming method developed and patented by Unilever is based on the principle that the nonhydratable gums present in the rice bran oil can be converted to a hydratable form by heating the oil to 70–80 °C and treating it with citric acid for about 20 min [Ringer et al. (1977)]. The hydratable gums formed are allowed to crystallize as calcium and magnesium salts, waxes, and glycerol. Final neutralization gives oil with a phosphorus content of less than 5 ppm. Buchold (1993) proposed a method of treating the nonhydratable gums by using enzymatic degumming. In this method, the enzyme used is phospholipase, which hydrolyzes the ester bond of the phospholipids at the oil-water interface thereby converting the nonhydratable gums into fully hydratable gums and free fatty acid. The hydratable gums are then removed by conventional water degumming [Roy et al. (2002) and Narayan et al. (2006)].

2). Deacidification or Neutralization

Sodium hydroxide is used in this procedure to remove free fatty acids and other impurities such as phosphatides, proteinaceous, and mucilaginous substances. Slight excess sodium hydroxide was mixed with the heated oil at the temperature about 60-80 °C and left for the aqueous phase to settle. The aqueous phase is known as soapstock which is removed from the neutral oil by washing with hot water, followed by settling or centrifugation. Even though the main purpose of the deacidification step is to remove of free fatty acid, the process also causes a reduction in phospholipid and coloring matter content [Belitz et al. (1987)].

3). Winterization or Dewaxing

This step is designed to remove high melting point waxes in rice bran oil. Winterization is a process whereby the wax is crystallized and removed from the oil

by filtration to avoid clouding of the liquid fraction at cooler temperature conditions by using temperature to control crystallization. In a conventional process, the oil is heated to around 90 °C to destroy any existing nuclei. After that, the oil is cooled with stirring to around 20 °C and then allowed to mature for a minimum of 4 h. Wax is separated by filtration in plate and frame filters.

4). Bleaching

Bleaching of rice bran oil is somewhat more difficult than most other vegetable oils primarily due to the high chlorophyll and red pigment content, and also due to the oxidized products of tocopherols and metallic salts of fatty acids. Generally, bleaching of rice bran oil is done after deacidification step by using adsorbents called bleaching earths (Fuller's earth, acid activated monmorillonite clays or activated carbon) to absorb coloring components. During bleaching, gums, soaps and some oxidation product are also adsorbed along with the pigments [Belitz et al. (1987)]. Bleaching rice bran oil prior to deacidification step is sometimes recommended because chlorophyll tends to be stabilized by alkali and heat, which then is more difficult to be removed [Cowan (1976)]. In addition to removing pigments, bleaching under high vacuum and at a temperature of around 110 °C helps reduce the amounts of oxidation products as the catalytic activity of activated earth at this temperature decomposes hydro-peroxides and the ion exchange properties of bleaching earth promote metal removal [Ghosh (2007)].

5). Deodorization

The aim of this step is to remove volatile compounds mainly aldehydes and ketones. These compounds have low threshold values for detection by taste or smell. Deodorization is essentially a steam distillation process carried out at temperature between 200 and 220 °C and pressure 6-10 mmHg. This treatment unfortunately also resulted in the removal of some of the natural protectants of oils such as tocopherols and sterols. As a result, the addition of citric acid is often made to chelate traces of pro-oxidant metals, hence diminish their activity that imparts to the oils, even at the diminished tocopherol content, thus increases the oil stability [Belitz et al. (1987)].

The chemical refining process has major drawbacks in that it always incurs an oil loss of an average three times the free fatty acid value. Moreover, most nutritional components present in rice bran oil are destroyed or removed during deacidification step [Ghosh (2007)]. Figure 2.2 shows the loss of two nutritional components: tocopheral and oryzanol, during each step of the chemical refining process. High loss of tocopheral can be observed at deodorization step as it is removed into deodorizer distillate. The highest oryzanol loss is found during deacidification step as it is removed into soapstock [Krisnangkura et al. (2000)].

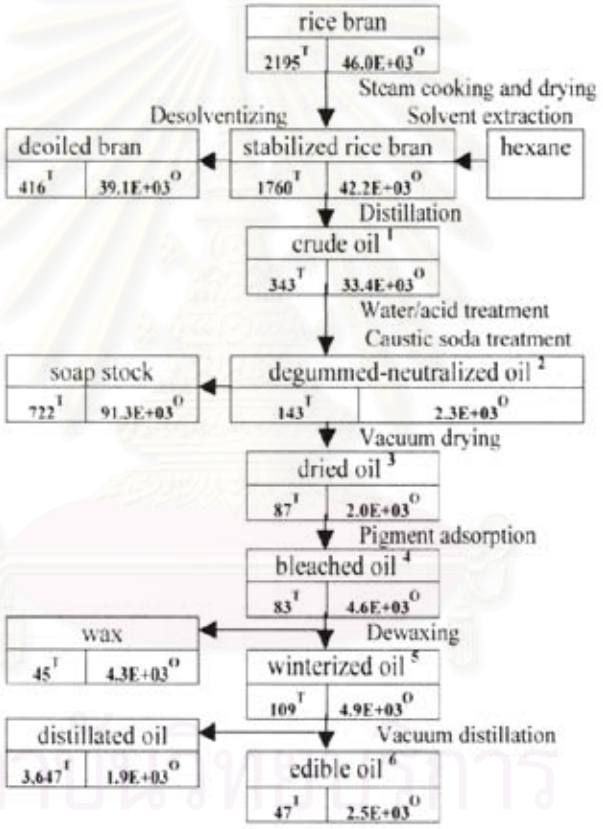


Figure 2.2 Tocopherols (T) and oryzanols (O) content in rice bran oil (RBO) processing (mg/kg)
Source: Krisnangkura et al. (2000)

Krishna et al. (2001) also report that alkali treatment removes considerable amount of oryzanol (83-95 %) while degumming and dewaxing do not affect the content of oryzanol in the oil considerably.

Table 2.2 Oryzanol content in rice bran oil after process of purifying crude rice bran oil

	Oryzanol content in refined oil (%)	Loss (%)
Control RBO (FFA, 6.8%)	1.86	—
Degumming	1.84	1.10
Dewaxing	1.75	5.90
Control RBO + alkali treatment	0.10	94.60
Degummed RBO + alkali treatment	0.11	94.10
Dewaxed RBO + alkali treatment	0.13	93.00

[a]Values are averages of samples from four determinations, and the coefficient of variation (CV) is <2% for all processing steps. FFA, free fatty acid.

Sorce**:** Krishna et al. (2001)

physical refining process usually comprises the following steps :

1). Degumming
2). Winterization
3). Bleaching
4). Deacidification / Deodorization

Physical refining process has similar steps to chemical refining except that for the removal of free fatty acid. In chemical refining, free fatty acid is removed by using alkali treatment but in physical refining, it is removed by steam distillation process similar to deodorization. In physical refining the removal low volatility of fatty acids (depending upon length) requires higher temperatures than those required for deodorization only. In practice, a maximum temperature of 240-250 °C is sufficient to reduce free fatty acids content to the levels of about 0.05-0.1 percent. A prerequisite for physical refining is that phosphatides be removed to a level below 5 ppm [FAO] as small amounts of phosphatides and iron are the probable cause of heat darkening during distillation [Ghosh (2007)]. In chemical refining, this level is easily achieved during deacidification step. However in physical refining, a special degumming technique is needed to achieve the desired phosphorus level mentioned

above. In addition to degumming, prebleaching is also necessary to remove color bodies and essentially all trace metals.

The economic of deacidification by using physical refining versus chemical refining normally favor physical refining only when high free fatty acid like rice bran oil are processed [Sullivan et al. (1976)]. Moreover, refined rice bran oil obtained from physical refining can retain most of important constituent oryzanol compared with chemical refining process [Krishna et al. (2001)].

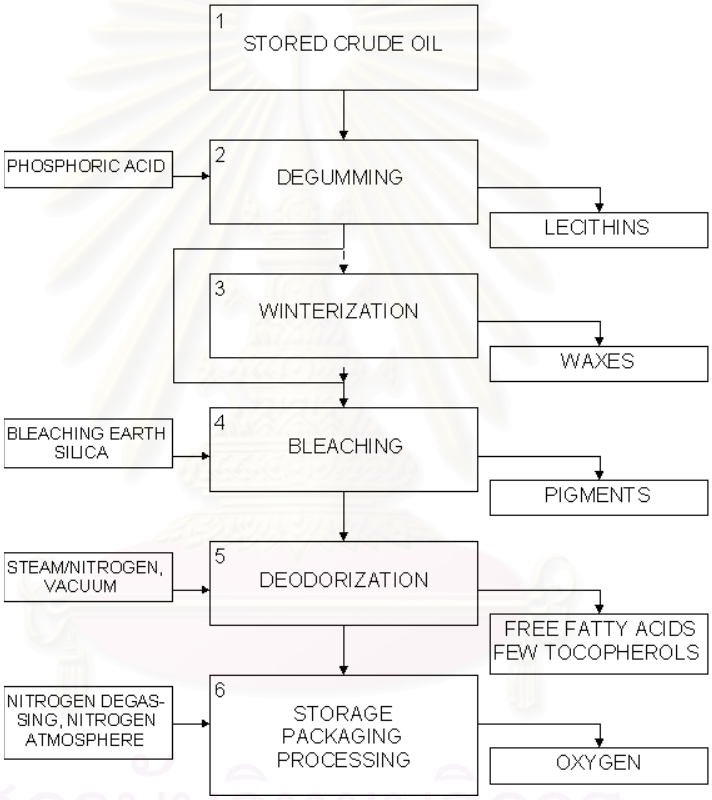


Figure 2.3 Rice bran oil production by physical refining.
Source: Belitz et al., 1987

Rice bran oil refining process produces many valuable by-products such as lecithin (degumming step), deodorizer distillate (deodorization step), and soapstock (deacidification step from chemical refining). Recovery of valuable compounds from these by products is considered an appropriate way to effectively utilize the bioresource and results in the reduction the overall cost of the oil production. In this research, we focus on the recovery of oryzanol from rice bran oil soapstock.

2.3 Rice bran oil soapstock

During chemical refining process, soap is formed in the deacidification step and significant amount of rice bran oil gets trapped within the soap. When the soap is removed by centrifugation, some oil is also removed with it. This by product is therefore called soapstock which is generally used as animal feed [Das et al. (1998)]. Often time, soapstock is reacidified by adding sulfuric acid to prepare acid oil which can be handled more easily and has longer shelf life.

2.3.1 Composition of soapstock

Soapstock consists of heavy alkali aqueous emulsion of lipids containing water as a major component. Conditions of refining and seasonal variation in oil seed composition dictate the type and quantity of impurities in the soapstock.

Table 2.3 Composition of soapstock

Composition of soapstock	
Water	65-70 % wt
Soap	20-22 % wt
Glycerides	2-2.5 % wt
Unsaponnified matter	7.75 % wt

Source: Narayan et al. (2006)

In addition to lipid loss during decidification step, a significant amounts of micronutrients especially oryzanol are also removed along with the oil into soapstock as its sodium salts (ranging 0.1-1.8 %) and is contained in unsaponifiable fraction whose composition is shown in Table 2.4 [Narayan et al. (2006)].

Table 2.4 Composition of unsaponified matter

Composition of unsaponified matter	
Sterols	42 % wt
Higher fatty acid alcohol	24 % wt
Oryzanol	20 % wt
Hydrocarbon	10 % wt
Unidentified compound	2 % wt

Source: Narayan et al. (2006)

2.4 γ-oryzanol

2.4.1 Structures

Oryzanol was first recovered from rice bran oil by Kaneko and Tsuchiya in 1954. It was then thought to be a single compound. Subsequent studies reveal that oryzanol is not a single compound but instead comprises a variety of ferulic acid esters called α-, β- and γ-oryzanol. Of these, γ-oryzanol has been the best characterized [Graf (1992)]. γ-oryzanol is a mixture of two molecules. The largest part is triterpene alcohols or plant sterols and the other part is ferulic acid. The individual components of γ-oryzanol whose molecular structures are shown in Figure 2.3, were identified as

- Δ^7-stigmastenyl ferulate
- stigmasteryl ferulate
- cycloartenyl ferulate
- 24-methylenecycloartanyl ferulate
- Δ^7-campestenyl ferulate
- campesteryl ferulate
- Δ^7-sitotenyl ferulate
- sitosteryl ferulate
- campestanyl ferulate
- sitostanyl ferulate

Three of these: cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate, are major components, accounting for 80% of γ-oryzanol in rice bran oil [Xu and Godber (1999)].

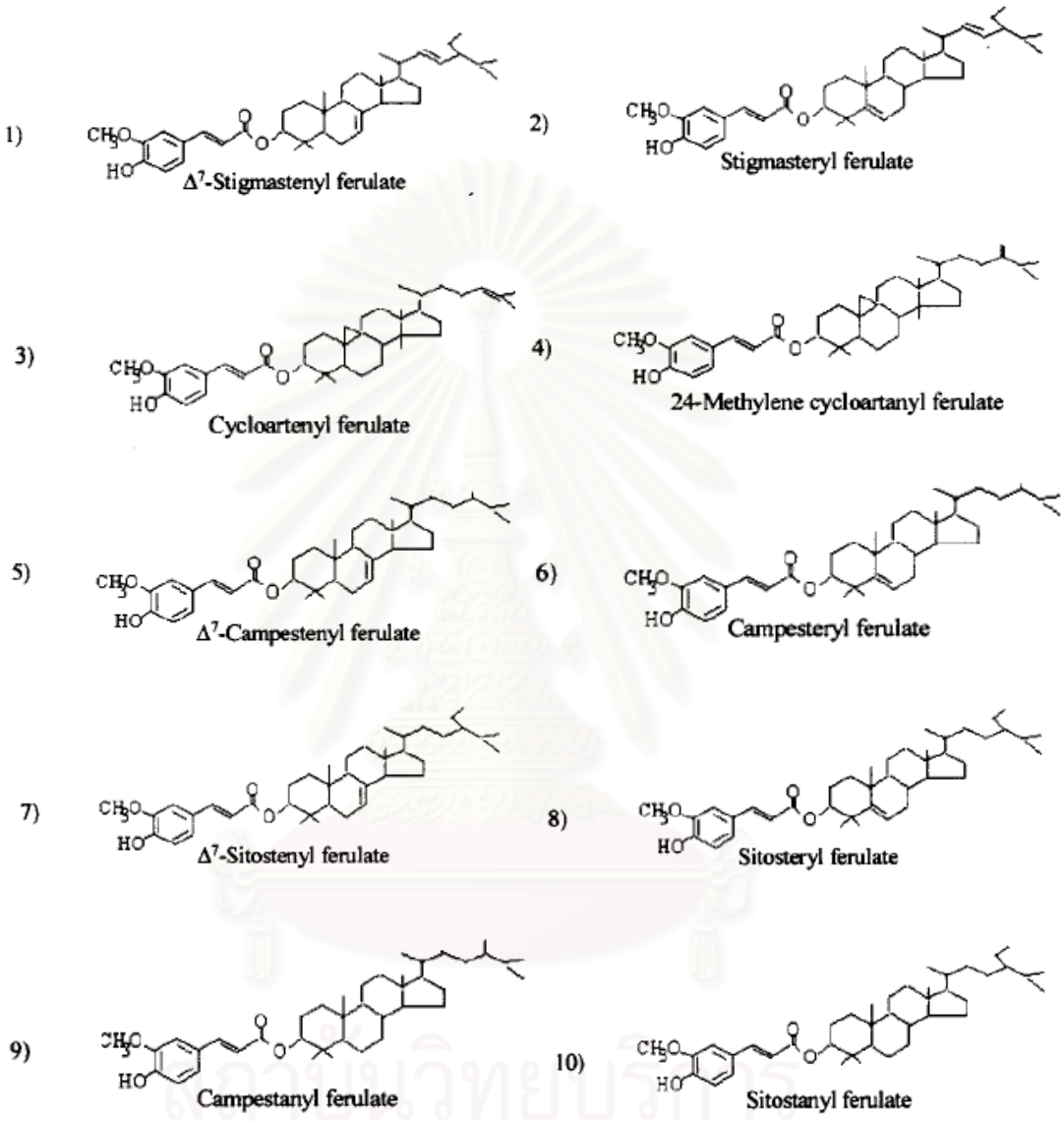


Figure 2.4 Molecular structures of identified components of γ-oryzanol
Source: Xu and Godber (1999)

2.4.2 Properties

γ-oryzanol ($C_{40}H_{58}O_4$) is a white or slightly yellow odorless crystalline powder and is stable at room temperature [Tamagawa et al. (1992)]. It has a melting point of 137.5-139.5 °C and shows absorption maxima at 231, 290 and 315 nm in heptane

[Tsuchiya and kaneko (1954)]. Oryzanol is insoluble in water, however, all of component of γ-oryzanol contains hydroxyl group in the ferlulate portion which gives rise to a relatively high polarity. Therefore, it may be soluble in more polar solvent such as isopropanol and ethyl acetate as well as non polar solvent such as n-heptane and hexane [Xu and Godber (2000)].

2.4.3 Physiological and biological effects

Over the past decades, a number of investigations have shown the beneficial physiological and biological effects associated with γ-oryzanol intake. These include:

1) γ-oryzanol emulsions are used as antioxidants and preservatives for cosmetics, food and etc. Such emulsions are effective in preventing color change [Minami and Morito (1982)].

2). γ-oryzanol containing pharmaceutical formulation is used in preventing motion sickness and in the treatment of nervous system disorders [Sakada and Hideharu (1982)].

3). γ-oryzanol exhibits hypochoresterolemic activity, which is associated with platelet aggregation. When added to a high cholesterol diet, it can significantly inhibit the platelet aggregation in rat [Seetharamajah et al. (1990)].

4). A plethora of γ-oryzanol containing transdermal pharmaceutical and moisturizing cosmetic preparations has been prepared for the treatment of skin disorders [Tokuda and Yasuaki (1989)].

5). Nail lacquers containing γ-oryzanol prevent discoloration of nails [Hayafune and Sato (1990)].

6). Lipid peroxidation can be prevented in the retina by γ-oryzanol due to its antioxidant property [Tadahisa et al. (1990)].

7). γ-oryzanol has been shown to be highly effective against lipogenic liver cirrhosis in sapontaneously hypertensive rats [Ito et al. (1992)].

8). Pharmaceutical preparations containing γ-oryzanol have been shown to reduce wrinkles in aged women [Sakai et al. (1993)].

9). Melanin formation accelerating topical preparations containing γ-oryzanol (1 wt %) have been shown to convert gray hair into natural black hair [Nakahara (1996)].

Oryzanol not only has been known for the therapeutic benefits, its safety to use as a drug has been demonstrated. For example, Tsushimoto et al. (1991) showed that oryzanol is non-genotoxic and non-inhibitory of cellular communication.

2.4.4 Applications of γ-oryzanol

γ-oryzanol has a focus on market potential and has been applied to a wide range of products. Cholesterol-lowering and antioxidant activity of oryzanol makes it suitable in food products such as cereal and margarine. It can also be put into frying oil for its stability and into food coating or packaging material for antioxidant effect to extend shelf life. Moreover, γ-oryzanol can be used in pharmaceutical products such as sun UV protection skin lotion and skin-care products for repairing dry and sensitive skin [Huang (2003)].

2.5 Impurities in rice bran oil soapstock

During rice bran milling, impurities get into the rice bran and subsequently rice bran oil and then rice bran oil soapstock after deacidification step. Knowledge of the types of impurities is essential for separation of γ-oryzanol. These impurities include:

2.5.1 Soap

Soap is sodium salts of free fatty acid that is contained in soapstock at approximately 20-22 % wt. Rice bran oil soapstock are highly to moderately soluble in water and methanol, but insoluble in acetone and ethyl acetate. This contrasting solubility behavior between soap and γ-oryzanol is the basis for separation of γ-oryzanol.

2.5.2 Glycerides

Glycerides present in soapstock in form of mono-glycerides, di-glycerides in minor amounts depend on the extent of hydrolysis of oil and mostly in form of tri-

glycerides. The amount of tri-glyceride in soapstock depends on operating condition during deacidification step. Tri-glycerides are soluble in organic solvent such as hexane, isopropanol, chloroform and ethyl acetate but mono-glycerides and di-glycerides have lower solubility in these solvent compare with tri-glycerides. A technique for removal of glycerides from soapstock is by converting them into soaps by saponification. The degree of saponification varies with conditions, but tri - glycerides saponify rapidly in aqueous alkali compared with the hydrolysis of oryzanol [Das et al. (1998)]. Oryzanol is then separated from the soap by extraction.

2.5.3 Phospholipids (gums)

Both hydratable and nonhydratable gums are present in soapstock and their proportions vary depending on the efficiency of the degumming step. The gums, especially the nonhydratable phospholipids, are the most interfering impurities affecting oryzanol separation from soapstock, probably owing to their high surface activity. During solvent extraction of soapstock, the gums stabilize the soapstock-solvent microemulsion and thereby decrease the rate of phase separation. To reduce their interference during the extraction step (solid-liquid and liquid-liquid) used for isolating oryzanol, efficient degumming of rice bran oil is desirable prior to the alkali-refining step.

2.5.4 Waxes

Waxes are esters of saturated fatty acid (C_{16} to C_{26}) and saturated fatty alcohols (C_{24} to C_{30}) and present in rice bran oil depends on extraction conditions. The common range in rice bran oil is 2–4 % with a level of <0.5 % being desirable. Rice bran waxes can be classified into two classes: soft waxes (m.p. < 75°C) and hard waxes (m.p. > 80°C). The majority of waxes (about two thirds) exist in polymeric form with the remainder being monomeric. During deacidification step, some wax gets into soapstock and tends to form stable emulsion. Waxes are insoluble in acetone, ethyl acetate, and isopropyl alcohol but soluble in hot hexane.

2.5.5 Sterol

Sterols are a major portion of the unsaponified matter of rice bran oil soapstock. Sterols in rice bran oil are mainly present as neutral sterols (free sterols and steryl esters), with some polar sterols (steryl glycosides and acylated steryl glycosides). The major sterols in rice bran oil are β-sitosterol, campesterol, and stigmasterol. During alkali refining, considerable amounts of steryl esters and steryl glycosides are extracted into the soapstock from the crude oil. The content of individual types of sterols in soapstock varies depending on processing conditions during alkali refining. Harsh processing conditions, such as high temperature and high pH during isolation of oryzanol, promote several chemical reactions of sterols such as hydrolysis, oxidation, dehydrogenation, and isomerization. Temperature, pH, and time of processing also contribute to the rate of sterol degradation. Alkaline hydrolysis cleaves the ester bond of steryl esters and releases its fatty acid into the reaction mixture along with the sterol. Acid hydrolysis cleaves the acetal (glycosidic) bond of steryl glycosides and releases the carbohydrate from the sterol or steryl ester. Neutral sterols (free sterols and steryl esters) are soluble in organic solvents such as acetone, chloroform, and ethyl acetate. Steryl glycosides contain carbohydrate moieties (sugar units) attached to sterol or steryl esters and therefore require relatively more polar solvents to solubilize them. This difference in solubility of esters and steryl glycosides forms the basis for the separation of sterol glycosides from steryl esters during the isolation process.

