Bio-active Compounds in Germinated Brown Rice and Its Application

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Food Technology
Prince of Songkla University
2010

Thesis Title	Bio-active Compounds in Germinated Brown Rice and Its Application
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ชื่อวิทยานิพนธ์ สารออกฤทธิ์ชีวภาพในข้าวกล้องงอกและการประยุกต์ใช้

ผู้เขียน นางสาวจิราภรณ์ บาลชื่น

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ปีการศึกษา 2552

บทคัดย่อ

การวิจัยนี้มีจุดประสงค์เพื่อศึกษาสารออกฤทธิ์ชีวภาพในข้าวกล้องงอก และการนำไปใช้ประโยชน์ในผลิตภัณฑ์เครื่องดื่มเพื่อสุขภาพ โดยศึกษาพันธุ์ข้าวที่เพาะปลูกในภาคใต้ จำนวน 3 สายพันธุ์ (ข้าวเหนียวดำเปลือกดำ ข้าวสังข์หยดพัทลุง และข้าวเฉี้ยงพัทลุง) การศึกษาสภาวะการงอกที่เหมาะสมของข้าวกล้องที่ให้ปริมาณแกมมา-อะมิโนบิวเทอริกเอซิด (สารกาบา) มากที่สุด โดยนำข้าวกล้องแช่ในสารละลายที่มีค่าพีเอชต่างๆ ได้แก่ พีเอช 7, 5, 3, 2 และน้ำกลั่น ที่อุณหภูมิห้อง (30±2 องศาเซลเซียส) เป็นเวลา 5 ชั่วโมง แล้วนำไปเพาะให้งอกในระบบเปิดและระบบปิด เป็นเวลา 12, 24, 36 และ 48 ชั่วโมง พบว่า สภาวะการเพาะที่ให้ปริมาณสารกาบามากที่สุด คือ การแช่ข้าวกล้องในสารละลายพีเอช 3 และทำให้งอกในระบบปิด โดยข้าวสังข์หยดพัทลุงและข้าวเฉี้ยงพัทลุง ใช้เวลางอก 36 ชั่วโมง ข้าวเหนียวดำเปลือกดำ ใช้เวลางอก 48 ชั่วโมง ปริมาณสารกาบาในข้าวกล้องงอกพันธุ์ข้าวเหนียวดำเปลือกดำ ข้าวสังข์หยดพัทลุงและข้าวเฉี้ยงพัทลุง มีค่าเพิ่มขึ้น 11.28, 16.74 และ 9.43 เท่า เมื่อเทียบกับข้าวกล้องที่ไม่ผ่านการงอก การวิเคราะห์องค์ประกอบทางโภชนาการ (โปรตีน, ไขมัน, เถ้า และใยอาหาร) และสารออกฤทธิ์ชีวภาพ (กรดเฟอรูลิก, ไฟเตท และแกมมา-ออไรซานอล) ในข้าวกล้องงอก พบว่า ปริมาณของโปรตีน ไขมัน ใยอาหารและกรดเฟอรูลิก มีค่าเพิ่มขึ้น ($p<0.05$) ปริมาณของแกมมา-ออไรซอล มีการเปลี่ยนแปลงเล็กน้อย ($p>0.05$) ส่วนปริมาณของไฟเตทมีค่าลดลง ($p<0.5$) เมื่อเทียบกับข้าวกล้องที่ไม่ผ่านการงอก ข้าวกล้องงอกพันธุ์สังข์หยดพัทลุง ซึ่งมีปริมาณของสารกาบามากที่สุด (44.53 มิลลิกรัม/100 กรัม) นำมาใช้ในการศึกษาต่อไป