2.5.6 Resinous material

Resinous materials in soapstock are thought to be formed by polymerization of wax components. The resinous materials can be saponified to triacontanol and soaps. The wax-like components are significant interfering impurities during the purification of oryzanol. These impurities interfere with oryzanol separation during chromatography because they strongly adsorb onto the support along with oryzanol, and interfere in crystallization of oryzanol by disrupting crystal growth. During crystallization of oryzanol from the unsaponified fraction of soapstock, the wax-like (mucilaginous) impurities precipitate out first when the temperature of solvent

miscella is reduced from about 60–70 °C (that is, the reflux temperature) to 25–30 °C. At this temperature (25–30°C), oryzanol also crystallizes, thus making the separation of wax and oryzanol difficult. Hence, the separation of supernatant miscella from mucilaginous impurities should be carried out judiciously.

2.5.7 Tocopheral derivative

Impurities that are carried out into soapstock include tocopherol derivatives (i.e., tocopherols and tocotrienols or vitamin E). They are a family of compounds possessing a hydroxychromane ring and a terpenoid side chain. These components have been separated from γ-oryzanol by normal-phase HPLC and reversed-phase HPLC. For normal-phase HPLC, 2.5 % ethyl acetate in isooctane as mobile phase and the elution order is tocopherol, tocotrienol and γ-oryzanol [Diak et al. (1994)]. Alternatively, the reversed-phase HPLC could be used in which acetonitrile/methanol/tert-butyl methyl ether (65:30:5) was used as a mobile phase. In this case, the elution order was tocotrienol, tocopherol, and then γ-oryzanol [Roger et al. (1993)]. In order to identify the major oryzanol components, subsequent analysis by CI-MS is needed.

2.5.8 Pigments

Crude rice bran oil contains pigments such as chlorophyll, carotenoids (lutein, xanthophylls), and protein degradation products. Among these pigments, concentrations of chlorophyll compounds are very high in crude rice bran oil. The content of peptides in rice bran oil varies depending on the degree of heating during stabilization of the rice bran [Gingras (2000)]. These impurities are removed by bleaching using adsorbents such as activated clay after beaching step. If these impurities are not removed during bleaching step, they are carried into the soapstock during alkali refining.

2.5.9 Glycolipids

Glycolipids (phosphoglycolipids) are critical interfering impurities affecting the purification of oryzanol. They interfere with the degumming step of rice bran oil refining as a result of their very high surface activity, which leads to high oil losses into soapstock during the deacidification step. It is recommended that glycolipids be kept to as low content as possible in crude rice bran oil [Narayan et al. (2006)].

2.6 Separation of γ-oryzanol

Tsuchiya and Kaneko (1954) detected some substance that exhibited a characteristic absorption in the ultraviolet region during their studies on rice bran oil. After searching for its source, a crystalline material was isolated and named oryzanol. The researchers obtained oryzanol by using sequence procedures, removed free fatty acids from rice bran oil with sodium carbonate, esterified the fats and removed the fatty acid ester using low-pressure distillation. Repeated chromatography of the residue and recrystalization afforded oryzanol. From the first recovery of oryzanol, many researchers attempted to separation γ-oryzanol from various materials that are derived from rice bran such as rice bran oil, rice bran oil soapstock and rice bran acid oil.

2.6.1 Separation of γ-oryzanol from rice bran

Commonly, hexane is used as a solvent to commercially extract oil from rice bran but it poses potential fire, healths and environmental hazards. Hu et al. (1996) proposed to compare isopropanol with hexane as extraction solvents for the enrichment of vitamin E and oryzanol in oil from stabilized rice bran. The researchers suggested that isopropanol is a promising alternative solvent to hexane for extraction. Beside organic solvent extraction, supercritical fluids have received attention for extraction of lipid. Xu et al. (2000) reported that the yield of γ-oryzanol by supercritical fluid extraction was higher than that obtained by solvent extraction. Limitation of this method is however fluctuations in the flow rate and pressure which

cause the variation in the results. Moreover, the equipment and installation are also expensive [Narayan et al. (2006)].

2.6.2 Separation of γ-oryzanol from rice bran oil

Many researchers have employed chromatographic methods for separation of γ-oryzanol from rice bran oil with the main objective to separate individual component of γ-oryzanol. Rogers et al. (1993) used semi preparative HPLC to fractionate rice bran oil oryzanol components into individual component analysis by chemical ionization-mass spectrometry. γ-oryzanol were isolated into five components (cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campestanyl ferulate, cycloartanyl ferulate, and β- sitosteryl ferulate) in C_{18} reverse phase column with a mixture of acetonitrile/methanol/isopropanol/water as the mobile phase. Later, Xu and Godber (1999) achieved to separate ten components of γ-oryzanol (Figure 2.3) that had not been previously reported. The researchers used normal phase HPLC for purification of γ-oryzanol, then separation of individual components of γ-oryzanol in reverse phase HPLC, and identification was conducted by a GC/MS. Although chromatographic method is an effective method to achieve high purity and recovery of γ-oryzanol, it is difficult to recover solvent and scale up.

2.6.3 Separation of γ-oryzanol from rice bran oil soapstock and acid oil

During rice bran oil refining, γ-oryzanol is one of the micronutrients that is removed in high quantity from rice bran oil after deacidification step of the chemical refining process. The by-product from this step, soapstock, was found to have high γ-oryzanol content therefore it is worth separating of γ-oryzanol from the soapstock. Seetharamajah et al. (1986) used four steps process to recover γ-oryzanol from rice bran oil soapstock. The process consists of liquid-liquid extraction, column chromatography, crystallization and recrystallization to obtain γ-oryzanol as show the following diagram (Figure 2.4).

However, this process involves several steps of extraction result the decrease in the yield of γ-oryzanol, and therefore is unsuitable for scale up. Later, Mingzhi and Yanyan (1999) proposed to recovery γ-oryzanol using second soapstock as a starting

material. Second soapstock was the soapstock fraction formed from the soapstock that once again underwent alkali refining by adding excess alkali. This material was subjected to multiphase fractional crystallization to obtain 98 % (w/w) pure γ-oryzanol, however the yield was only 1.9 % (w/w). γ-oryzanol recoverrd from this process has high purity but unsatisfied yield thus it can not be used in commercial scale.

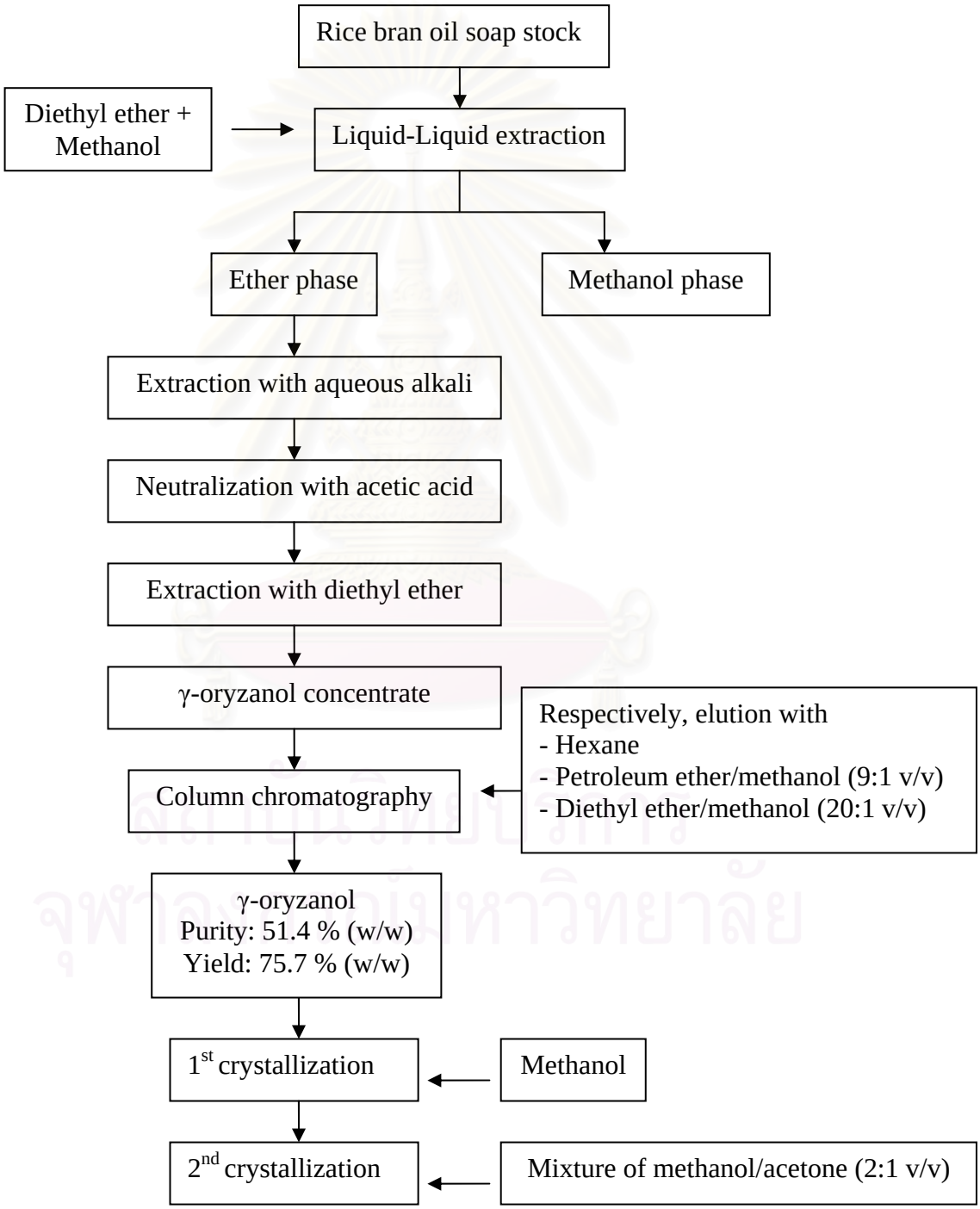


Figure 2.5 Seetharamajah et al.'s process

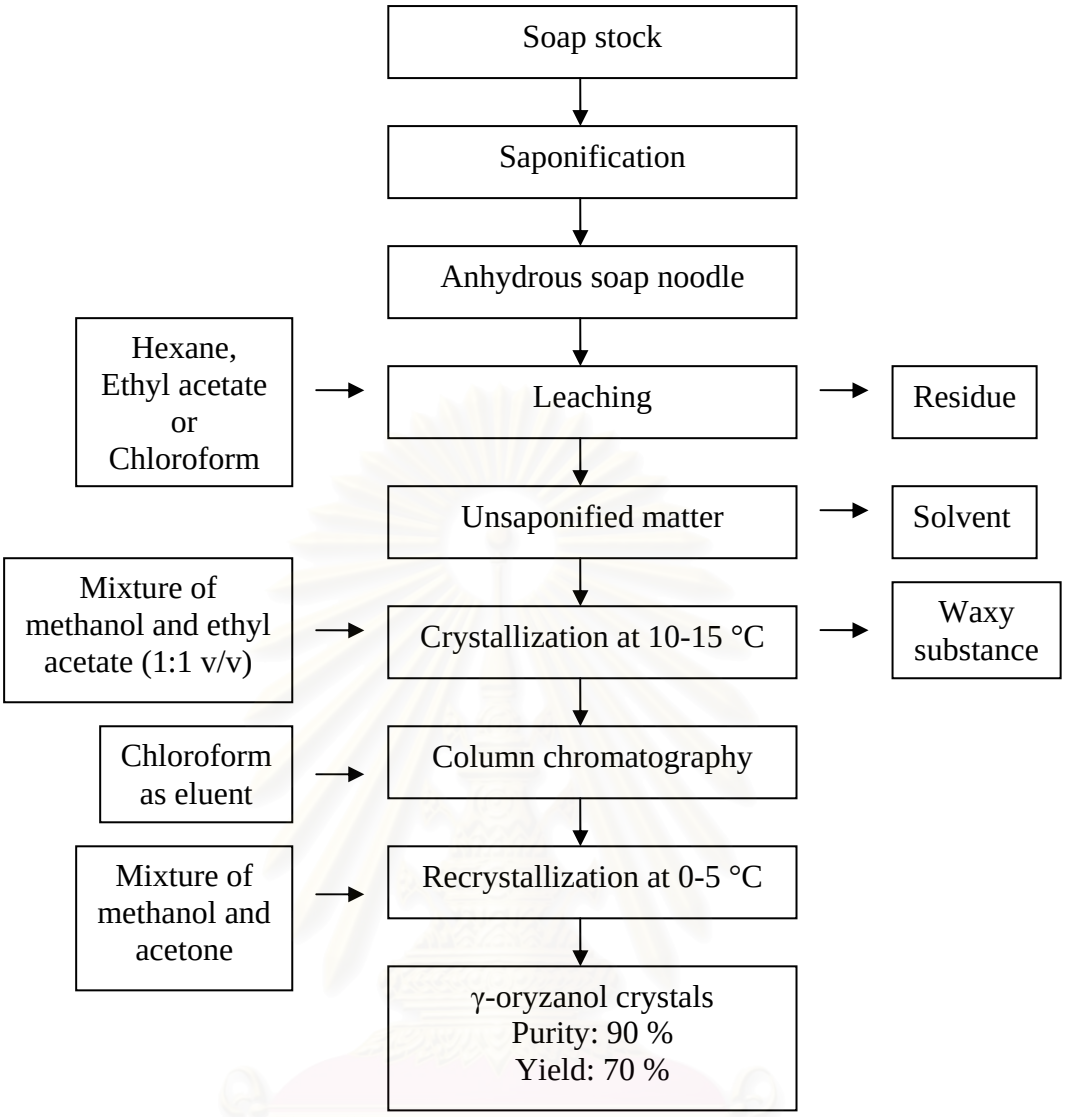


Figure 2.6 Rao et al.'s process

When developing into large scale process, several factors such as productivity, environment and health problems, process investment, and separation efficiency (purity and yield) must be considered [Narayan et al. (2006)]. Processes for isolation of γ-oryzanol have been continuously developed in order to improve the purity and yield. Rao et al. (2002) proposed a method that was easy to handle, store and extract soapstock by this converting the soapstock into soap noodle. After saponification, anhydrous soap noodle was conducted and was then subjected to process as shown in diagram (Figure 2.5). Although this process provided high purity and yield of γ-oryzanol, it has some disadvantages such as requiring tedious pretreatment and extrusion to form noodle, involving a large number of unit operations. Furthermore column chromatography is obstruction for commercial scale.

In 2005, Indira et al. propose an improved technique for saponification, dehydration, and leaching to obtain enriched fraction of γ-oryzanol. Saponification step is achieved by using mild operating condition LTST (low temperature and short time) to avoid degradation of γ-oryzanol. Dehydration was performed by using HTST (high temperature and short time) process such as drum drying. In addition, Indira et al. proposed a technique for improving selectivity during leaching by using pack bed mode. This process is summarized as shown in Figure 2.6. They obtained higher yield and purity of γ-oryzanol than extraction of porous anhydrous noodle soapstock proposed by Rao et al. (purity 30 % and yield 54 %). Related to Indira et al.'s process, Narayan et al. (2004) proposed a simple process for crystallization of γ-oryzanol from oryzanol enriched fraction. This process comprises of nonselective temperature assisted dissolution of oryzanol enriched unsaponifiable fraction in a suitable mixture, fractional precipitation of mucilaginous interfering impurities, and crystallization of oryzanol from supernatant as summarized in Figure 2.7. Due to rather high purity and yield and uncomplexity of both processes, it can be scaled up to commercial process.

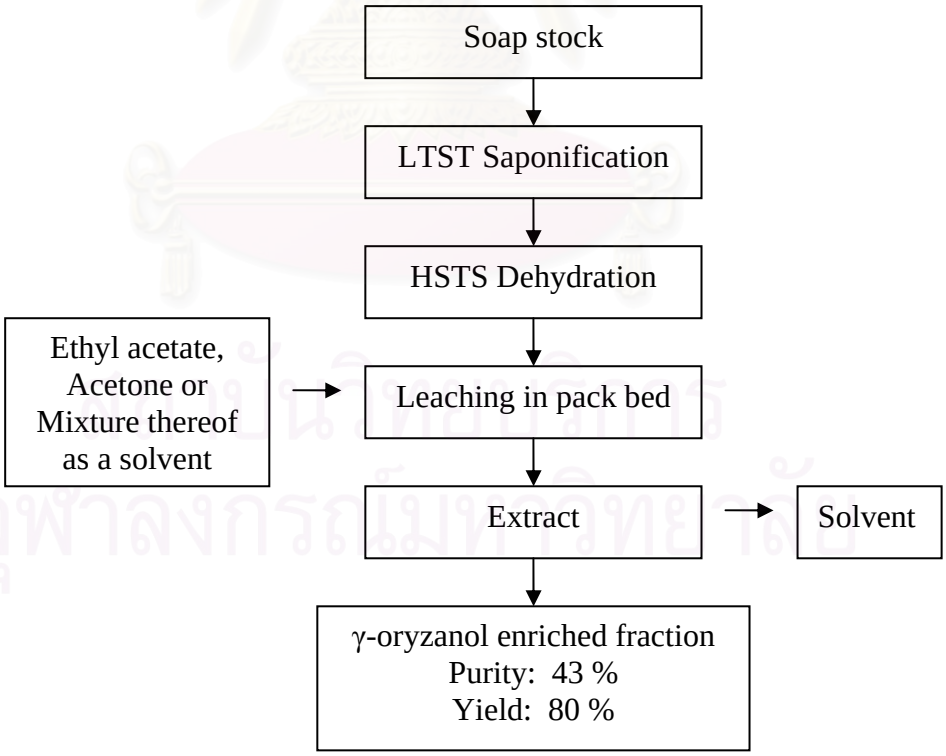


Figure 2.7 Indira et al.'s process

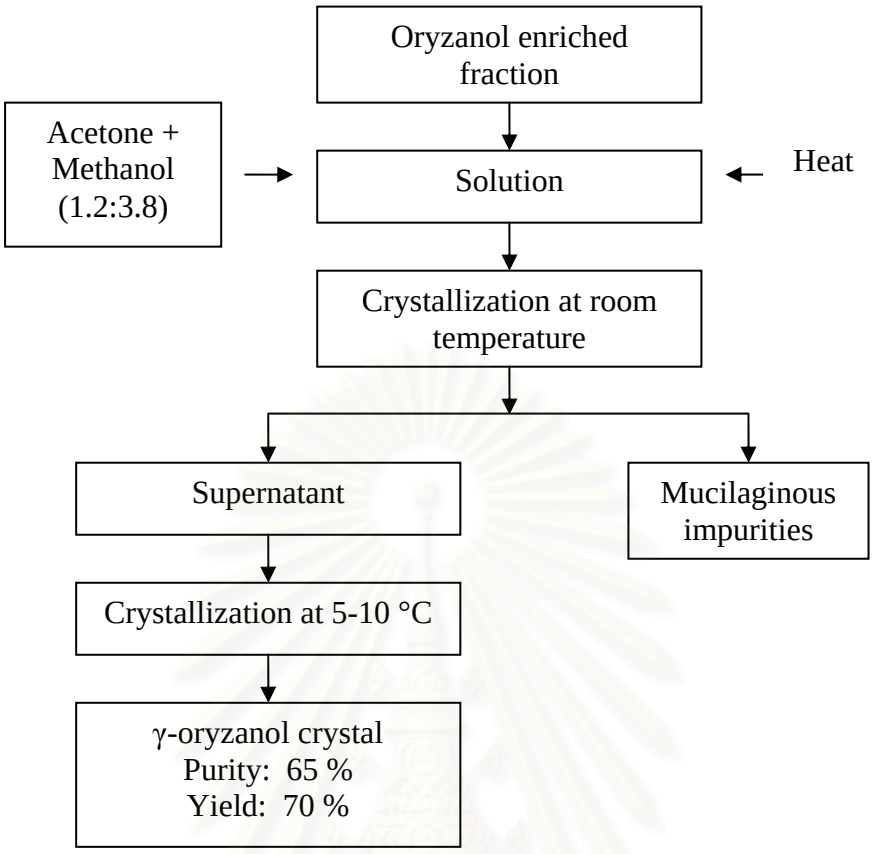


Figure 2.8 Narayan et al.'s process

In rice bran oil industry, soapstock are usually converted into acid oil immediately in order to make it easy to handle and to extend shelf life. Das et al. (1998) proposed to recovery γ-oryzanol from rice bran acid oil. The acid oil was subjected to conventional vacuum distillation for free fatty acid removal to obtain material called pitch which is used as a starting material for the process. The following steps are depicted in Figure 2.8. After purification by charcoal treatment, 96 % purity and 76 % yield of γ-oryzanol was obtained. Although this method obtained high yield and purity of γ-oryzanol, it is predominantly laboratory procedure and therefore was difficult to scale up to commercial process.

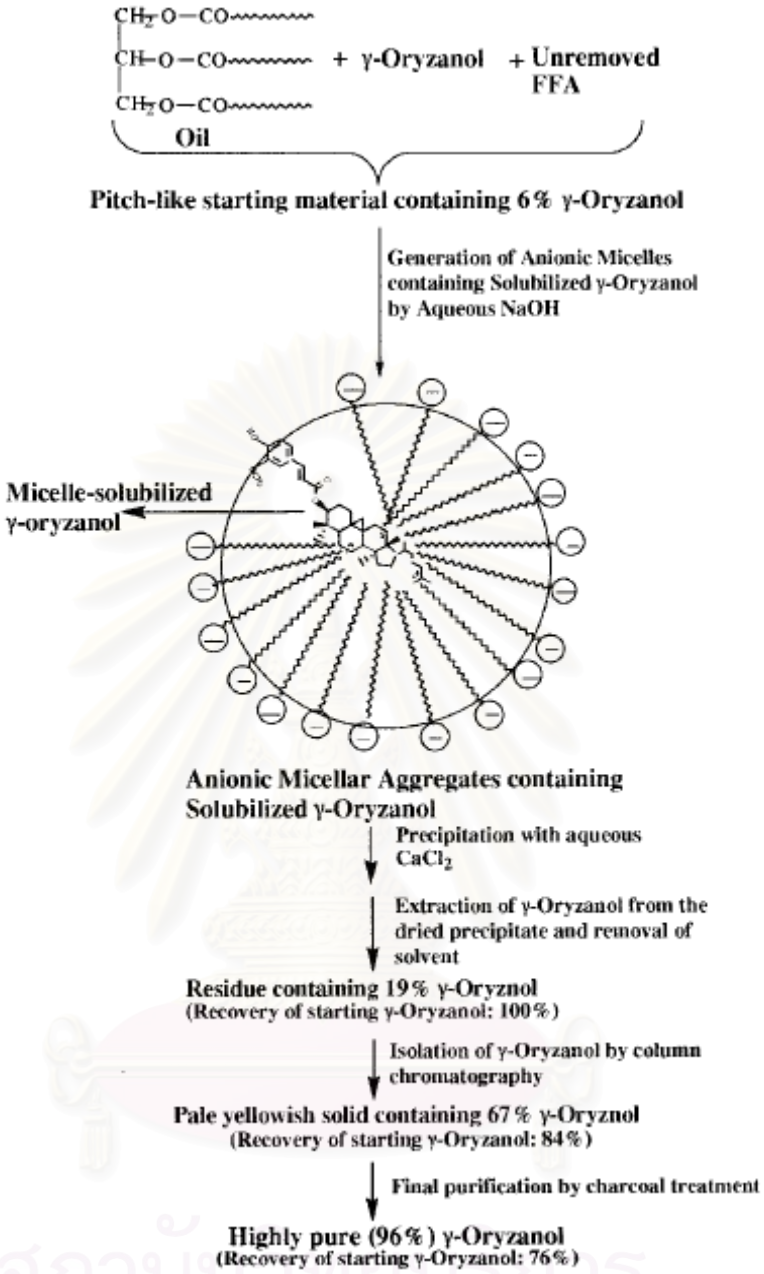


Figure 2.9 Das et al.'s process

From the above review of the γ-oryzanol recovery processes, we focus on those described by Rao et al., Indira et al. and Narayan et al. Those processes have potential application in commercial scale due to their uncomplexity and obtained high yield and purity.

CHAPTER III

MATERIALS & METHODS

3.1 Materials and chemicals

Rice bran oil soapstock was obtained from Thai Edible Oil Co., Ltd., Samutprakarn, Thailand. γ-oryzanol standard was purchased from Wako, Japan. Ethyl acetate, sodium hydroxide and sodium bicarbonate were purchased from APS fine chem, NSW, Australia. Acetone was purchased from VWR, UK. Methanol was purchased from Fisher scienctific, UK, and n-hexane was purchased from Lab scan, Ireland.

3.2 Characterization of soapstock

3.2.1 pH

5 g of soapstock was dissolved in 20 ml water. The mixture was then heated at 40 °C until the soapstock was completely dissolved. The pH of the mixture was measured using a pH meter (Hanna HI9813).

3.2.2 Moisture content

Moisture content of the soapstock was determined by drying the sample to constant weight in a vacuum oven at 105 ± 2 ˚C. The sample was allowed to cool to room temperature in desiccator and weighed.

3.2.3 γ-oryzanol content

The γ-oryzanol contents in the starting soapstock sample and the saponified sample were measured and used as a basis for the calculation of the yield and purity at each step of the separation process. For the determination of the γ-oryzanol content in either the original soapstock or the saponified soapstock, the soapstock sample (original or saponfied) was first dehydrated in oven at 105 ± 2 ˚C, after which 3 g of

the dehydrated soapstock was extracted with 200 ml ethyl acetate for 4 hr in a soxhlet apparatus. The quantification of γ-oryzanol in the extract was carried out using a UV-Visible spectrophotometer. After leaching, the sample residue was extracted with 3 volumes of 50 ml of ethyl acetate under sonication at 40 ˚C. The extracts are combined and analyzed for the amount of γ-oryzanol by a UV-Visible spectrophotometer.

3.2 Separation of γ-oryzanol

The procedures for separation γ-oryzanol from soapstock consisted of three main steps: saponification, leaching, and crystallization as depicted in the Figure 3.1

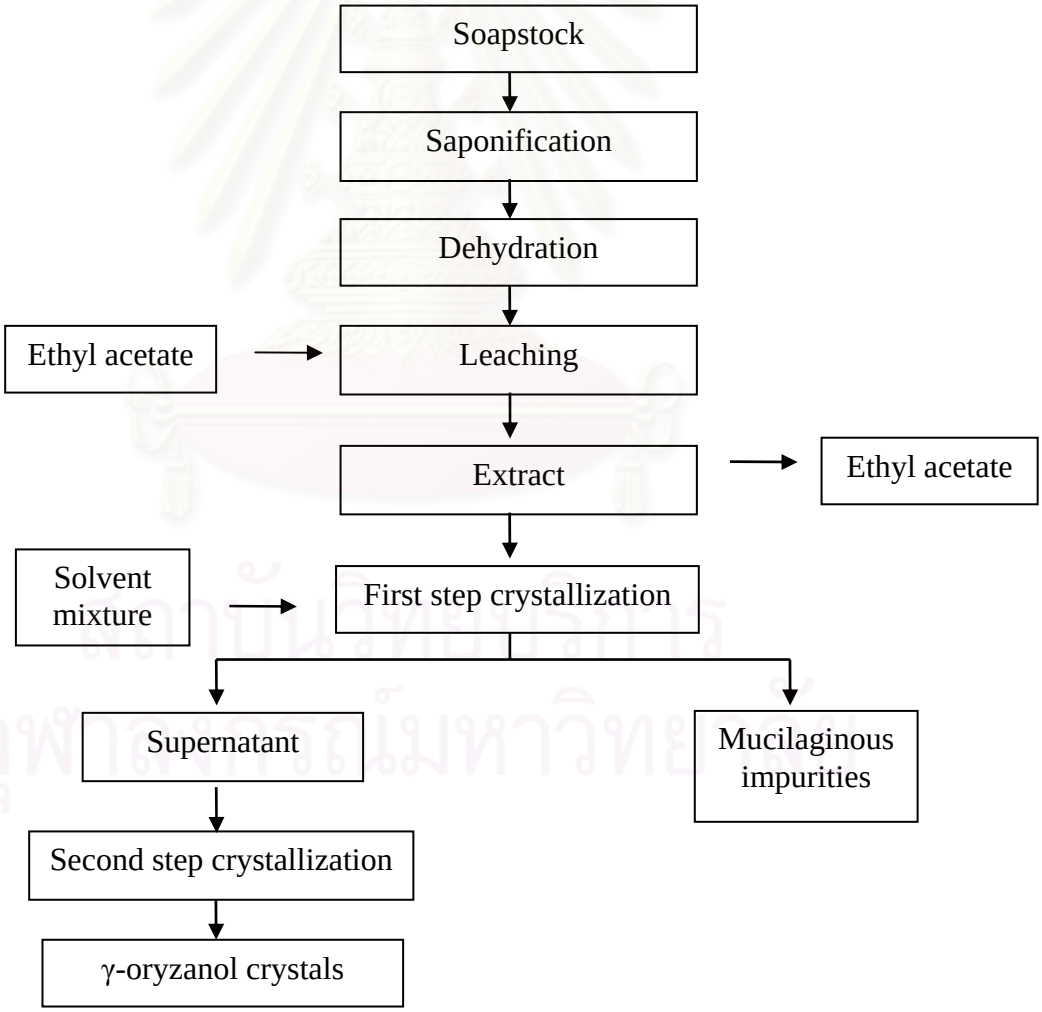


Figure 3.1 Procedures for separation γ-oryzanol from soapstock

3.2.1. Saponification

The purpose of the saponificant step is to completely convert the fatty matter remained in the soapstock sample obtained from industry into soap. In this step, 100 g of soapstock was weighed and transferred into a 600 ml beaker in which the soapstock was saponified by adding NaOH whose exact quantity required for the reaction could be calculated based on the *saponification value* determined using A.O.C.S official method, Cd 3b-76, 1989 (Appendix A). The reaction was carried out at 100 °C and constant stirred over a period of 2 h. After the reaction was completed, the excess alkali was neutralized with sodium bicarbonate and the saponified soapstocks were dehydrated by using vacuum oven at 90 °C for 2-3 h.

3.2.2 Leaching

The dehydrated saponified soapstock was ground, weighed, and transferred into a thimble (33×100 mm), which was then placed in holder of soxhlet extractor. Leaching was then carried out using 100 ml of ethyl acetate. Leaching was carried out over a period of 4 hr using various ratios of sample to ethyl acetate: 3g/200 ml, 6g/200 ml, 10g/200 ml and 15g/200ml to determine the suitable ratio. After the 4 h leaching process, the majority of ethyl acetate was evaporated from extract using a rotary vacuum evaporator (EYELA rotary evaporator N-1000). The remaining ethyl acetate was then dried under nitrogen flow to obtain γ-oryzanol enriched fraction. The γ-oryzanol enriched fraction was then weighed and quantified for the γ-oryzanol content using a UV-Visible spectrophotometer. The suitable ratio was selected such that the maximum extraction yield could be obtained per unit volume of solvent, and this ratio was then used to prepare the γ-oryzanol enriched fractions for subsequent study for the determination of suitable crystallization conditions.

3.2.3. Crystallization

To determine the suitable binary solvent mixture system for crystallization, acetone: methanol (15, 25 and 35 % v/v acetone) or ethyl acetate: methanol (10, 20 and 30 % v/v of ethyl acetate) were examined. About 1 g of γ-oryzanol enriched

fraction obtained from the previous leaching step was added to the 5 ml of the solvent different solvent mixture in a 250 ml flask. The mixture was refluxed at elevated temperature to facilitate the complete dissolution of γ-oryzanol enriched fraction. Then, the temperature of hot solution was gradually lowered to room temperature (25 or 30 °C) and was settled at this temperature for a time period of 1 or 2 h to precipitate the mucilaginous impurities. The clarified supernatant phase was carefully decanted into a 50 ml test tube and was subjected to crystallization at 2-10 °C for 8-24 h in a cooling water bath (EYELA cool ace CA-1111). Yellowish crystal obtained from final crystallization was filtered using a filter paper No.1 and quantified for γ-oryzanol content using a UV-spectrophotometer and HPLC, from which the yield and purity of γ-oryzanol in the final product could be calculated.

In this study, the effects of the key factors that affect the yield and purity of the γ-oryzanol was determined. The ranges of these variables are summarized in Table 3.1.

Table 3.1 Ranges of variables

Variables	Ranges
Leaching	
Soapstock: solvent (ethyl acetate)	3g/200 ml, 6g/200 ml, 10 g/ 200 ml and 15 g/200 ml
First crystallization step	
• Type and compositions of solvent mixtures	• acetone: methanol (15, 25 and 35 % v/v of acetone) • ethyl acetate: methanol (10, 20 and 30 % v/v of ethyl acetate)
• Crystallization temperature	25 and 30 °C
• Crystallization time	1 and 2 hr.
Second crystallization step	
• Crystallization temperature	2, 5 and 10 °C
• Crystallization time	8, 16 and 24 hr

3.3 Quantification of γ-oryzanol

3.3.1 UV-spectrophotometric analysis

For quantification of γ-oryzanol content in the sample, UV-spectrophotometric analysis was used. Absorbance of the sample solution in ethyl acetate was measured at the wavelength of 320 nm in 1-cm cell using UV-2540 UV-Visible spectrophotometer. γ-oryzanol (purity 98 %) was used as a standard.

3.3.2 HPLC analysis

HPLC analysis for the quantification of γ-oryzanol in the sample obtained from each step of separation was carried out with a reverse phase HPLC. The HPLC apparatus consisted of a pump (All tech model 626, USA), equipped with ELSD detector (All tech ELSD 2000ES). The detector condition was set at the tube temperature of 60 °C, nitrogen gas flow of 1.7 L/min and the impactor was off. The analysis was carried out at room temperature on Alltima C18, 250mm × 4.60 mm I.D. column. The mobile phase consisted of methanol:isopropanol (70:30 v/v) and the flow rate was controlled at 1.2 ml/min and 5 μl injection volume. The concentration of γ-oryzanol was obtained by summing all individual components [Chusuwan et al. (2001)].

CHAPTER IV

RESULTS & DISCUSSION

4.1 Physical and chemical properties of rice bran oil soapstock

The rice bran oil soapstock used in this study was provided by Thai Edible Oil Co., Ltd. The soapstock sample was yellow-brown viscous fluid having strong rice odor (Figure 4.1). The major component of soapstock was water and the rest were soap, glycerides, and unsaponifiable matters, which include sterol, higher fatty alcohol, hydrocarbon, vitamin E, and γ-oryzanol [Narayan et al. (2006)]. The composition of different components in the sample would normally depend on various factors such as the rice species, the growing conditions, as well as the conditions employed for the rice bran oil production. In this study, the pH, the water content, as well as the γ-oryzanol content of the soapstock sample were determined and the results are summarized in Table 4.1. The moisture content of the soapstock sample used in this study was found to be approximately 56.96 wt %, thus the other 43.04 wt % was therefore soap, glycerides, and unsaponifiable matters. Of particular interest, the γ-oryzanol content in the soapstock was measured to be approximately 4.9 wt % based on the wet basis, or 7.7 wt % based on the dry basis, while the literature values vary from 1.5-6.5 wt % on the wet basis [Rao et al. (2002), Indira et al. (2005), and Narayan et al. (2006)].

Figure 4.1 Rice bran oil soapstock sample

Table 4.1 Physical and chemical properties of rice bran oil soapstock

Physical and Chemical properties	
pH	10.5
Moisture content[a]	56.7 % wt
Saponification value[b]	24 mg NaOH/g soapstock
γ-oryzanol content[c]	4.9 % wt wet basis 7.7 % wt dry basis
γ-oryzanol content in saponified and dehydrated soapstock[d]	5.83 % wt dry basis

a see appendix B-1 b see appendix B-2

c see appendix B-4 d see appendix B-5

For the separation of γ-oryzanol from soapstock, water was first eliminated to reduce the operational scale of the system. The resulted partially dried sample contains some amount of glycerides, which if remained in the sample, would be extracted into ethyl acetate along with γ-oryzanol, making the subsequent separation step difficult. For this reason, prior to the leaching of γ-oryzanol, the remaining glycerides need to be converted into insoluble soap via saponification [Narayan et al. (2006)]. The suitable quantity of sodium hydroxide was estimated based on the saponification value which could be determined using a standard method (AOCS Cd 3b-76, 1989). Based on this value, approximately 24 mg NaOH/g soapstock or 2.4 % wt of soapstock was required for the saponification process. The high performance liquid chromatograpy (HPLC) analysis of the extract showed that the soapstock that was saponified with 2.4 wt % of NaOH no longer contained glycerides as shown in Figure 4.2 and 4.3, indicating that the complete conversion of triglyceride could be achieved. Subsequently, the saponified soapstock was dehydrated and extracted with ethyl acetate in a soxhlet apparatus.

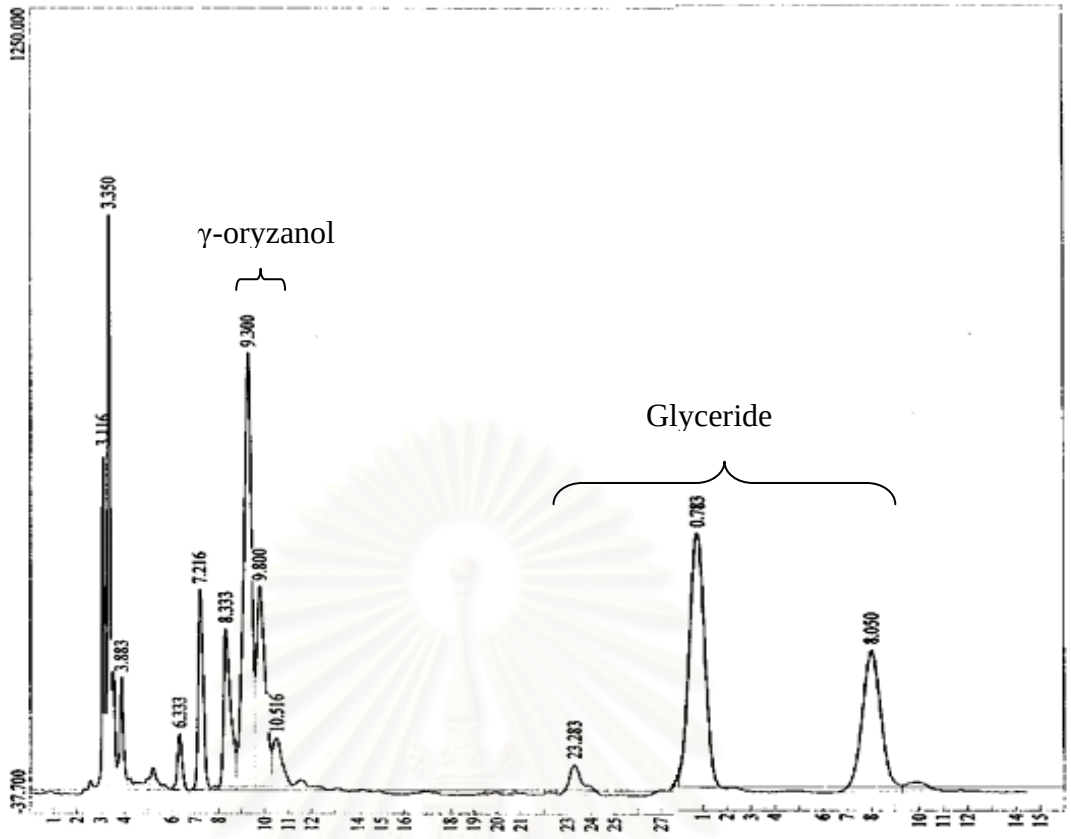


Figure 4.2 Chromatogram dehydrated soapstock extract obtained by leaching with ethyl acetate

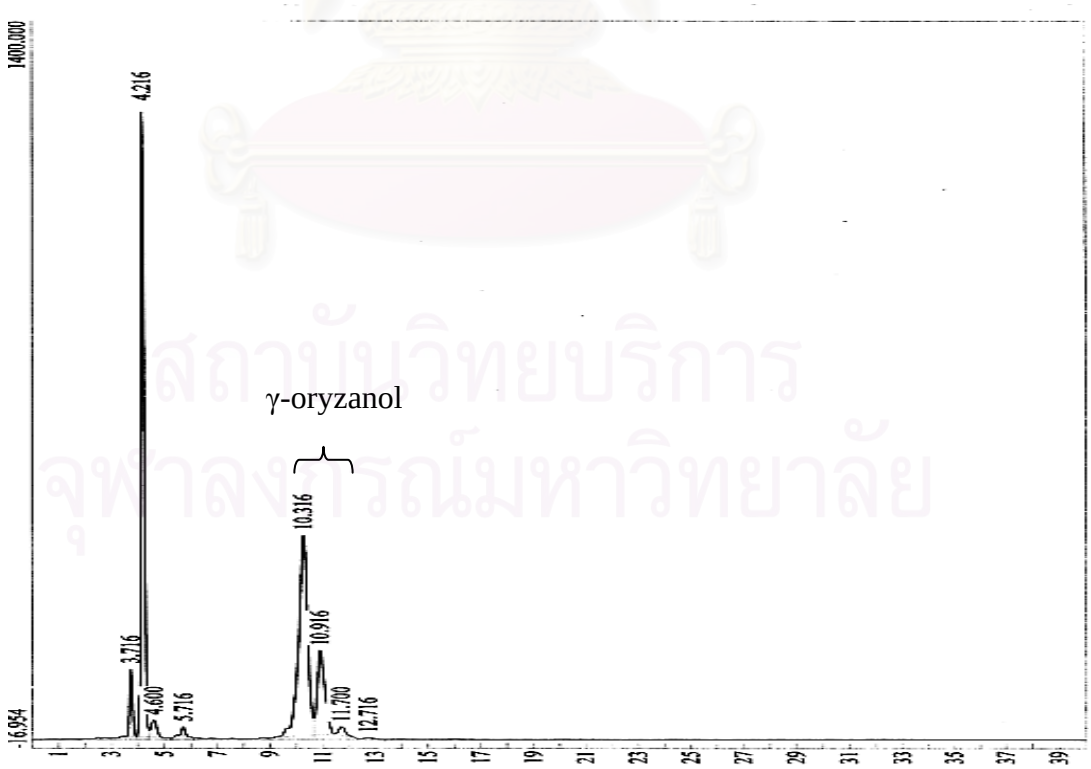


Figure 4.3 Chromatogram dehydrated saponified soapstock extract obtained by leaching with ethyl acetate

After saponification, the γ-oryzanol in the dehydrated saponified soapstock was measured and was found to decrease by about 40.5 % from 7.7 % wt γ-oryzanol in the original sample to 5.83 % dry weight in the saponified sample (Table 4.1). The calculation for the 40.5 % perctent of γ-oryzanol loss can be found in Appendix B-3. This loss of γ-oryzanol could be due to the fact that, even though γ-oryzanol is often classified as an unsaponifiable matter, the compound could undergo saponification and could be converted into ferulic acid and sterols [Krishna et al. (2003)]. Moreover, some γ-oryzanol loss could occur during the transfer of viscous material in the saponification process.

Based on these results, in preparation of the soapstock for the subsequent experiment to determine the suitable separation process conditions, the soapstock was first saponified with NaOH 2.4 wt. %. The reaction product was then dehydrated and ground into fine particles. The amount of γ-oryzanol contained in the dried saponified material is taken to be 100% and the yield and purity of the γ-oryzanol obtained during each separation step would be determined based on this value.

4.2 Effect of dehydrated saponified soapstock to ethyl acetate ratio in leaching step

In the leaching step, γ-oryzanol was extracted from the dehydrated saponified soapstock with ethyl acetate in a soxhlet apparatus and the effect of the raw material to solvent ratio was investigated. Figure 4.4, shows that the purities and yields of γ-oryzanol in the extracts obtained with various sample to solvent ratios (3g/200ml, 6g/200ml, 10g/200 ml and 15 g/200 ml) were not significantly different. The results indicated that soxhlet extraction was an effective method for leaching of γ-oryzanol from the dehydrated saponified soapstock as a portion of fresh solvent was repeatedly brought into contact with the sample, thereby mass transfer was enhanced. In addition, the high temperature of the system (at the boiling temperature of the solvent) could increase the extractability. Thus as high as 97-98 % yield was achieved within the extraction time of 4 h.

The purity of γ-oryzanol or γ-oryzanol content in extract was determined spectrophotometrically to be about 30-40 % wt, which was much higher than the original content in the saponified soapstock raw material (about 5.83 % dry wt). Since γ-oryzanol was the main component being removed by the solvent in leaching step,

the leaching step thus allowed the compound to be concentrated. However, other undesirable compounds were extracted along with γ-oryzanol and the extraction with high ratio of ethyl acetate (i.e., 3g/200) was found to give slightly lower γ-oryzanol content than the extraction carried out with lower ratio of the solvent (15g/200 ml).

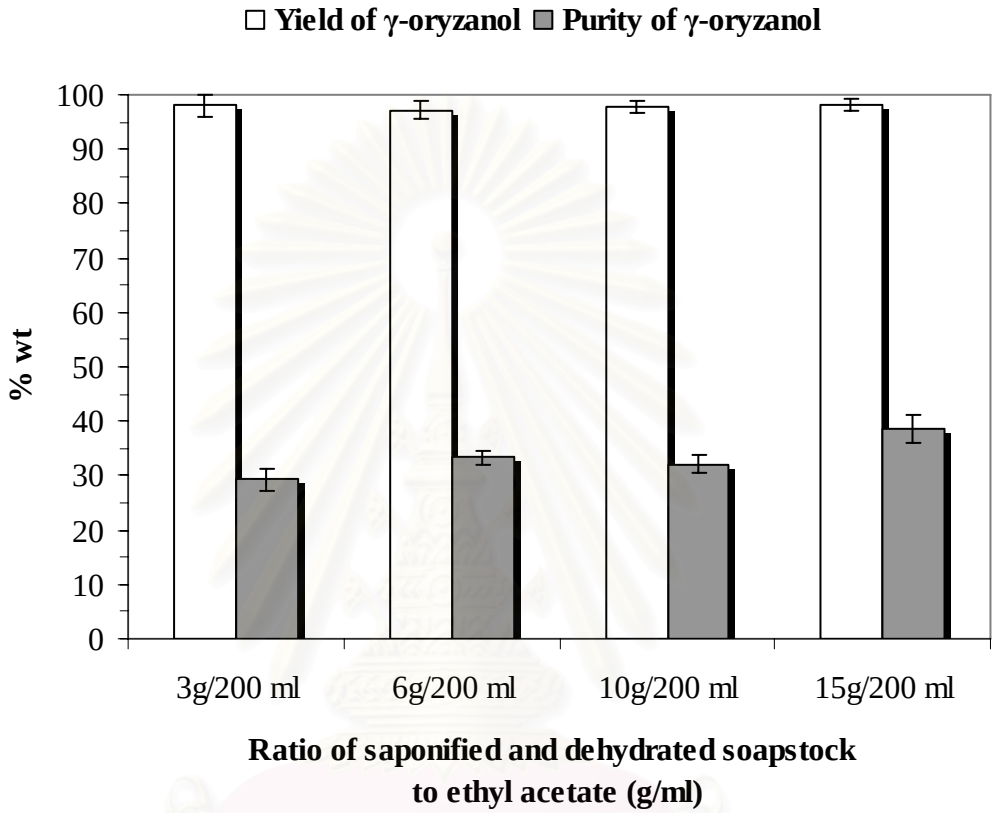


Figure 4.4 Percent purity and yield of γ-oryzanol in extracts at various samples to ethyl acetate ratio.

Based on these results, the suitable ratio of saponified and dehydrated soapstock to ethyl acetate for leaching was 15g/200 ml and this ratio was used for the preparation of the γ-oryzanol enriched fraction for the subsequent study on the crystallization process.

4.3 Effect of variables for crystallization step on yield and purity of γ -oryzanol

From the previous step, after solvent evaporation, γ-oryzanol enriched fraction was obtained (Figure 4.5), which was then purified further though two-step crystallization. The first crystallization step was to precipitate the mucilaginous

impurities including waxes and gums from the extract mixture. The supernatant was crystallized again in the second step to obtain γ-oryzanol crystals with increased purity.

In first crystallization step, the γ-oryzanol enriched fraction was dissolved in the crystallization solvent under reflux to ensure complete solubilization. The temperature of the solution was then reduced from the reflux temperature to 25-30 °C, to allow the precipitation of the mucilaginous impurities that would otherwise, by disrupting the crystal growth, interfere with the crystallization of γ-oryzanol in the second step. At this first step however, some γ-oryzanol also crystallized and was lost during the process. For this reason, it was therefore crucial to determine the suitable polarity of crystallization solvent to reduce γ-oryzanol loss, and to obtain the product with high yield and purity.

Figure 4.5 Dehydrated saponified soapstock

Crystallization is based on the principles of solubility; compounds (solutes) tend to be more soluble in hot liquids (solvents) than they are in cold liquids. If a saturated hot solution is allowed to cool, the solute is no longer soluble in the solvent and forms the crystals. The solvent systems for crystallization should be such that the solubility could be adjusted during the process either by changing temperature or by adjusting the composition of the solvent mixture, as in the crystallization with anti-solvent. In this study, the means which the solubility was adjusted was by using temperature reduction. Two systems of solvent mixture at different composition were selected for the crystallization study. These are acetone: methanol in the range of 15-35 % v/v of acetone and ethyl acetate: methanol in the range of 10-30 % v/v of ethyl acetate. The reason for choosing such systems is that γ-oryzanol is generally soluble

in acetone or ethyl acetate at room temperature but is nearly insoluble in methanol. The range of the compositions investigated was selected such that the solubility of γ-oryzanol in resulted mixture could be adjusted by controlling the crystallization temperature. Table 4.2 shows the dielectric constants of the crystallization solvent and the solvent mixtures used in this study. These values of dielectric constants lie in the range between 24 and 31, which covered the suitable range of polarity of crystallization suggested by Narayan et al. (2004), who reported the mixture of acetone: methanol at the ratio in the ranges between 20 % to 25 % v/v of acetone to be most suitable, and whose dielectric constants lie in the range of 29.6-30.2.

Table 4.2 Polarity of solvent or solvent mixtures for crystallization step

Solvent	Polarity (dielectric constant)
Methanol	32.6
Ethyl acetate	6
Acetone	20.7
10 % v/v of Ethyl acetate	29.94
20 % v/v of Ethyl acetate	27.28
30 % v/v of Ethyl acetate	24.62
15 % v/v of Acetone	30.815
25 % v/v of Acetone	29.625
35 % v/v of Acetone	28.435

4.3.1 Effect of solvent mixture composition on first step crystallization

The effect of different composition of the solvent mixtures on the yield (the percentage of the amount of γ-oryzanol in the supernatant to the amount of γ-oryzanol in the dehydrated saponified soapstock) and the percentage of γ-oryzanol loss (the percentage of the amount of γ-oryzanol precipitated with the mucilaginous impurities during the first crystallization step) are shown in Figure 4.6. The results were determined based on the UV-spectroscopic analysis of the samples. As seen from the figure, the γ-oryzanol loss decreased with increasing percent of acetone or ethyl acetate and the yield of γ-oryzanol in the supernatant increased with increasing

percent of acetone or ethyl acetate. The highest γ-oryzanol loss was obtained at 15 % of acetone and 10 % of ethyl acetate due to the poor solubility of γ-oryzanol in these solvent mixtures, causing it to precipitate with the impurities when the temperature was reduced from reflux temperature (about 80 °C) to 25 °C. At higher percentages of acetone and ethyl acetate such as in the 35 % of acetone and 30 % of ethyl acetate mixtures with methanol, the solubility of γ-oryzanol was high, and thus the γ-oryzanol loss was smaller as it would still remain soluble in the supernatant. As the yield is related to the γ-oryzanol loss, the result of γ-oryzanol yield after the first crystallization step then would proceed in the opposite direction. In other words, the percentage of γ-oryzanol in the supernatant should be the lowest when the percentages of acetone or ethyl acetate in the methanol were low, where the γ-oryzanol was the smallest.

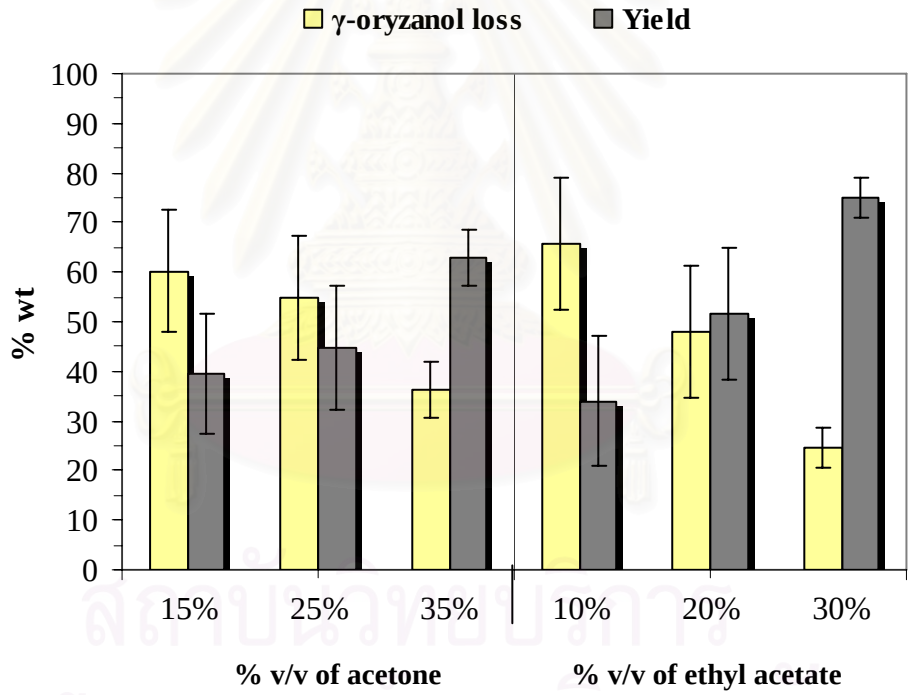
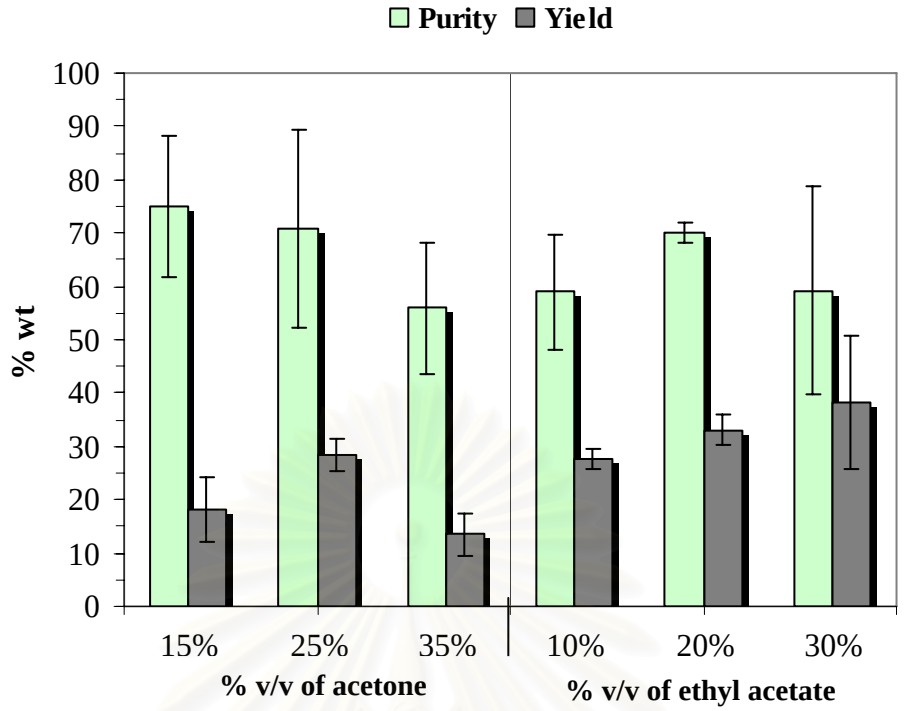
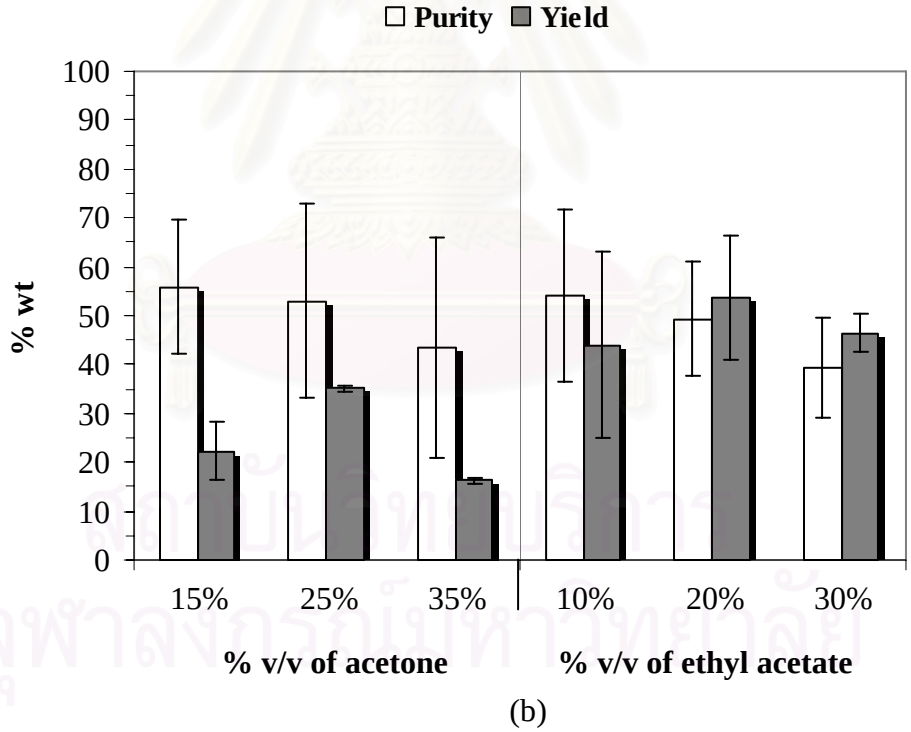


Figure 4.6 Percent γ-oryzanol loss and yield of γ-oryzanol after first crystallization step at 25 °C, for 1 h by using solvent mixtures of various ratios; UV-Visible spectrophotometrically measurement was employed for γ-oryzanol analysis

After mucilaginous impurities were separated, the supernatant was then allowed to crystallize again in the second step at 5±1 °C for 16 h and the yield and the purification of the γ-oryzanol crystals were determined.



(a)



(b)

Figure 4.7 Percent purity and yield of γ-oryzanol after second crystallization step at temperature 5±1 °C for 16 h.; (a) analyzed by UV-Visible spectrophotometer and (b) analyzed by HPLC

The quantification of γ-oryzanol yield and purity of the crystallized samples obtained in the second step was carried out both by using UV-visible spectrophotometer and HPLC. The results are shown in Figure 4.7 (a) and 4.7 (b).

For UV-visible spectrophotometric analysis (Figure 4.7 (a)) of the crystallization system of acetone: methanol solvent mixture system, it can be observed that at 15 % v/v of acetone, although high purity of γ-oryzanol could be obtained, low yield was resulted as a large amount of γ-oryzanol was lost with the impurities in the first crystallization step. At 35 % v/v of acetone, both the yield and the purity were low due to the high solubility of γ-oryzanol in this solvent mixture, thus causing poor crystallization. Moreover, the mucilaginous impurities retained in supernatant after crystallization step 1 also crystallized in this step when temperature was reduced to 5±1 °C and resulted low purity. The mixture of 25 % v/v of acetone in methanol was found to be the most suitable. This result is consistent with that reported by Narayan et al. (2004).

Other than acetone: methanol mixture, Rao et al. (2002) reported the use of ethyl acetate: methanol (50 % v/v of ethyl acetate) as a solvent mixture for removal of mucilaginous impurities from γ-oryzanol extract at 10-15 °C, however, the investigation of the system of ethyl acetate:methanol for γ-oryzanol crystallization has never been reported. Such system is investigated here and the yield of γ-oryzanol after the second crystallization step was found to increase with increasing percentage of ethyl acetate. The highest purity obtained from this system was found for the solvent mixture with 20 % of ethyl acetate and this mixture was found to be the most suitable solvent mixture. Since at 20 % v/v of ethyl acetate, the yield of γ-oryzanol was slightly higher than that obtained at 25 % of acetone which was the most suitable solvent mixture for acetone:methanol system while purity was comparable, we selected this solvent (20 % v/v of ethyl acetate) as the suitable solvent mixture. Nevertheless, due to the large uncertainty in the experimental results, the selection of the most suitable condition based on the spectroscopic analysis alone is not straightforward, therefore the HPLC analysis was employed and the results were compared.

The yield and purity of the crystallized products analyzed based on the HPLC analysis is shown in Figure 4.7 (b). For acetone: methanol solvent mixture system, it can be observed that the purity slightly decreased with increasing percentage of acetone while the highest yield was found at 25 % of acetone. This mixture was also

found to be the most solvent mixture based on the UV-spectrophotometer results. For ethyl acetate: methanol system, the HPLC purity was slightly decreased with increasing percentage of ethyl acetate while the highest yield was found at 20 % of ethyl acetate. Considering the yield and purity of γ-oryzanol achieved, 20 % of ethyl acetate was the most suitable for this system. Compared with acetone: methanol system at 25 % of acetone, the HPLC analysis indicated that, the purity of γ-oryzanol obtained by 20 % of ethyl acetate (ethyl acetate: methanol mixture) showed no significant difference, while the yield was higher. Therefore, 20 % v/v of ethyl acetate) was considered the suitable solvent mixture.

It is now worth to compare the results determined based on UV-spectroscopic analysis and HPLC analysis, which show similar behavior for the yield and purity for the γ-oryzanol crystallization in acetone: methanol solvent mixture. That is, the purity was decreased with increasing percentage of acetone while the highest yield was obtained at the same proportion, 25 % of acetone. Unlike acetone: methanol system, rather different behaviors were observed for the yield and purity of γ-oryzanol in ethyl acetate: methanol system. For the UV-visible spectrophotometric analysis, the purity was the highest at 20 % ethyl acetate and the yield increased with increasing percentage of ethyl acetate, while based on the HPLC analysis, the purity was found to decrease with increasing percentage of ethyl acetate and the highest yield was found at 20 % of ethyl acetate. Due to the higher accuracy of the technique compared with the UV-spectrophotometric analysis, HPLC analysis would often be recommended, and the results here suggested that the mixture of 20 % of ethyl acetate in methanol was the most suitable. One of the other advantages of using ethyl acetate: methanol system is that ethyl acetate was used in the previous leaching and crystallization processes, which make it solvent handling simple if fewer solvents are used. Moreover, ethyl acetate is widely used in food and pharmaceutical processing.

4.3.2 Effect of first step crystallization temperature on yield and purity of γ-oryzanol

The effect of temperature for the first crystallization was studied by using 20 % v/v of ethyl acetate as a crystallization solvent mixture and the crystallization time of 1 h. The temperatures in which the process was compared were 25 and 30 °C. The

Lower temperature was not considered since γ-oryzanol could crystallize along with the mucilaginous impurities, causing a large γ-oryzanol loss.

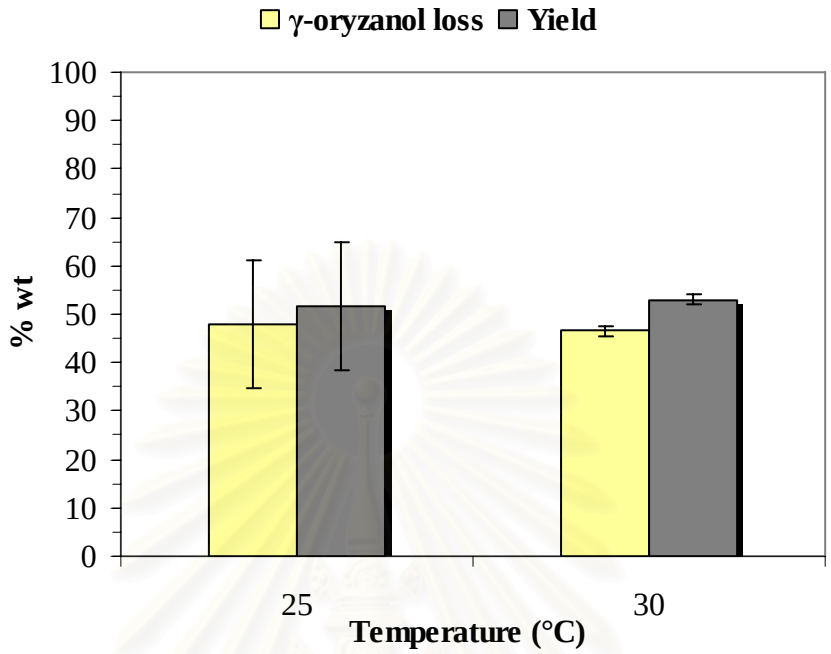
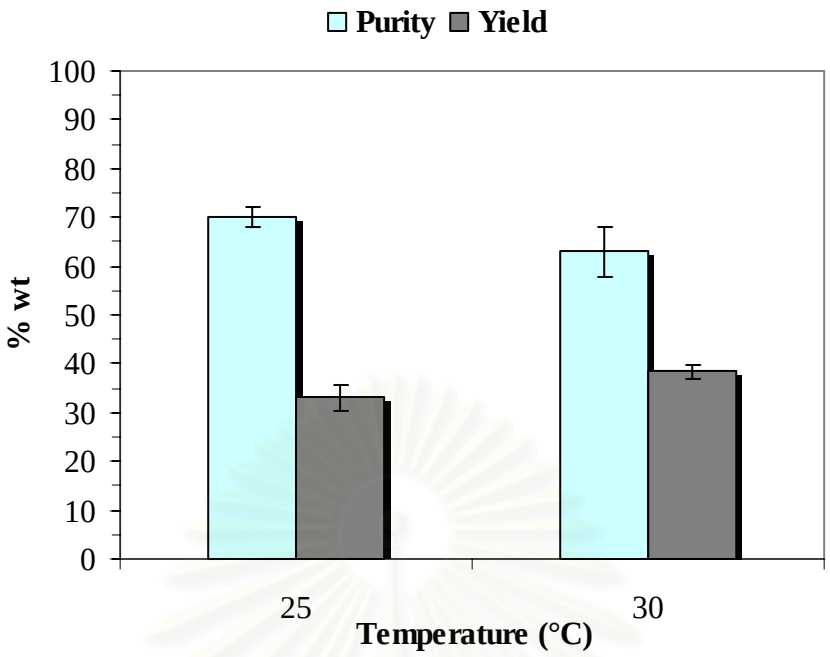


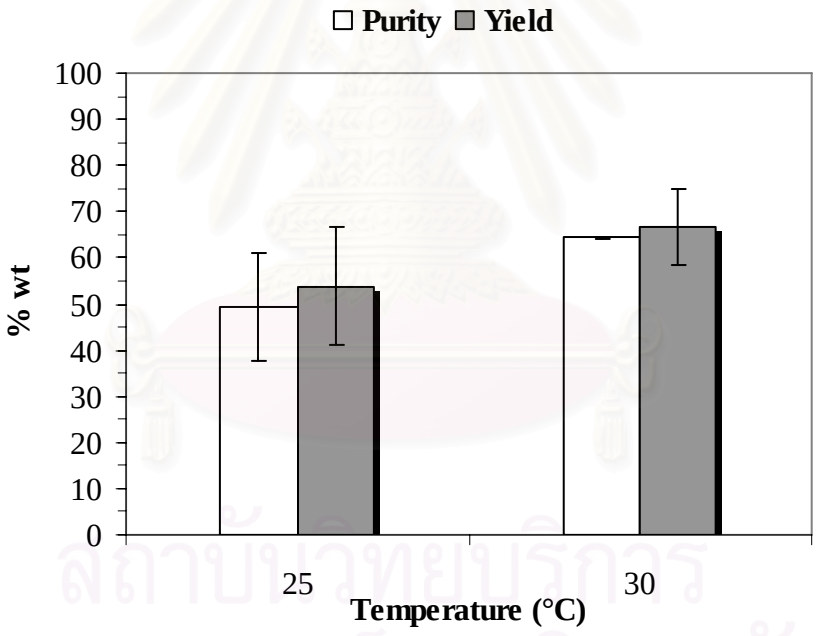
Figure 4.8 Percent oryzanol loss and yield of γ-oryzanol after first crystallization step using 20 % ethyl acetate in methanol for1 h at 25 and 30 °C, analyzed by UV-visible spectrophotometer

The result in Figure 4.8 shows the two temperatures gave no significant difference in the percentage of γ-oryzanol loss (25 °C: 47.98 % and 30 °C: 46.55 %) and the percentage of γ-oryzanol in the supernatant after the first crystallization step (25 °C: 51.62 % and 30 °C: 53.04 %).

When the supernatants taken from the first step, both at 25 and 30 °C, were further crystallized in the second step at 5±1 °C for 16 h, the resulting crystal obtained from this step was quantified γ-oryzanol by using both UV-visible spectrophotometer and HPLC for the calculation of yield and purity (Figure 4.9 (a) and 4.9 (b)). The results obtained by both methods of analysis showed no significant difference in the yields and purities obtained at different temperature. Therefore, the suitable first step crystallization time was 25 °C and this condition was used for subsequent study.



(a)



(b)

Figure 4.9 Percent purity and yield of γ-oryzanol after second crystallization step at 5±1 °C for 16 h; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC

It should be noted from the results in Figure 4.9 (a) and 4.9 (b) that the yields quantified by HPLC were higher than that obtained by UV-visible spectrophotometer. The difference in the yields determined by the two methods of analysis was due to the

different accuracies of these methods. This was because in a rather purified sample, UV-spectrophotometer could be accurately used for the quantification of the compound of interest. This is also seen in the comparable results of the purity obtained from both analytical methods shown in Figure 4.9 (a) and 4.9 (b). However, when considering the content of γ-oryzanol in the starting saponified soapstock, which contained several impurities, the results of γ-oryzanol contents determined by the two methods were different: 5.83 wt % by UV-Spectrophotometer and 3.46 wt % by HPLC, respectively. The yields which were calculated from the ratio of the content of γ-oryzanol in crystallized product to that in the starting saponified soapstock, were therefore higher for that determined by HPLC analysis, which were thus more accurate.

4.3.3 Effect of first step crystallization time on yield and purity of γ-oryzanol

The effect of the first step crystallization time (1 and 2 h) was investigated for crystallization system of 20 % v/v of ethyl acetate in methanol at 30 °C. The results in Figure 4.10 show that no significantly difference in γ-oryzanol loss (1h: 46.55 %, 2 h: 44.12 %) and yield (1 h: 53.04 %, 2 h: 55.45 %).

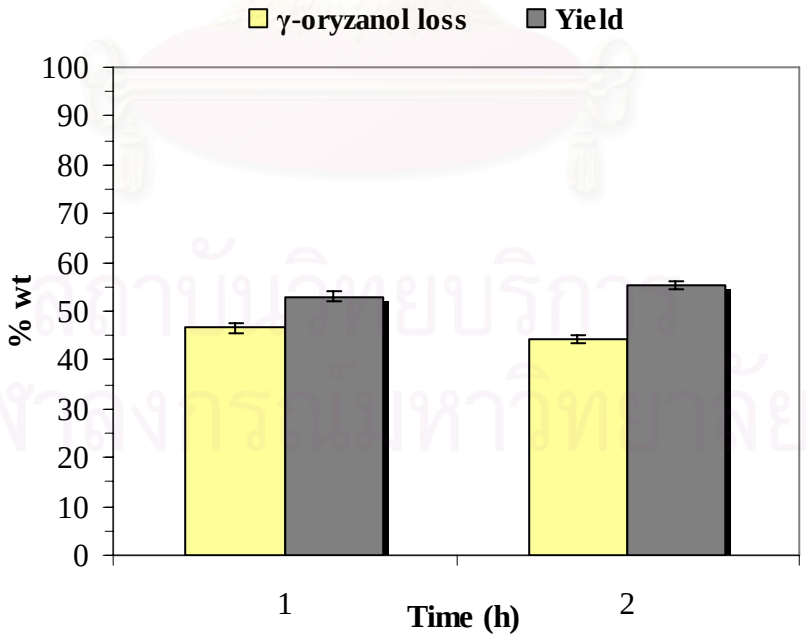


Figure 4.10 Percent of γ-oryzanol loss and yield of γ-oryzanol after first crystallization step using 20 % ethyl aceate in methanol at 30 °C for 1 and 2 h, analyzed by UV-visible spectrophotometer

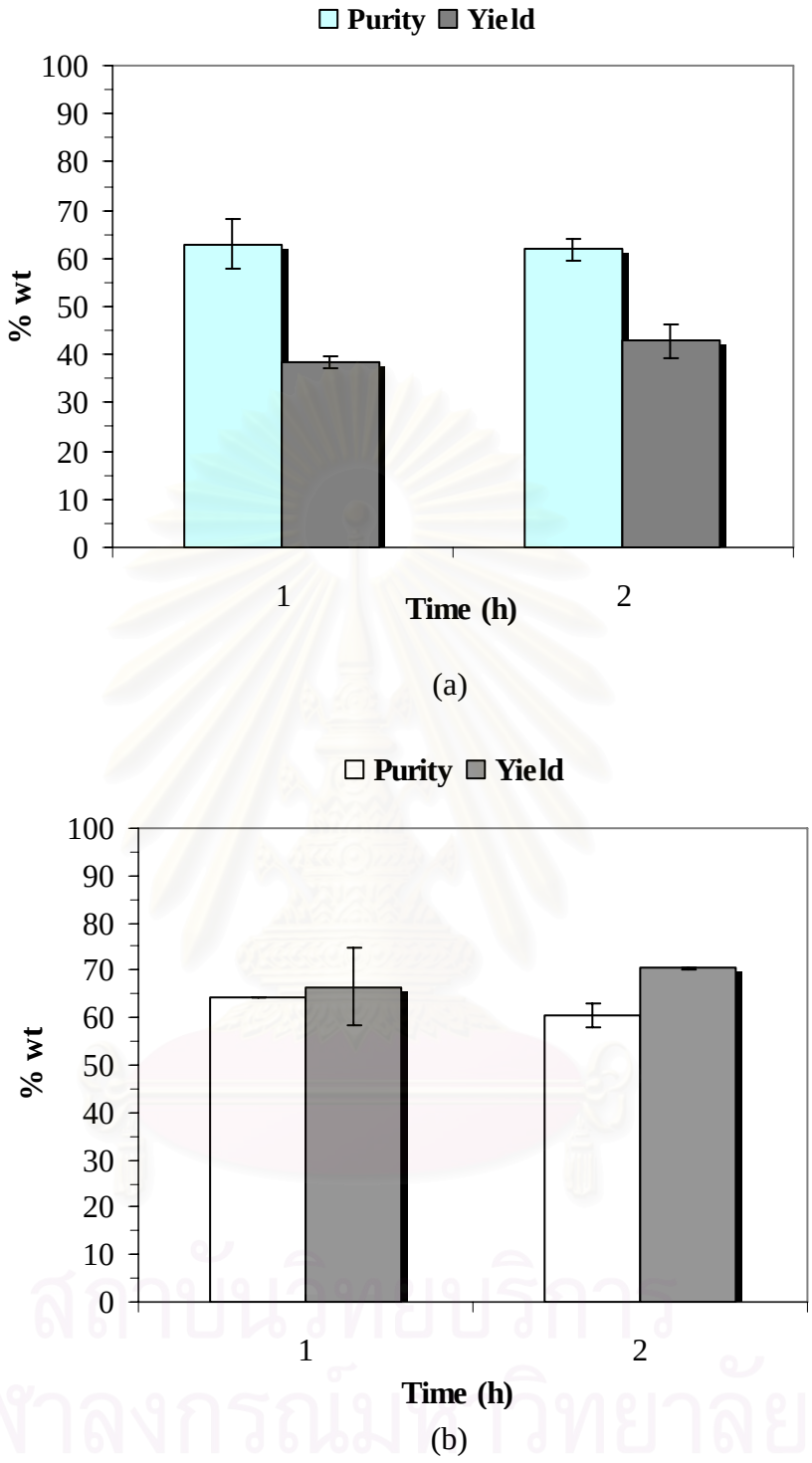


Figure 4.11 Percent purity and yield of γ-oryzanol after second crystallization step at 5±1 °C for 16 h.; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC

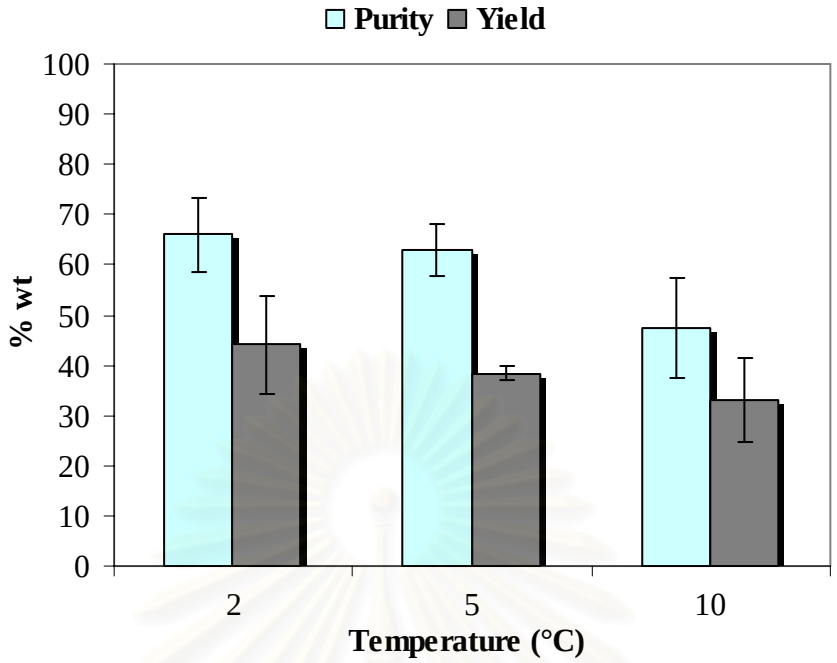
The supernatant from the first step was then separated and allowed to crystallize in the second step at 5±1 °C for 16 h, the purity and yield of γ-oryzanol are

shown in Figure 4.11 (a) and 4.11 (b) obtained by UV-visible spectrophotometric and HPLC analysis, respectively. The results from these Figures indicated that crystallization time in the range of this study again did not affect the yield and purity of γ-oryzanol. Therefore, crystallization time for 1 h was sufficient to separate mucilaginous impurities and this was used for the subsequent study for the second crystallization step.

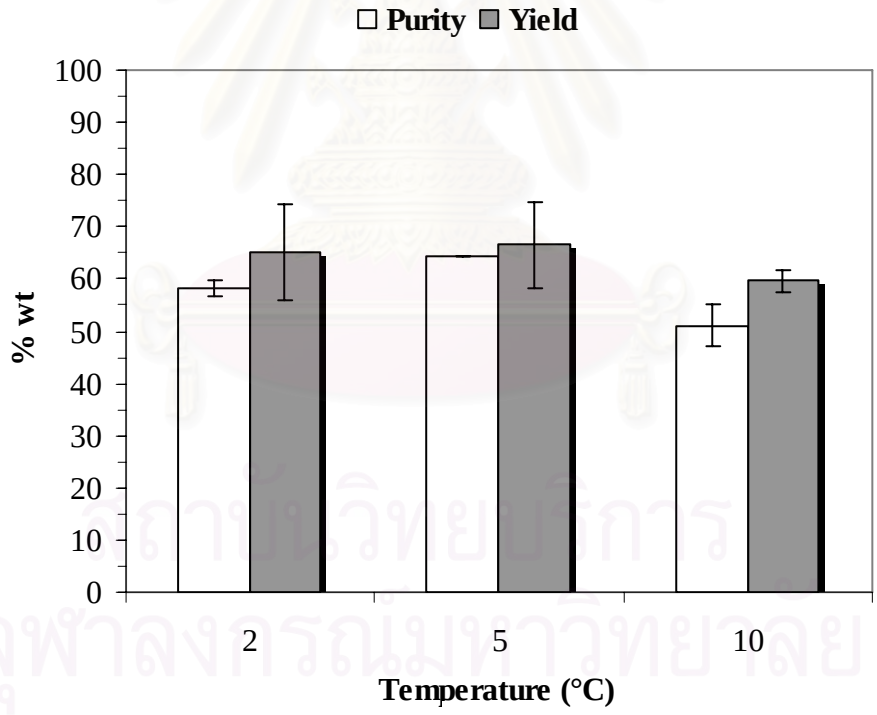
The results here again showed that the yield obtained by HPLC analysis was higher than that obtained by UV-visible spectrophotometric analysis, while the purities were comparable. Nevertheless, both cases gave the same conclusion about no significant effect of the first step crystallization tiem between 1 and 2 h.

4.3.4 Effect of second step crystallization temperature on yield and purity of γ-oryzanol

The effect of the second step crystallization temperature was determined by using the suitable condition previously determined, that is, 20 % v/v of ethyl acetate in methanol at 30 °C and 1 h of crystallization time were used for the first step. For the second crystallization step, the temperatures of 2, 5 and 10 °C were examined in which the crystallization was allowed to take place for 16 h in the cooling water bath. The result in Figure 4.12 (a) analyzed by UV-visible spectrophotometer shows that the yield of γ-oryzanol slightly decreased with increasing temperature (2 °C: 44.07%, 5 °C: 38.43 % and 10 °C: 33.04 %). The purity obtained at 2 and 5 °C were not significantly different (2 °C: 65.97 % and 5 °C: 62.96 %) but at 10°C, the purity was found to be lower (47.47 %). These results were also consistent with that obtained from HPLC analysis (Figure 4.12 (b)) in which the yield was 65.25 %, 66.57 % and 59.58 % and the purity was 58.26, 64.31 and 51.12 % at 2, 5 and 10 °C, respectively. From these results 5 °C was considered the suitable temperature which gave no different results compared with those achieved at 2 °C. Thus, the temperature of 5 °C was therefore used for the subsequent experiment to determine the suitable time for the second step crystallization.



(a)



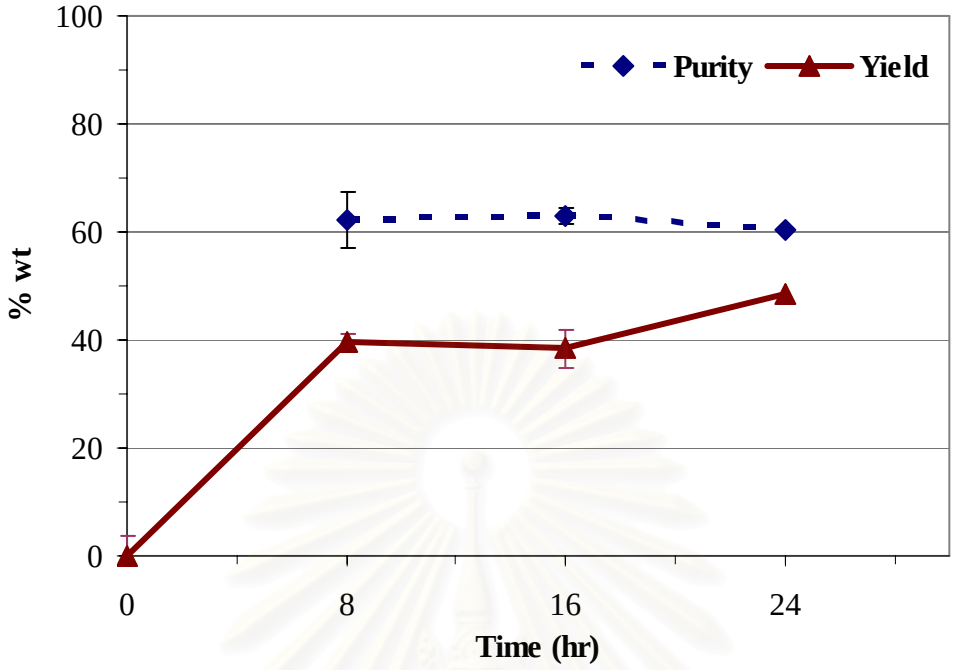
(b)

Figure 4.12 Percent purity and yield of γ-oryzanol after second crystallization step after 16 h at 2±1, 5±1 and 10±1°C; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC

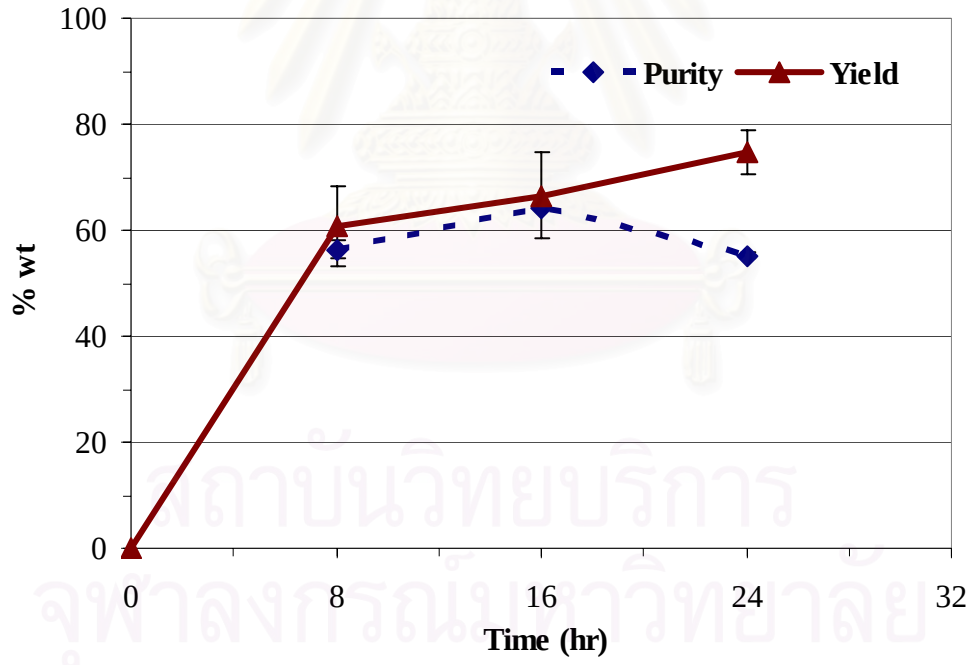
4.3.5 Effect of second step crystallization time on yield and purity of γ-oryzanol

The effect of the second step crystallization time was investigated using the suitable condition obtained from the previous study. After the first crystallization step in 20 % v/v of ethyl acetate in methanol, at 30 °C for 1 h, the supernatant was allowed to crystallize at 5 °C for 8, 16 and 24 h. Quantification of γ-oryzanol in the crystal samples were carried out both by using UV-visible spectrophotometer and HPLC and the results are shown in Figure 4.13 (a) and 4.13 (b). From the Figure 4.13 (a), it was observed that percent purity was not significantly different for the range of crystallization time employed in this study (62.11 %, 62.96 % and 60.51 %, respectively). The yield of γ-oryzanol was found not to be significantly different when the time was increased from 8 to 16 h. Nevertheless, the γ-oryzanol yield increased as the time increased from 16 to 24 h.

When HPLC analysis was used to quantify the γ-oryzanol content in the crystal sample, the results of γ-oryzanol purity and yield are shown in Figure 4.13 (b). The same trend was observed with that in Figure 4.13 (a), that is, the purity was comparable for different crystallization time while the yield increased with increasing time from 8 to 24 h from 60.6 % to 74.6 %. The crystallization phenomenon can be considered as composing of two steps, nucleation followed by crystal growth [Lin and Koseoglu, 2003]. The result from Figure 4.13 (b) indicated that the rate of nucleation was higher than the rate of crystal growth. Due to the slow rate of crystal growth, crystallization time over 16 h or overnight would be required [Seetharamaiah et al. (1986), Das et al. (1999), Rao et al. (2002), Narayan et al. (2004)]. In this study, the second step crystallization was carried out up to 24 h and the yield of γ-oryzanol obtained was higher than the value reported by Narayan et al. (2004), which was in the range of 56-60 %, and while the purity was comparable to that obtained by the same investigator (60-65 %purity).



(a)



(b)

Figure 4.13 Percent purity and yield of γ-oryzanol after second crystallization step at 5±1 °C for 8, 16 and 24 h; (a) analyzed by UV-visible spectrophotometer and (b) analyzed by HPLC

It could be observed again in this study that the yields quantified by HPLC were noticeably higher than that obtained by UV-visible spectrophotometer. Considering the content of γ-oryzanol in crystallized products, both UV-spectrophotometer HPLC methods gave comparable results (Figure 4.14). This was because in a rather purified sample, UV-spectrophotometer could be accurately used for the quantification of the compound of interest as mentioned above. This was also seen in the comparable results on the purity obtained from both analytical methods shown in Figure 4.13(a) and 4.13(b). Nevertheless, UV-visible spectrophotometric analysis is less accurate than HPLC analysis but it is a more widely used method that give reasonable quantification of γ-oryzanol for the initial investigation for the effect separation conditions, and is therefore an acceptable method used for the analysis of γ-oryzanol in rice bran oil [Das et al. (1998), Rao et al. (2002), Krishna et al. (2001)].

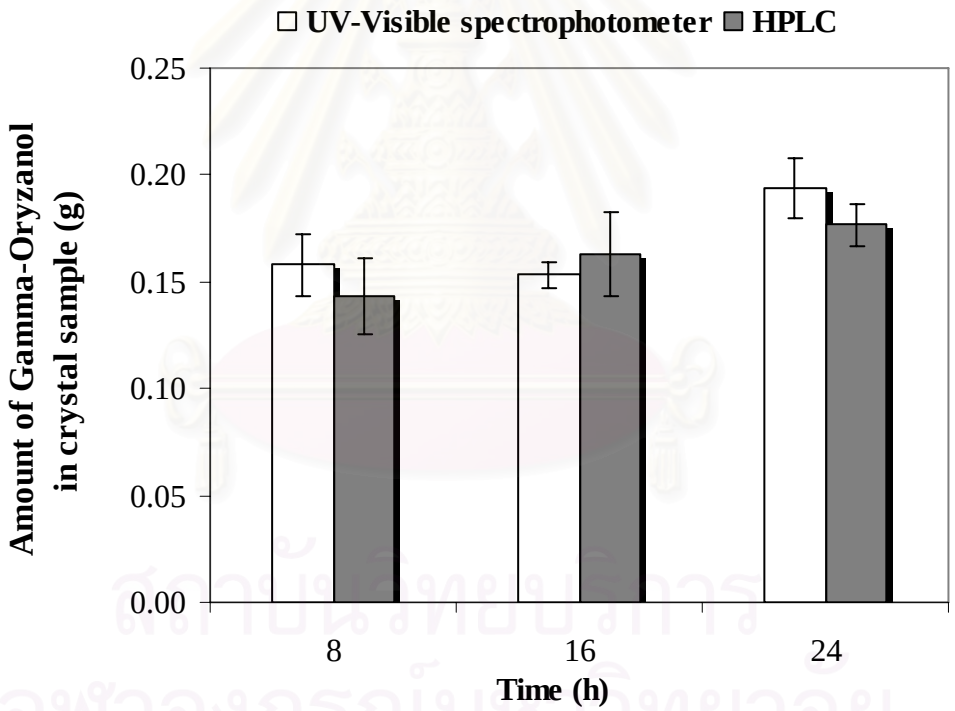


Figure 4.14 Amount of γ-oryzanol in crystal sample after second crystallization step at 5±1 °C for 8, 16 and 24 h; analyzed by UV-visible spectrophotometer and HPLC

CHAPTER V

CONCLUSIONS & RECOMMENDATIONS

5.1 Conclusions

The objective of this study is to determine the suitable conditions for separation of γ-oryzanol from rice bran oil soapstock by leaching and crystallization. The yield and purity of γ-oryzanol from each step were important factors for the selection of suitable conditions and the suitable steps for γ-oryzanol separation and purification are summarized in Figure 4.13. First, the soapstock containing 4.9 % wt of γ-oryzanol was saponified with NaOH 2.4 % wt to convert the remaining glycerides into soap. The saponified soapstock was then dried in a vacuum oven and ground into fine particles. The amount of γ-oryzanol contained in this dried saponified material was 5.83 % wt of the dried sample and the yield and purity of γ-oryzanol obtained at each separation step were calculated based on this quantity. After saponification, dried saponified material was leached by ethyl acetate, in which the suitable ratio of raw material to ethyl acetate was determined. The suitable ratio was found to be 15 g materials/ 200 ml of solvent. The yield and purity of γ-oryzanol of the extract were 99.23 and 39.6 % wt, respectively, as determined by UV-visible spectrophotometric analysis. After the solvent was evaporated, the extract was crystallized in the first crystallization step for 1 h to separate the mucilaginous impurities using 20 % v/v of ethyl acetate mixture in methanol at 30 °C. Subsequently, the supernatant obtained was allowed to crystallize in the second step to obtain purer γ-oryzanol crystals. The suitable temperature and time for the second crystallization step were 5 °C and 24 h. The yield and purity of γ-oryzanol obtained from this step (74.6 % yield and 55.17 % purity) were comparable with those reported in literature (56-60 % yield and 60-65 % purity).

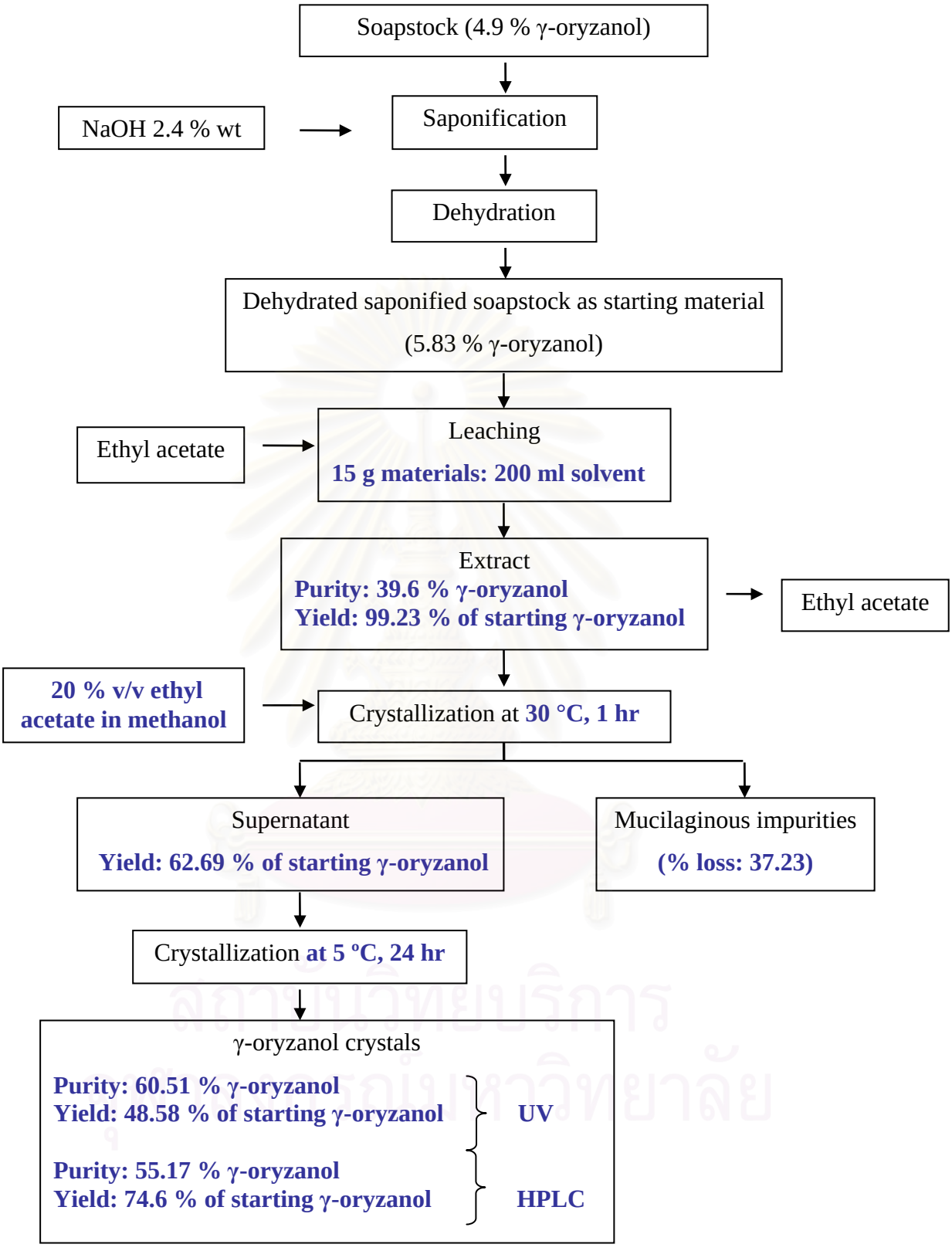


Figure 4.13 The suitable conditions for separation of γ-oryzanol from soapstock

5.2 Recommendations

This research investigates the production of value added γ-oryzanol product from soapstock by-product of rice bran oil processing. Leaching and crystallization were considered key steps of the separation and therefore the suitable conditions for these steps were determined. Two leaching systems of solvent mixtures investigated which are acetone:methanol and ethyl acetate:methanol mixtures. The first was suggested by Narayan et al. (2004), while the latter system was proposed in this study and the effect of parameters such as temperature and crystallization time were described. Recommendations for future study were as follows.

1. As the composition of the starting soapstock itself was one of the important factors for the separation process of γ-oryzanol, more detailed information about the composition of the soapstock sample should be determined. Moreover, the analysis of the resulted products from each step should be also be conducted using an appropriate method of analysis in order to gain better understand about the process yield and purity of γ-oryzanol obtained at each step.

2. In this study, there observed significant loss of γ-oryzanol during the saponification step, which should therefore be minimized. This could be achieved by improving the mixing system such that the minimum amount of sodium hydroxide actually would be needed to complete saponification.

3. The solubility of γ-oryzanol in solvent mixtures of interest should be examined at various temperatures in order to predict the crystallization behavior.

4. The higher purity of γ-oryzanol could further be obtained by using column recrystallization or chromatography. For the recrystallization, the supersaturation could be achieved by the temperature reduction similar to the first two steps of crystallization. Alternatively, the use of anti-solvent crystallization is another possibility that could be worth investigating. For the purification achieved by chromatography, several studies have reported the use of alumina or silica chromatography column with appropriate eluents as effective purification methods [Seetharamajah et al. (1986), Das et al. (1999), Rao et al. (2002)] and the process is thus recommended for the future study.

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APPENDICES

APPENDIX A

EXPERIMENTAL DATA FOR ANALYSIS

A-1 Standard calibration curve of γ-oryzanol from UV-Visible spectrophotomeric analysis

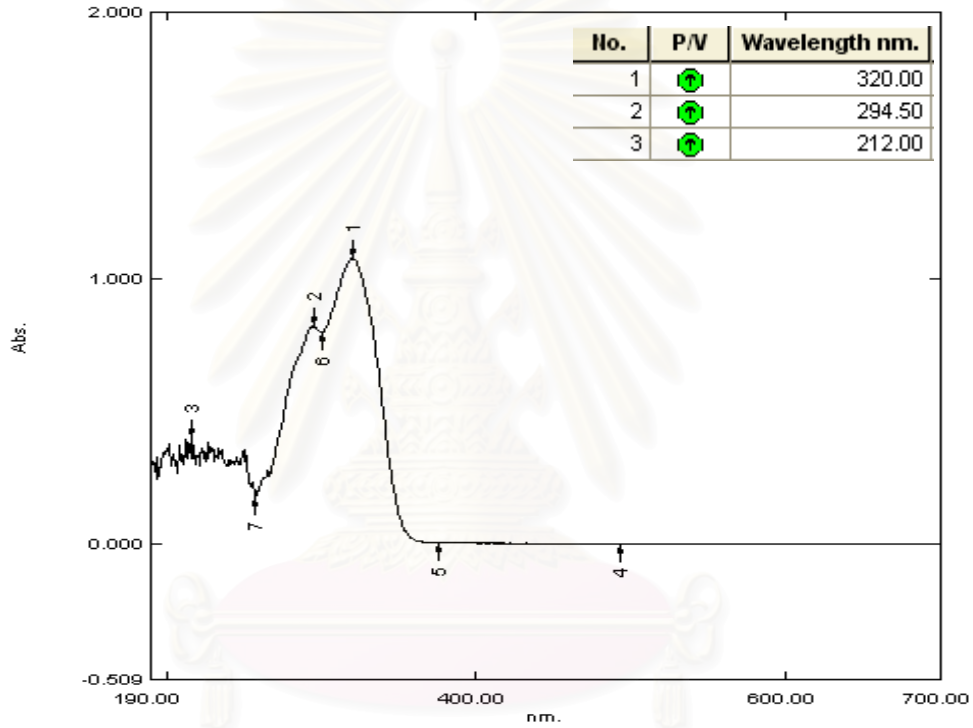

Figure A-1.1 Spectrum peak scan of γ-oryzanol standard in ethyl acetate

Table A-1 Standard calibration curve data of γ-oryzanol from UV-Visible spectrophotomeric analysis

Concentration of γ-oryzanol (μg/ml)	Absorbance at 320 nm			
	Exp 1	Exp 2	Exp 3	average
0.98	0.036	0.036	0.036	0.036
2.94	0.117	0.117	0.118	0.117
4.9	0.196	0.197	0.197	0.197
6.86	0.276	0.276	0.276	0.276
9.8	0.394	0.394	0.394	0.394
14.7	0.607	0.609	0.609	0.608
19.6	0.777	0.778	0.78	0.778
24.5	0.979	0.98	0.98	0.98

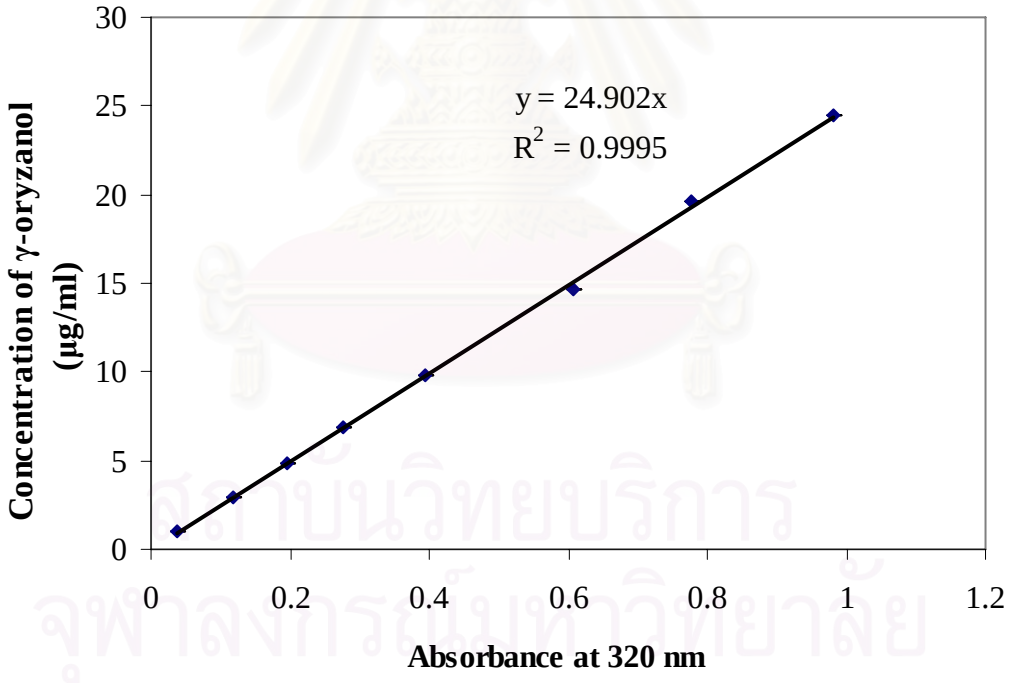

Figure A-1.2 Standard calibration curve of γ-oryzanol from UV-Visible spectrophotomeric analysis

A-2 Standard calibration curve of γ-oryzanol HPLC analysis

Table A-2.1 Standard calibration curve of γ-oryzanol HPLC analysis

Concentration of γ-oryzanol (mg/ml)	98 % assay	Retention time	Peak area	Total peak area
0.1001	0.098098	10.250	344.747	498.527
		11.083	103.390	
		11.600	50.390	
0.4004	0.392392	10.283	1678.949	2034.913
		11.066	255.387	
		11.666	100.577	
2	1.96	9.283	19943.114	24287.739
		10.000	2936.674	
		10.466	1407.951	
2.5025	2.45245	10.250	26592.057	30017.076
		11.033	2509.933	
		11.616	915.086	
4	3.92	10.300	47659.111	54320.1195
		11.083	4903.6845	
		11.683	1757.324	
6	5.88	10.366	63320.345	75725.801
		11.050	9132.956	
		11.633	3272.500	

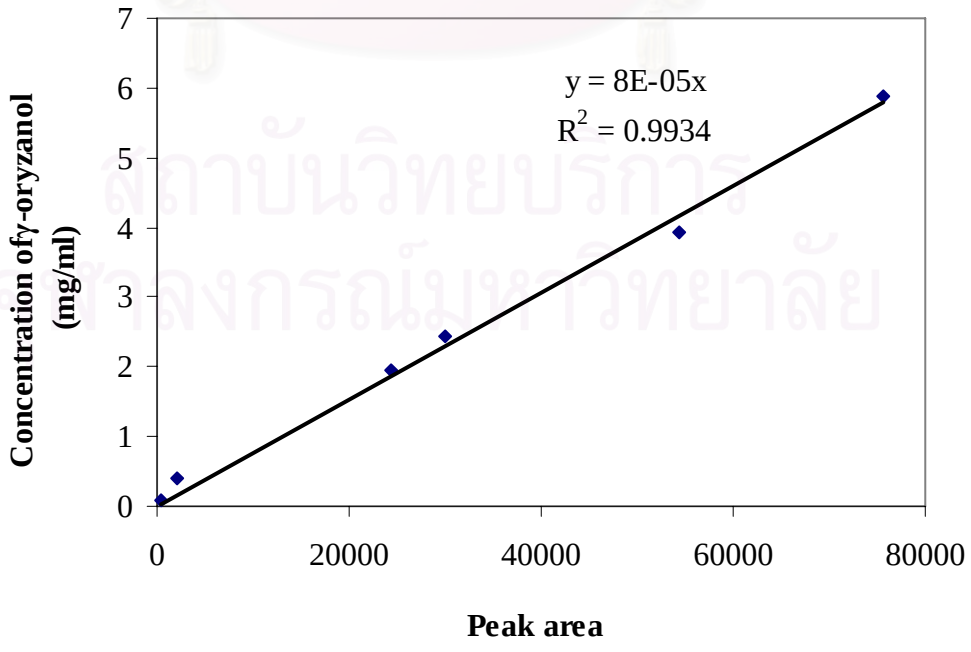

Figure A-1.1 Standard calibration curve of γ-oryzanol analyzed by HPLC

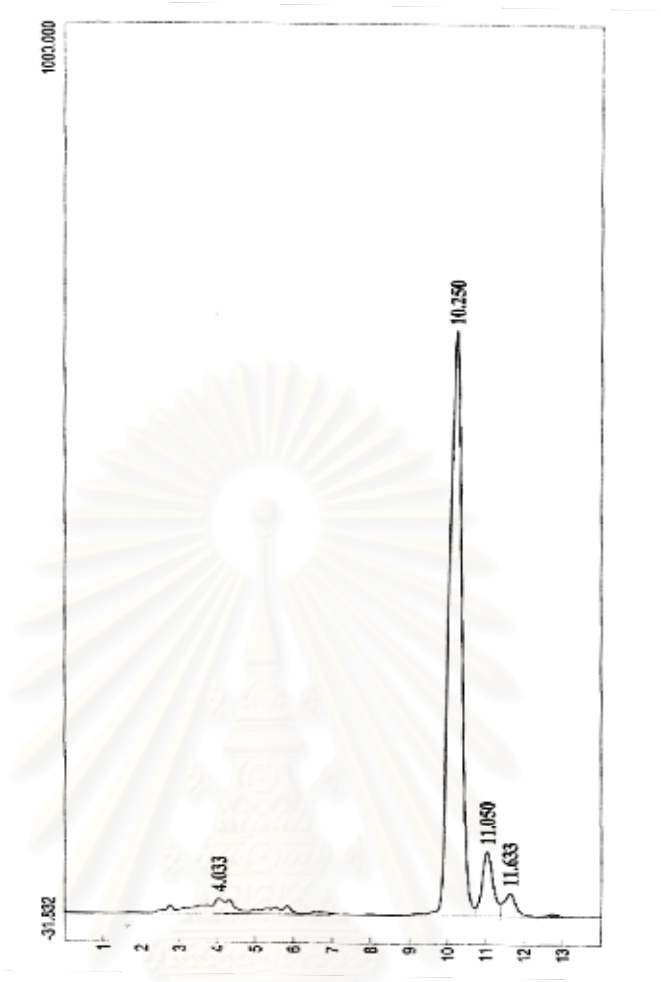

Figure A-1.2 Chromatogram of γ-oryzanol standard

APPENDIX B

EXPERIMENTAL DATA OF PHYSICAL AND CHEMICAL PROPERTIES OF RICE BRAN OIL SOAPSTOCK

B-1 Moisture content of rice bran oil soapstock

Table B-1 Moisture content data

Sample	Weigh of sample (g)	Weigh of sample after drying (g)	Moisture content (%)
1	3.0073	1.2915	57.05
2	3.0067	1.2931	56.99
3	3.0027	1.2961	56.84
average	3.0056	1.2936	56.96

B-2 Saponification value of rice bran oil soapstock

The saponification value is the number of mg of potassium hydroxide (KOH) to saponify the esters in 1 g of the sample and neutralize the free acids in 1 g of a sample (A.O.C.S official method, Cd 3b-76, 1989).

Procedure

Unless otherwise specified, proceed as follows: Weigh accurately about 1 g of the sample, transfer into an Erlenmeyer flask, add 40 ml of ethanol, and dissolve while warming if necessary. Add 20 ml of ethanolic potassium hydroxide TS, accrately measured, equip the flask with a reflux condenser, and heat in a water bath for 30 minutes while shaking the flask occasionally. Cool, add a few drops of phenolphthalein TS, and immediately titrate excess potassium hydroxide with 0.5

mol/l hydrochloric acid. Perform a blank test, and calculate the saponification value by the formula

$$\text{Saponification value} = \frac{(a-b)\times 28.05}{Weight\,(g)\,of\ sample} \quad \text{mg KOH/ 1g sample}$$

Where

a = volume (ml) of 0.5 mol/l hydrochloric acid consumed in the blank test

b = volume (ml) of 0.5 mol/l hydrochloric acid consumed in the test.

Table B-2 Saponification value data

No.	Blank	Sample	Saponification value (mg KOH/ 1g sample)	Saponification value (mg NaOH/ 1g sample)	% wt NaOH
1	42.6	40.2	33.66	24	2.4
2	42.6	40.3	32.2575	23	2.3
3	42.6	40.2	33.66	24	2.4
avg	42.6	40.23	33.19	23.7	2.4

*** 1 mgNaOH = 1.4025 mgKOH

B-3 γ-oryzanol loss in saponification step

Starting material, soapstock	540	g
γ-oryzanol in soapstock	4.9	% wt
so, γ-oryzanol in 540 g of soapstock	26.46	g

After saponification and dehydration;

dehydrated saponified soapstock	240	g
γ-oryzanol in dehydrated saponified soapstock	5.83	% wt
so, γ-oryzanol in 240 g of dehydrated saponified soapstock	15.741	g

γ-oryzanol loss in saponification step $\left(\frac{26.46-15.74}{26.46}\right)\times 100 = 40.5$ % wt

B-4 γ-oryzanol content in rice bran oil soapstock

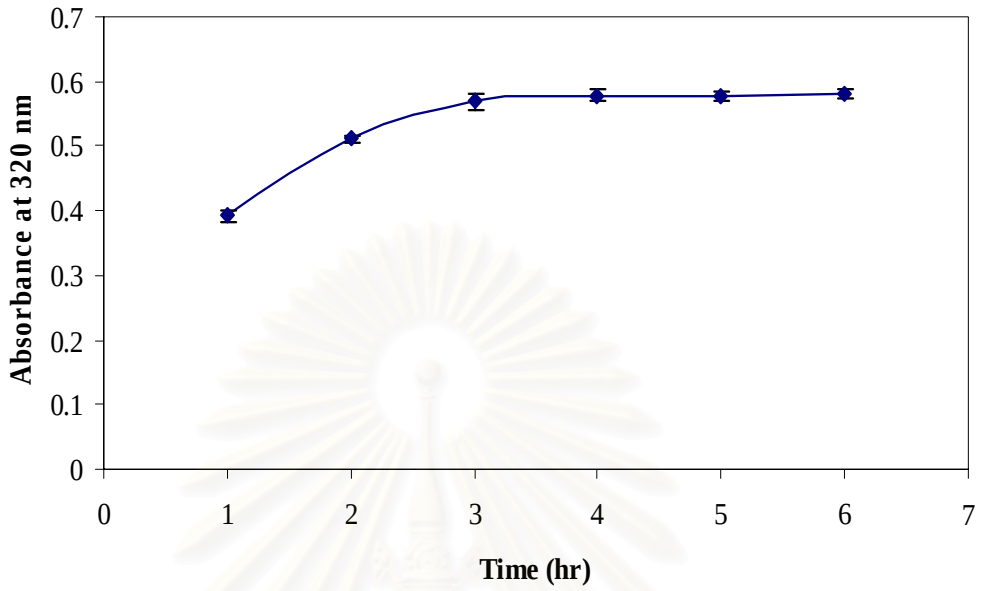

Figure B-4 Absorbance of γ-oryzanol at 320 nm in extract extracted from dehydrated soapstock (3g/200 ml solvent) after soxhlet extraction at various times.

Table B-4 γ-oryzanol content from dehydrated soapstock

Sample	Absorbance	γ-oryzanol (g)	% γ-oryzanol (dry basis)	% γ-oryzanol (wet basis)
1	0.584	0.2327	7.76	4.94
2	0.577	0.2299	7.66	4.88
		average	7.71	4.91

B-5 γ-oryzanol content in dehydrated saponified rice bran oil soapstock.

Table B-5.1 γ-oryzanol content in saponified and dehydrated rice bran oil soapstock analyzed by UV-Visible spectrophotometer

Sample	γ-oryzanol content (g)*
γ-oryzanol content in extract	15.6169
Recovery 15g/200 ml Run #1	0.0246
Recovery 15g/200 ml Run #2	0.0197
Recovery 15g/200 ml Run #3	0.0144
Recovery 15g/200 ml Run #4	0.0076
Recovery 15g/200 ml Run #5	0.0054
Recovery 15g/200 ml Run #6	0.0088
Recovery 15g/200 ml Run #7	0.0062
Recovery 15g/200 ml Run #8	0.0028
Recovery 15g/200 ml Run #9	0.0039
Recovery 15g/200 ml Run #10	0.0031
Recovery 15g/200 ml Run #11	0.0028
Recovery 15g/200 ml Run #12	0.0037
Recovery 15g/200 ml Run #13	0.0034
Recovery 15g/200 ml Run #14	0.0052
Recovery 15g/200 ml Run #15	0.0012
Recovery 15g/200 ml Run #16	0.0015
Recovery 15g/200 ml Run #17	0.0010
Recovery 15g/200 ml Run #18	0.0056
Total recovery	0.1208
Total γ-oryzanol content recovery in dehydrated saponified soapstock	15.7377
% γ-oryzanol content	5.83 %

* γ-oryzanol content was determined by soxhlet extraction for 4 hr. from dehydrated saponified soapstock 270 g (18 runs) and then the residue was extracted again by sonication 1 hr at 40 °C with 50 ml ethyl acetate for 3 times, analyzed by UV-Visible spectrophotometer.

Table B-4.2 γ-oryzanol content in saponified and dehydrated rice bran oil soapstock analyzed by HPLC.

Sample	γ-oryzanol content (g)
γ-oryzanol in extract	9.2763
Total recovery	0.0656
Total γ-oryzanol content recovery in dehydrated saponified soapstock	9.3419
% γ-oryzanol content	3.46 %

B-5 Chromatogram of sample from each step of separation

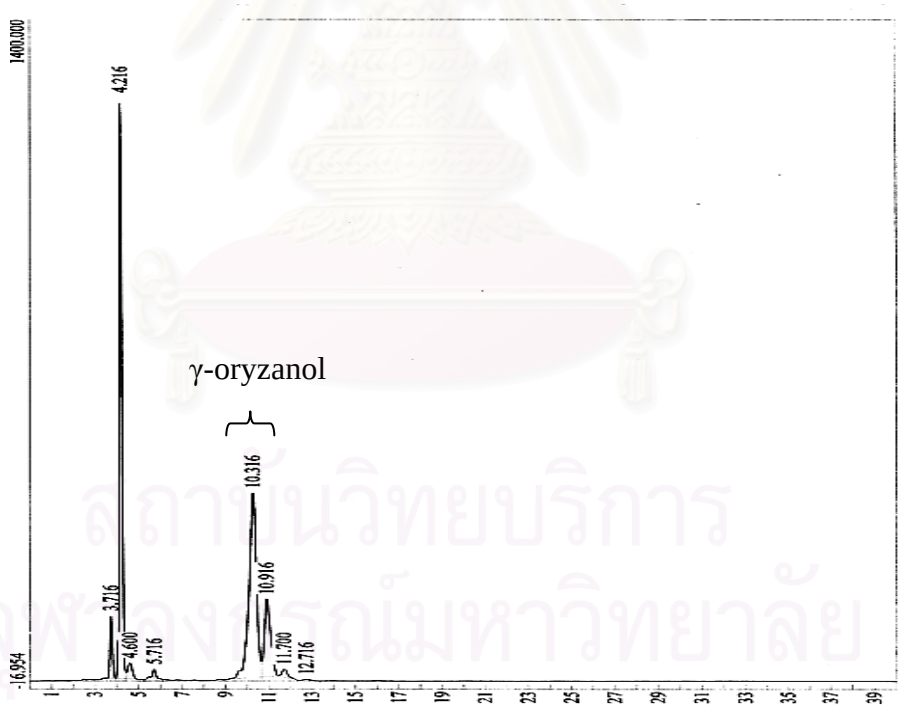

Figure B-5.1 Chromatogram of ethyl acetate extract from dehydrated saponified soapstock

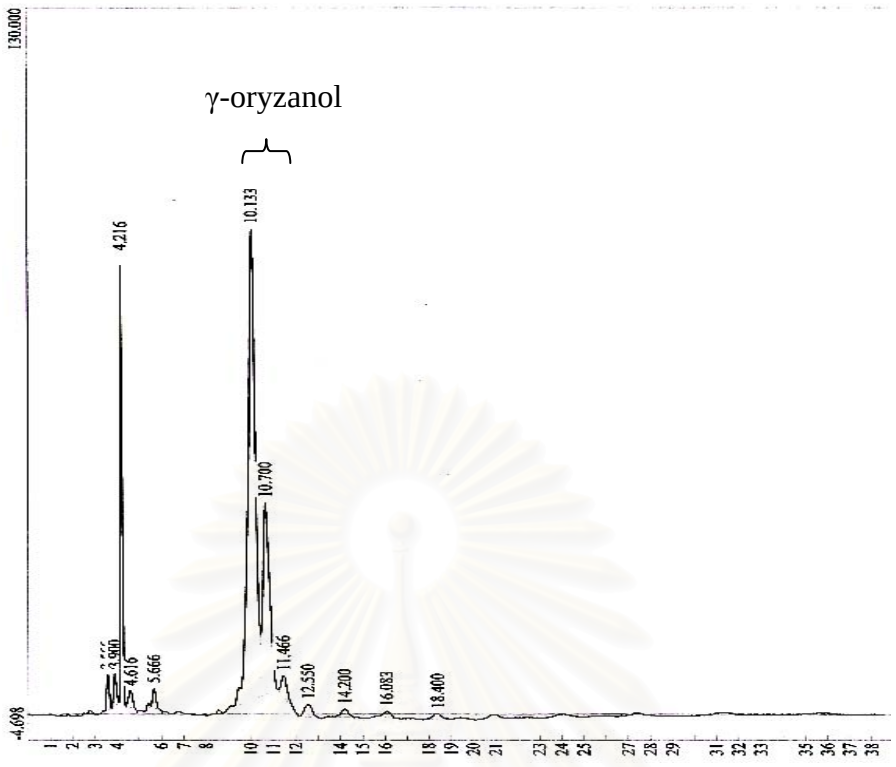

Figure B-5.2 Chromatogram of mucilaginous impurities sample from first step crystallization

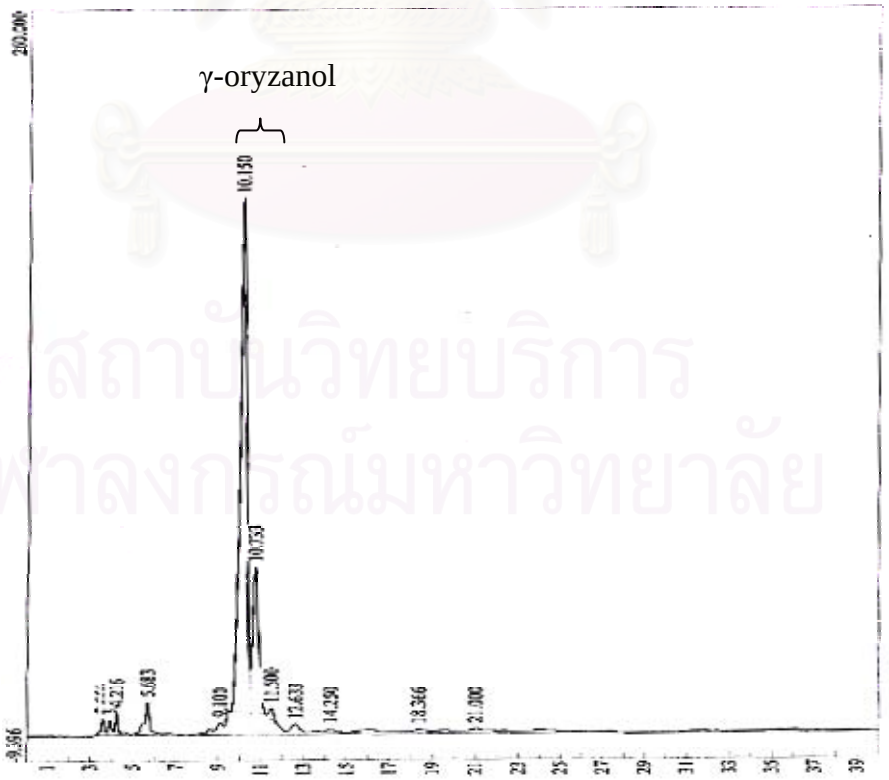

Figure B-5.3 Chromatogram of crystal sample from second step crystallization

APPENDIX C

EXPERIMENTAL DATA

C-1 Calculation of yield and purity of γ-oryzanol in the sample

$$\%\ \gamma-oryzanol\ loss = \frac{Amount\ of\ \gamma-oryzanol\ in\ mucilaginous\ impurities}{Amount\ of\ \gamma-oryzanol\ in\ extract}\times 100$$

$$\%\ purity\ of\ \gamma-oryzanol = \frac{Amount\ of\ \gamma-oryzanol\ in\ sample}{Weigh\ of\ sample}\times 100$$

$$\%\ yiled\ of\ \gamma-oryzanol = \frac{Amount\ of\ \gamma-oryzanol\ in\ sample}{Amount\ of\ \gamma-oryzanol\ in\ dehydrated\ saponified\ soapstock}\times 100$$

C-2 Experimental data for leaching

Table C-2 Percent yield and purity of γ-oryzanol from leaching step

Sample	Run	γ-oryzanol (g)	Total recovery	Extract (g)	Yield (%)	SD	Purity (%)	SD
3 g/200 ml	1	0.1534	0.0008	0.5237	99.49	2.00	29.29	1.91
	2	0.1685	0.0058	0.5266	96.65		32.00	
	average				98.07		29.29	
6 g/200ml	1	0.2978	0.0031	0.8884	98.98	1.65	33.52	1.13
	2	0.2737	0.0095	0.8539	96.63		32.05	
	3	0.3135	0.0131	0.9148	95.79		34.27	
	average				97.13		33.28	
10 g/200 ml	1	0.4822	0.0154	1.5564	96.91	1.12	30.98	1.64
	2	0.5857	0.0090	1.7586	98.49		33.3	
	average				97.7		32.14	
15 g/200 ml	1	0.7461	0.0246	2.1320	96.81	1.13	34.99	2.51
	2	0.8691	0.0197	2.1489	97.79		40.44	
	3	0.8965	0.0076	2.2299	99.16		40.20	
	4	0.9555	0.0088	2.4758	99.09		38.57	
	average				98.21		38.55	

C-3 Experimental data for the study of suitable solvent mixture system

Table C-3.1 Weigh of samples from crystallization step

Solvent system	Run	Extract (g)	Mucilaginous impurities (g)	Crystal (g)
15% acetone	1	1.0059	0.5872	0.0656
	2	1.0088	0.5027	0.1375
25% acetone	1	1.0062	0.4967	0.1255
	2	1.0094	0.4359	0.2134
35% acetone	1	1.0062	0.3436	0.0666
	2	1.0096	0.3003	0.1387
10 % ethyl acetate	1	1.0000	0.5152	0.1741
	2	1.0011	0.4988	0.204
20 % ethyl acetate	1	1.0060	0.455	0.2588
	2	1.0030	0.3699	0.2594
30 % ethyl acetate	1	1.0021	0.2088	0.324
	2	1.0023	0.2316	0.2503

Table C-3.2 Percent γ-oryzanol loss and yield from first step crystallization

Solvent system	Total γ-oryzanol* (g)	% γ-oryzanol loss	SD	% Yield	SD
15% acetone	0.2741	68.81	12.31	30.95	12.21
	0.2054	51.41		48.23	
	average	60.11		39.59	
25% acetone	0.2537	63.67	12.51	36.06	12.41
	0.1838	45.98		53.61	
	average	54.82		44.83	
35% acetone	0.1612	40.46	5.65	59.09	5.61
	0.1298	32.47		67.02	
	average	36.46		63.06	
10 % ethyl acetate	0.2976	75.16	13.23	24.65	13.13
	0.2238	56.45		43.22	
	average	65.81		33.93	

20 % ethyl acetate	0.2288	57.42		42.25	
	0.1531	38.54	13.35	60.99	13.25
	average	47.98		51.62	
30 % ethyl acetate	0.1089	27.44	4.11	72.01	4.08
	0.0858	21.62		77.78	
	average	24.53		74.90	

* Total γ-oryzanol (g) in mucilaginous impurities

Table C-3.3 Percent purity and yield of γ-oryzanol from second step crystallization

Solvent system	Total γ-oryzanol* (g)	% Purity	SD	% Yield	SD
15% acetone	0.0553	84.23	13.19	13.77	6.11
	0.0902	65.58		22.40	
	average	74.90		18.08	
25% acetone	0.1052	83.84	18.47	26.21	3.10
	0.1232	57.73		30.59	
	average	70.79		28.40	
35% acetone	0.0431	64.68	12.29	10.73	3.93
	0.0656	47.30		16.29	
	average	55.99		13.51	
10 % ethyl acetate	0.1161	66.68	10.86	29.09	2.04
	0.1047	51.32		26.21	
	average	59.00		27.65	
20 % ethyl acetate	0.1245	71.54	2.05	31.03	2.80
	0.1400	68.63		34.98	
	average	70.08		33.00	
30 % ethyl acetate	0.1889	72.98	19.54	47.05	12.49
	0.1176	45.35		29.39	
	average	59.16		38.22	

* Total γ-oryzanol (g) in crystal after second step crystallization

C-4 Experimental data for the study of suitable temperature for first step crystallization

Table C-4.1 Weigh of samples from crystallization step

Temperature (°C)	Run	Extract (g)	Mucilaginous impurities (g)	crystal (g)
25	1	1.0060	0.4550	0.2588
	2	1.0030	0.3699	0.2594
30	1	0.9969	0.3794	0.2235
	2	0.9996	0.3864	0.2653

Table C-4.2 Percent γ-oryzanol loss and yield from first step crystallization

Temperature (°C)	Total γ-oryzanol (g)	% γ-oryzanol loss	SD	% Yield	SD
25	0.2288	57.42	13.35	42.25	13.25
	0.1531	38.54		60.99	
	average	47.98		51.62	
30	0.1863	47.20	0.92	52.40	0.91
	0.1817	45.90		53.69	
	average	46.55		53.04	

Table C-4.3 Percent purity and yield of γ-oryzanol from second step crystallization

Temperature (°C)	Total γ-oryzanol (g)	% Purity	SD	% Yield	SD
25	0.1245	71.54	2.05	31.03	2.80
	0.1400	68.63		34.98	
	average	70.08		33.00	
30	0.1489	66.61	5.17	37.42	1.43
	0.1573	59.30		39.44	
	average	62.96		38.43	

C-5 Experimental data for the study suitable time for second step crystallization

Table C-5.1 Weigh of samples from crystallization step

Time (hr)	Run	Extract (g)	Mucilaginous impurities (g)	crystal (g)
1	1	0.9969	0.3794	0.2235
	2	0.9996	0.3864	0.2653
2	1	1.0005	0.3162	0.2682
	2	1.0005	0.3322	0.2851

Table C-5.2 Percent γ-oryzanol loss and yield from first step crystallization

Time (hr)	Total oryzanol (g)	% γ-oryzanol loss	SD	% Yield	SD
1	0.1863	47.20	0.92	52.40	0.91
	0.1817	45.90		53.69	
	average	46.55		53.04	
2	0.1727	43.58	0.77	55.99	0.76
	0.1770	44.67		54.91	
	average	44.12		55.45	

Table C-5.3 Percent purity and yield of γ-oryzanol from second step crystallization

Time (hr)	Total oryzanol (g)	% Purity	SD	% Yield	SD
1	0.1489	66.61	5.17	37.42	1.43
	0.1573	59.30		39.44	
	average	62.96		38.43	
2	0.1615	60.20	2.25	40.44	3.41
	0.1807	63.39		45.27	
	average	61.79		42.85	

C-6 Experimental data for the study of suitable temperature for second step crystallization

Table C-6.1 Weigh of samples from crystallization step

Temperature (°C)	Run	Extract (g)	Mucilaginous impurities (g)	crystal (g)
2	1	1.0004	0.3443	0.2437
	2	1.0004	0.2916	0.2863
5	1	0.9969	0.3794	0.2235
	2	0.9996	0.3864	0.2653
10	1	1.0005	0.3162	0.2682
	2	1.0005	0.3322	0.2851

Table C-6.2 Percent γ-oryzanol loss and yield from second step crystallization

Temperature (°C)	Total oryzanol (g)	% γ-oryzanol loss	SD	% Yield	SD
2	0.1515	38.24	3.56	61.29	3.53
	0.1316	33.21		66.28	
	average	35.73		63.79	
5	0.1863	47.20	0.92	52.40	0.91
	0.1817	45.90		53.69	
	average	46.55		53.04	
10	0.1437	36.26	0.43	63.25	0.43
	0.1461	36.87		62.65	
	average	36.57		62.95	

Table C-6.3 Percent purity and yield of γ-oryzanol from second step crystallization

Temperature (°C)	Total oryzanol (g)	% Purity	SD	% Yield	SD
2	0.1482	60.81	7.29	37.12	9.82
	0.2036	71.13		51.01	
	average	65.97		44.07	
5	0.1489	66.61	5.17	37.42	1.43
	0.1573	59.30		39.44	
	average	62.96		38.43	
10	0.1085	40.45	9.92	27.18	8.29
	0.1553	54.48		38.90	
	average	47.47		33.04	

C-7 Experimental data for the study of suitable time for second step crystallization analyzed by UV-Visible spectrophotometer

Table C-7.1 Weigh of samples from crystallization step

Time (hr)	Run	Extract (g)	Mucilaginous impurities (g)	crystal (g)
8	1	0.9995	0.3112	0.2714
	2	0.9995	0.3428	0.2373
16	1	0.9969	0.3794	0.2235
	2	0.9996	0.3864	0.2653
24	1	1.0004	0.3443	0.3304
	2	1.0004	0.2916	0.3102

Table C-7.2 Percent γ-oryzanol loss and yield from first step crystallization

Time (hr)	Total oryzanol (g)	% γ-oryzanol loss	SD	% Yield	SD
8	0.1385	35.00	2.29	64.51	2.28
	0.1514	38.24		61.29	
	average	36.62		62.90	
16	0.1863	47.20	0.92	52.40	0.91
	0.1817	45.90		53.69	
	average	46.55		53.04	
24	0.1618	40.83	5.09	58.72	5.05
	0.1332	33.63		65.86	
	average	37.23		62.29	

Table C-7.3 Percent purity and yield of γ-oryzanol from second step crystallization

Time (hr)	Total oryzanol (g)	% Purity	SD	% Yield	SD
8	0.1684	62.05	0.09	42.22	3.70
	0.1475	62.17		36.99	
	average	62.11		39.61	
16	0.1489	66.61	5.17	37.42	1.43
	0.1573	59.30		39.44	
	average	62.96		38.43	
24	0.2038	61.67	1.64	51.04	3.48
	0.1841	59.35		46.12	
	average	60.51		48.58	

C-8 Experimental data for the suitable time for second step crystallization analyzed by HPLC

Table C-7.1 Percent purity and yield of γ-oryzanol from second step crystallization

Time (hr)	Total oryzanol (g)	% Purity	SD	% Yield	SD
8	0.1561	57.51	1.66	65.93	7.52
	0.1309	55.16		55.29	
	average	56.33		60.61	
16	0.1435	64.22	0.12	60.79	8.17
	0.1708	64.39		72.34	
	average	64.31		66.57	
24	0.1837	55.59	0.59	77.51	4.12
	0.1699	54.76		71.68	
	average	55.17		74.60	

APPENDIX D

The 17 th Thai Chemical Engineering and Applied Chemistry Conference

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Value Added Products from By-products of Rice Bran Oil Processing

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Value Added Products from By-Products of Rice Bran Oil Processing

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1. Introduction

Rice is a major agricultural product of Thailand with annual productivity about 29 million tons due to suitable topography and climate nature (www.oae.go.th). Commonly, consumers prefer white rice therefore brown rice is subjected to milling process. Rice bran is an important by product of the milling process which is enriched in fibre, proteins, oil, and important antioxidants such as vitamin E and γ-oryzanol. Currently, rice bran is mostly used as animal feed, while a small amount is used to produce value added edible cooking oil. Although rice oil is considered nutritious oil and is becoming popular especially in USA and Europe, during rice bran oil processing, various by products are produced and large amount of nutrition was lost with them. Here we are interested in two major by products of the rice oil processing: deoiled bran and soapstock. Deoiled bran is rich in proteins and amino acid and is obtained after oil extraction. Soapstock is obtained after deacidification by alkali treatment of crude rice bran oil during the refining process. It has a significant amount of γ-oryzanol, which is important component that show many health benefits such as reduction of cholesterol in the blood and anti-aging effect etc. At present, majority of both deoiled bran and soapstock is used as animal feed, and it is the objective of this study to increase the value of these by products.

Generally, proteins and amino acid that present in deoil bran can be extracted by chemical method which is alkai or acid hydrolysis, followed by acid precipitation. However, this method obtained low protein yield due to degradation at extreme pH condition. Alternatively, enzymatic process has been studies but it takes a long time and high cost of enzymes make the process commercially uneconomical. Subcritical water or pressurized water at temperature between boiling point (100 °C) and its critical temperature (374.15 °C) is an interesting alternative. At such condition, water polarity decreases, thus make it better solvent for extraction of several organic bioactive substances.

Separations of γ-oryzanol from rice bran oil soapstock have been investigated for many decades. In the development of these processes into commercial scale, several factors such as productivity, environment and health problems, process investment, and separation efficiency (purity and yield) must be considered. Furthermore, isolation procedure developed for one soapstock does not necessarily work well with another soapstock [Narayan et al., 2006]. Consequently, in this study, we selected some processes from literature and further investigate the experimental separation of γ-oryzanol from domestic soapstock.

In this study we divided our investigation into two parts. Part 1: Subcritical water hydrolysis of deoiled rice bran and part 2: Separation of γ-oryzanol from rice bran oil soapstock.

2. Materials and Methods

Part 1: Subcritical water hydrolysis of deoiled rice bran

1. Materials. Deoiled rice bran was obtained from Thai Edible Oil Co., Ltd., Ayuthaya, Thailand.

2. Subcritical water hydrolysis. The hydrolysis reaction was carried out in closed batch reactor in which the effect of temperature in the range 200-220 °C, reaction time of 10-30 min and raw material to water weight ratio of 1:5 and 2:5 on the yield of protein, amino acid and reducing sugar.

3. Analytical method

3.1 Analysis of protein. Protein content of soluble portion was assayed using Lowry's method (Lowry et al., 1951), using bovine serum albumin (BSA) as a standard.

3.2 Analysis of amino acids. Amino acids content was analyzed by Ninhydrin assays using L-Glutamic acid as a standard.

3.3 Analysis of reducing sugar. Reducing sugars content was assayed by dinitrosalicylic colorimetric method, using D-Glucose as a standard, using dinitrosalicylic reagent that developed by Sumner and co-worker.

Part 2: Separation of γ-oryzanol from rice bran oil soapstock by using selected process

1. Materials Rice bran oil soapstock was obtained from Thai Edible Oil Co., Ltd., Ayuthaya, Thailand.

2. Separation of γ-oryzanol The selected processes [Rao et al., 2002, Indira et al., 2004, Narayan et al., 2005] were applied to separate γ-oryzanol from soapstock. This process includes the following steps, saponification, dehydration, leaching and crystallization.

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3. Quantification of γ-oryzanol γ-oryzanol contents was determined by spectrophotometric method.

3. Results and Discussion

Part 1: Subcritical water hydrolysis of deoiled rice bran

The optimum condition for deoiled rice bran extraction by using subcritical water hydrolysis was 20-30 min of reaction time and proper weight ratio of deoiled bran to water was 1:5. The reaction temperature suitable for producing protein and amino acid was 220 °C as shown in Fig.1, Fig.2 and Fig.3.

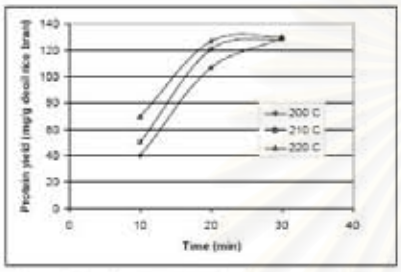

Fig.1 Protein yield after hydrolysis of raw materials at different temperature and time

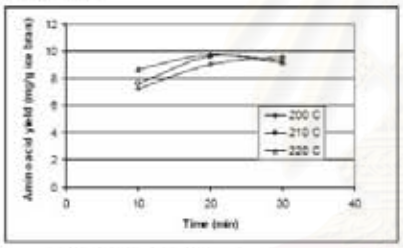

Fig.2 Amino acid yield after hydrolysis of raw materials at different temperature and time

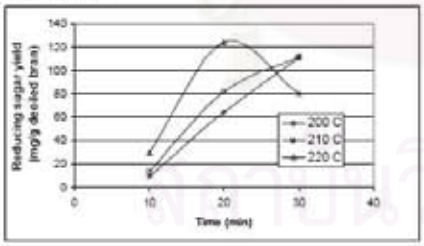

Fig.3 Reducing sugar yield after hydrolysis of raw materials at different temperature and time

Part 2: Separation of γ-oryzanol from rice bran oil soapstock by using selected processes

The separation γ-oryzanol from soapstock by selected processes was found to be appropriate for the acquired soapstock. Quantification γ-oryzanol in the sample that obtained after recrystallization could be achieved by using UV spectrophotometer. It was found that spectrum peak of the sample was comparable to that of the standard with the same maximum absorption wave length as shown in Fig. 4. Details on the effects of process conditions will be discussed further.

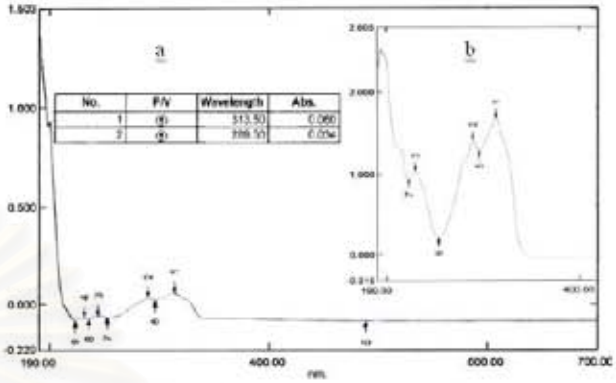

Fig. 4 Spectrum peak scanning report for sample comparable to γ-oryzanol standard. Fig 4-a: γ-oryzanol standard and Fig 4-b: sample.

4. Conclusions

The results in the present study suggested that subcritical water could be used to potentially hydrolyze deoiled rice bran into more valuable products. The selected processes from literature could be applied for separation of γ-oryzanol from soapstock.

5. Acknowledgements

We thank Thai Edible Oil Co., Ltd for providing raw materials (deoiled rice bran and rice bran oil soapstock).

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Contributed Paper

Value Added Products from By-products of Rice Bran Oil Processing

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ABSTRACT

This study deals with the production of value-added product derived from rice bran oil processing by-products. The investigation was divided into two parts, Part I: subcritical water (SW) hydrolysis of deoiled rice bran and Part II: separation of γ-oryzanol from rice bran oil soapstock. In Part I SW hydrolysis reaction was carried out in a closed batch 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 of protein, amino acid, reducing sugars, and antioxidant activity. The results in the present study suggested that subcritical water could be used to potentially hydrolyze deoiled rice bran into more valuable products. The suitable condition for protein and amino acids production from deoiled rice bran by subcritical water hydrolysis was 1:5 at 30 min and hydrolysis temperature of 220 °C. At this condition, the protein: 130.17 mg/g rice bran, amino acid: 9.14 mg/g rice bran. In Part II, separation of γ-oryzanol from rice bran oil soapstock was conducted following the selected processes from literatures. The processes involve saponification, dehydration, leaching and crystallization. The content of γ-oryzanol was analyzed by using UV spectrophotometer. The selected processes were demonstrated as suitable means for separating γ-oryzanol from soapstock obtained locally.

Keywords: Subcritical water, rice bran oil, deoiled rice bran, soapstock, γ-oryzanol.

1. INTRODUCTION

Rice is a major agricultural product of Thailand with annual productivity of about 29 million tons due to suitable topography and climate nature [1]. Commonly, consumers prefer white rice therefore brown rice is subjected to milling process. Rice bran is an important by product of the milling process which is enriched with fibre, proteins, oil, and important antioxidants such as vitamin E and γ-oryzanol. Currently, rice bran is mostly used as animal feed (60%), while the rest (40%) is used to produce value added edible cooking oil. Although rice oil is considered nutritious oil and is becoming popular especially in USA and Europe, during rice bran oil processing, a large amount of bran nutrition was lost along with the by products. Here we are interested in two major by products of the

rice oil processing: deoiled bran and soapstock. Deoiled bran is rich in proteins and amino acid and is obtained after oil extraction. Soapstock is obtained after deacidification by alkali treatment of crude rice bran oil during the refining process. It has a significant amount of γ-oryzanol, which is an important component that shows many health benefits such as reduction of cholesterol in the blood and anti-aging effect etc. At present, the majority of both deoiled bran and soapstock is used as animal feed. The objective of this study is therefore to increase the value of these by products.

Generally, proteins and amino acid that are presented in deoiled bran can be extracted by chemical method which is alkai or acid hydrolysis, followed by acid precipitation. However, low protein yield was obtained due to degradation at extreme pH condition. Alternatively, enzymatic process has been studied but the process takes a long time and the high cost of enzymes make the process commercially uneconomical. Subcritical water or pressurized water at the temperature between boiling point (100 °C) and its critical temperature (374.15 °C) is an interesting alternative. At such condition, water polarity decreases, thus make it better solvent for extraction of several organic bioactive substances.

Separations of γ-oryzanol from rice bran oil soapstock have been investigated for many decades. In the development of these processes into commercial scale, several factors such as productivity, environmental and health concerns, process investment, and separation efficiency (purity and yield) must be considered. Furthermore, isolation procedure developed for one soapstock does not necessarily work well with another soapstock [2]. Consequently, in this study, we employed selected processes from literatures and further investigated the experimental separation of γ-oryzanol from domestic soapstock.

In this study we divided our investigation into two parts. Part I: Subcritical water hydrolysis of deoiled rice bran and Part II: Separation of γ-oryzanol from rice bran oil soapstock.

2. MATERIAL AND METHOD

Part I: Subcritical water hydrolysis of deoiled rice bran

2.1.1 Materials. Deoiled rice bran was obtained from Thai Edible Oil Co., Ltd., Ayuthaya, Thailand.

2.1.2 Subcritical water hydrolysis. The hydrolysis reaction was carried out in closed batch reactor in which the effect of temperature in the range 200-220 °C, reaction time of 10-30 min and raw material to water weight ratio of 1:5 and 2:5 on the yield of protein, amino acid and reducing sugar.

2.1.3 Analytical method

1) Analysis of protein. Protein content of soluble portion was assayed using Lowry's method [3], using bovine serum albumin (BSA) as a standard.

2) Analysis of amino acids. Amino acids content was analyzed by Ninhydrin assays using L-Glutamic acid as a standard.

3) Analysis of reducing sugar. Reducing sugars content was assayed by dinitrosalicylic colorimetric method, using D-Glucose as a standard using dinitrosalicylic reagent developed by Sumner (1921) [4].

4) $ABTS^{+}$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 [5]. 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 absorbance of the reference, respectively.

Part II: Separation of γ-oryzanol from rice bran oil soapstock

2.2.1 Materials Rice bran oil soapstock was obtained from Thai Edible Oil Co., Ltd., Ayuthaya, Thailand.

2.2.2 Separation of γ-oryzanol The selected processes [6, 7, 8] were applied to separate γ-oryzanol from soapstock. This process includes the following steps: saponification, dehydration, leaching, and crystallization.

2.2.3 Quantification of γ-oryzanol γ-oryzanol contents was determined by spectrophotometric method.

3. RESULTS AND DISCUSSIONS

Part 1: Subcritical water hydrolysis of deoiled rice bran

3.1.1 Protein of soluble products

The amounts of protein in the soluble products obtained by SW hydrolysis of deoiled rice bran at various conditions are shown in Figure 1. The highest protein (130.17 ± 2.48 mg/g deoiled rice bran) was obtained from the 220 °C hydrolysis for 30 min. Based on the amount in the original bran reported by NIR (15.53 %wt) this account for the protein recovery of 84%.

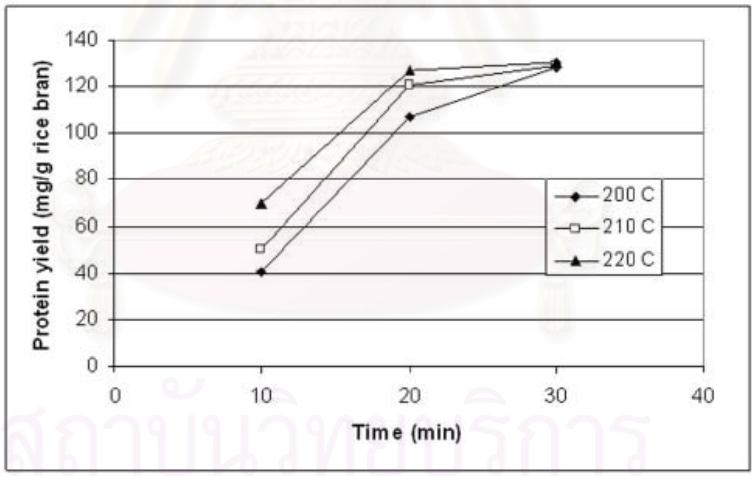

Figure 1. Protein yield after hydrolysis of deoiled rice bran at different temperature and time.

3.1.2 Amino acid of soluble product

The amino acids of soluble products obtained at various hydrolysis conditions are shown in Figure 2. The highest yield was 9.74 ± 50.08 μg/g raw rice bran, which was obtained at 220 °C for 20 min. This relatively low yield indicates that the rate of amino acids decomposition to smaller molecules of organic acids or other products was faster than the amino acid production. The temperature and reaction time do not have significant effects on the amount of total amino acids. At this condition, the result indicated that the rate of amino acid production was comparable to the rate of amino acid decomposition into smaller organic acids.

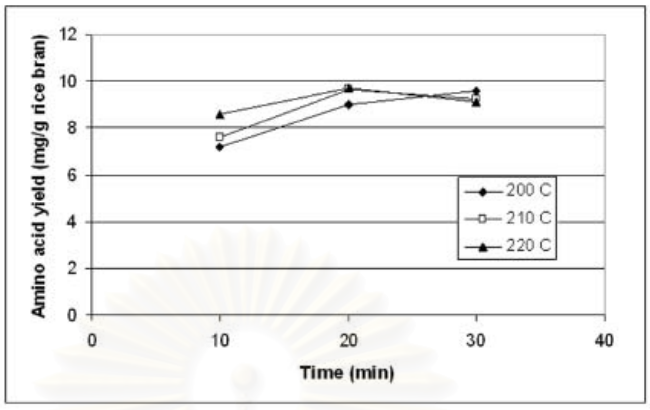

Figure 2. Amino acid yield after hydrolysis of raw materials at different temperature and time

3.1.3 Reducing sugar in soluble products

When carbohydrate reacts with hydronium and hydroxide ions, reducing sugars are produced. The reducing sugar of the soluble hydrolysis products at different temperature and time are shown in Figure 3. The reducing sugar content in the soluble products increased with increasing temperatures and times of reaction, except for the hydrolysis product obtained at 220°C and 30 min, whose reducing sugar content decreased. This result again indicated that at this condition, the rate of reducing sugar decomposition into smaller molecules of organic carbon was high, and that the decomposition of reducing sugar to other product was favored over the production of reducing sugar.

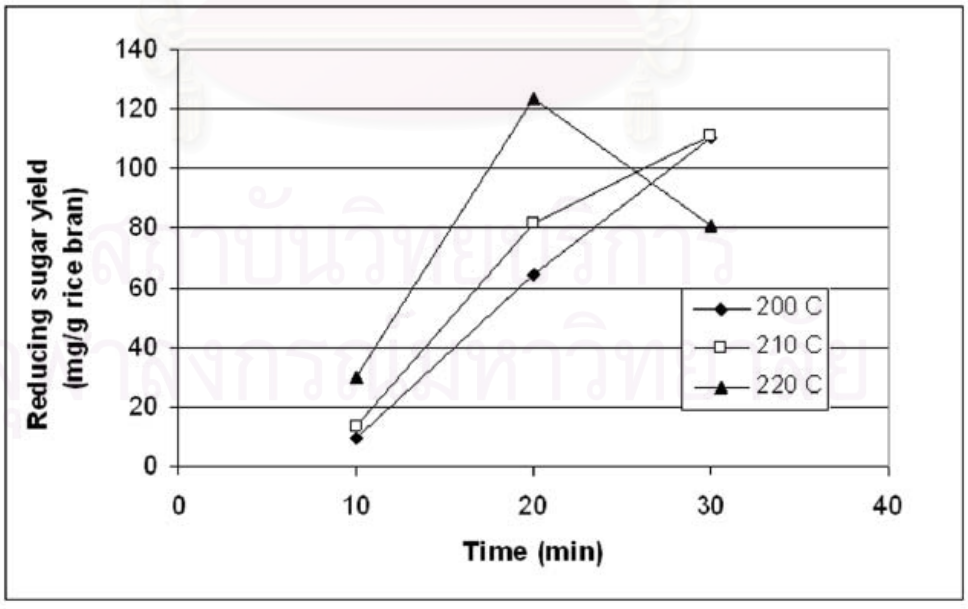

Figure 3. Reducing sugar yield after hydrolysis of raw materials at different temperature and time

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

The effect of the ratio of raw material-to-water on extraction yield was studied by comparing the product obtained from two different ratios of deoiled rice bran-to-water, 1:5 and 2:5 for reaction conditions at 210 °C and 30 min (Figure 4). It was found that the of protein, amino acids and reducing sugar decreased when the ratio of raw material-to-water was increased from 1:5 to 2:5. This is mainly because the high content of raw material increases the density and viscosity of the mixture, therefore caused the 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 of raw material-to-water of 1:5 was therefore more suitable.

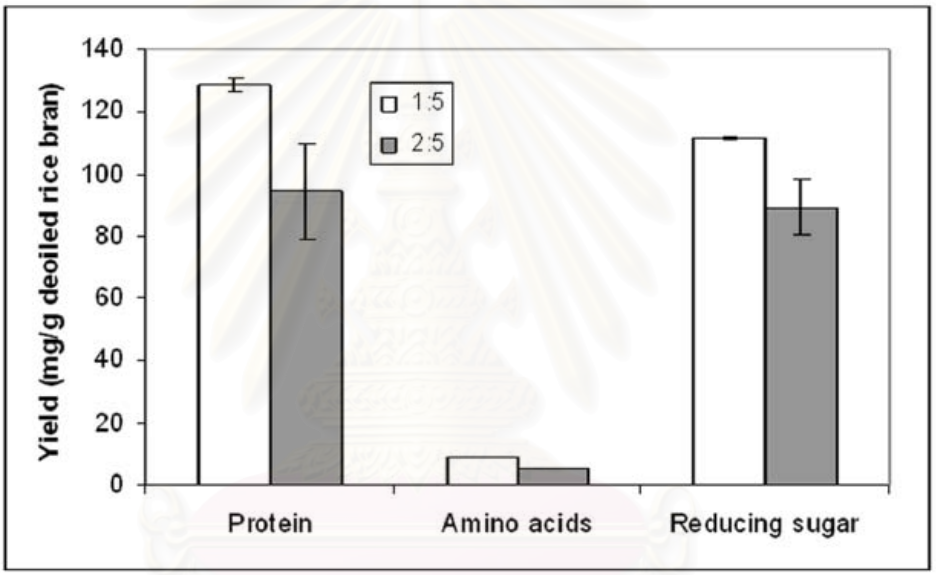

Figure 4. Effect of ratio of deoiled rice bran-to-water (1:5 and 2:5) for 210 °C and 30 min

3.1.5 Antioxidant activity

In this study, the antioxidant activity of soluble products obtained with SW was evaluated with $ABTS^{\bullet+}$ scavenging assay. Antioxidant activity was represented by (IC_{50}) index which is the concentration of sample producing 50% reduction of the radical absorbance. The antioxidant activity was measured at hydrolysis temperature of 200°C and 220 °C and hydrolysis time at 20 min and 30 min. These conditions were found to be suitable and were selected for the antioxidatant test. The results in Figure 5 indicated that with the hydrolysis time of 20 min, antioxidant activity increased as temperature increased from 200°C to 220°C. 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 but 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 better able to dissolve in water, thus the soluble products exhibited higher antioxidant activity. At long exposure time with high temperature, however, some antioxidative compounds might be degraded, thus the activity might decrease [8]. It is recommended that in the future study, analysis should be carried out to identify the antioxidant compounds obtained in the soluble products.

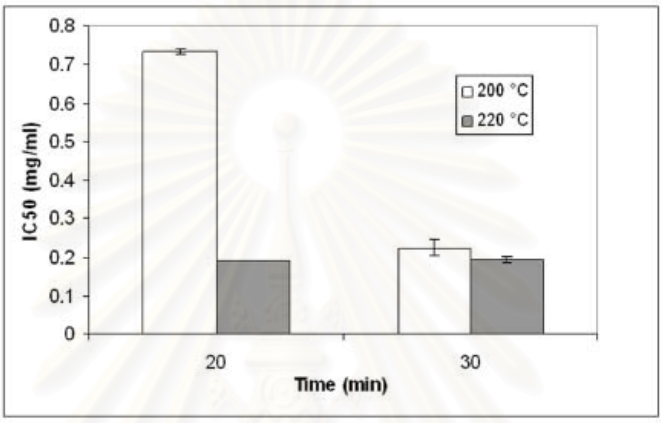

Figure 5. Antioxidant activity (IC_{50}) of the soluble products at hydrolysis times of 20 and 30 min and temperature of 200 °C and 220 °C.

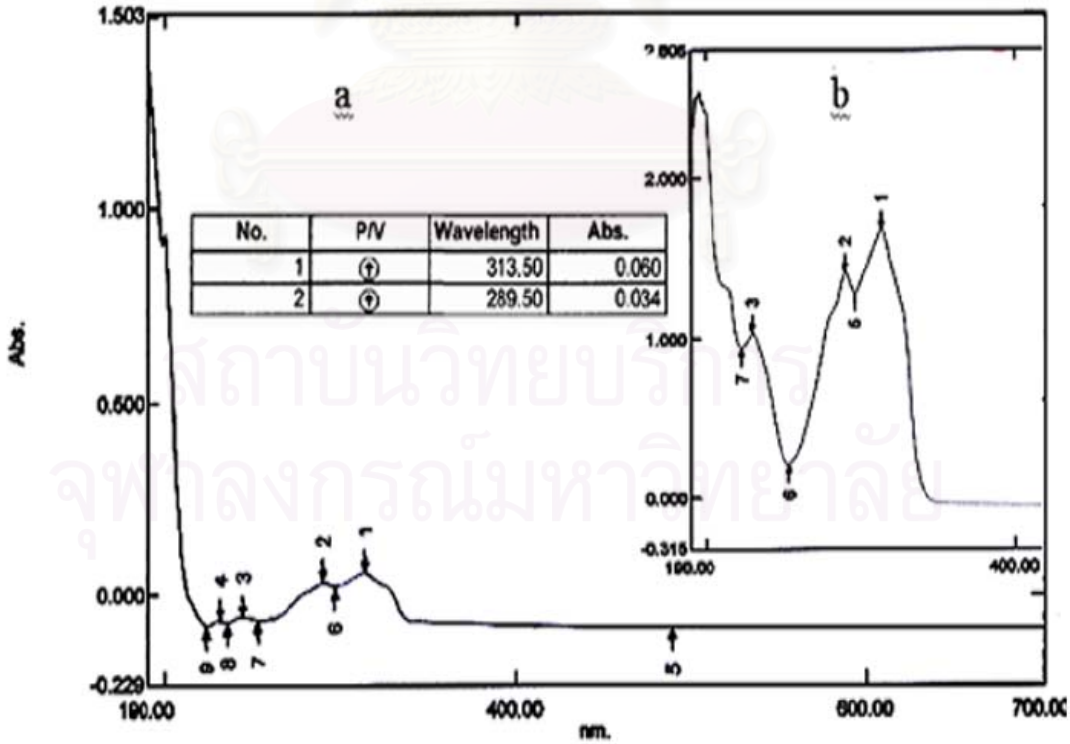

Figure 6. Spectrum peak scanning report for sample comparable to γ-oryzanol standard. (a) γ-oryzanol standard, (b) sample.

Part II: Separation of γ-oryzanol from rice bran oil soapstock by using selected processes

The separation γ-oryzanol from soapstock by selected processes was found to be appropriate for the acquired soapstock. Quantification γ-oryzanol in the sample obtained after recrystallization could be achieved by using UV spectrophotometer. It was found that spectrum peak of the sample was comparable to that of the standard with the same maximum absorption wave length as shown in Figure 6. Details on the effects of process conditions will be discussed further.

4. CONCLUSIONS

The results in the present study suggested that subcritical water could be used to potentially hydrolyze deoiled rice bran into more valuable products. The suitable condition for protein and amino acids production from deoiled rice bran by subcritical water hydrolysis was 1:5 at 30 min and hydrolysis temperature of 220 °C. At this condition, the protein: 130.17 mg/g rice bran, amino acid: 9.14 mg/g rice bran. The selected processes from literature could be applied for separation of γ-oryzanol from locally obtained soapstock.

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