นำข้าวกล้องงอกมาขัดสีและแยกเป็นส่วนของจมูกข้าว รำข้าวและข้าวขาว นำแต่ละส่วนมาสกัดสารกาบาด้วยตัวทำละลายชนิดต่างๆ (เอทานอลร้อยละ 75, เอทานอลร้อยละ 50 และน้ำกลั่น) ที่อุณหภูมิ 30, 40, 50 และ 60 องศาเซลเซียส เป็นเวลา 3, 6, 12 และ 24 ชั่วโมง พบว่าสภาวะการสกัดที่มีปริมาณของสารกาบามากที่สุด ในข้าวกล้องงอก จมูกข้าว รำข้าว และ ข้าวขาว คือ การสกัดโดยใช้น้ำกลั่นที่อุณหภูมิห้อง เป็นเวลา 6, 6, 24 และ 12 ชั่วโมง ตามลำดับ ฉะนั้นจึงคัดเลือกสภาวะนี้เพื่อใช้ในการศึกษาต่อไป

การพัฒนาผลิตภัณฑ์เครื่องดื่มเพื่อสุขภาพจากข้าวกล้องงอก ทำการสำรวจพฤติกรรมการบริโภคเครื่องดื่มเพื่อสุขภาพและความคิดเห็นต่อการพัฒนาเครื่องดื่มเพื่อสุขภาพจากข้าวกล้องงอก โดยใช้แบบสอบถามในการสำรวจกลุ่มผู้บริโภค จำนวน 300 คน ในเขตมหาวิทยาลัย หน่วยงานราชการ และห้างสรรพสินค้า ในจังหวัดสงขลา ผลการศึกษา พบว่า ผู้บริโภคส่วนใหญ่ดื่มเครื่องดื่มเพื่อสุขภาพ 3-4 ครั้ง/สัปดาห์ โดยเครื่องดื่มที่นิยมมากที่สุด 3 ลำดับ คือ ผลิตภัณฑ์นม น้ำผักหรือน้ำผลไม้ และผลิตภัณฑ์จากธัญพืช ปัจจัยหลักในการตัดสินใจเลือกซื้อเครื่องดื่มเพื่อสุขภาพ คือ รสชาติ คุณค่าทางโภชนาการ และราคา ความคิดเห็นของผู้บริโภคต่อการพัฒนาผลิตภัณฑ์เครื่องดื่มเพื่อสุขภาพจากข้าวกล้องงอก พบว่า ผู้บริโภคต้องการเครื่องดื่มชนิดพร้อมดื่ม บรรจุในขวดแก้ว และมีรสหวาน ซึ่งข้อมูลนี้นำมาใช้ในการศึกษาหาสูตรที่เหมาะสมของผลิตภัณฑ์เครื่องดื่มเพื่อสุขภาพจากข้าวกล้องงอก สำหรับการศึกษาสูตรที่เหมาะสมในการผลิตเครื่องดื่มเพื่อสุขภาพใช้เทคนิคแบบส่วนผสม และประเมินความสัมพันธ์ในรูปรีเกสชันต่อผลการทดสอบทางประสาทสัมผัสในด้านลักษณะปรากฏ สี ความหนืด เนื้อสัมผัส กลิ่นรส รสชาติ และความชอบรวม โดยใช้โปรแกรมวิเคราะห์สำเร็จรูป (Design-expert 7.1.6) พบว่า สูตรที่เหมาะสม ประกอบด้วย สารสกัดจากข้าวกล้องงอกร้อยละ 83.3 ถั่วเหลืองร้อยละ 10.0 น้ำตาลร้อยละ 5.0 และ นมผงร้อยละ 1.7 เครื่องดื่มเพื่อสุขภาพที่พัฒนาขึ้นได้รับการยอมรับจากกลุ่มผู้บริโภค คุณค่าทางโภชนาการต่อ 1 หน่วยบริโภค (240 มิลลิลิตร) ของเครื่องดื่มเพื่อสุขภาพจากข้าวกล้องงอก ประกอบด้วย พลังงาน 170 กิโลแคลอรี โปรตีน 10 กรัม ไขมัน 4.5 กรัม คาร์โบไฮเดรต 22 กรัม ใยอาหาร 1 กรัม น้ำตาล 17 กรัม โซเดียม 35 มิลลิกรัม วิตามินบีหนึ่ง 0.14 มิลลิกรัม วิตามินบีสอง 0.07 มิลลิกรัม แคลเซียม 65 มิลลิกรัม เหล็ก 1.0 มิลลิกรัม และสารกาบา 41.30 มิลลิกรัม

การศึกษาอายุการเก็บรักษาของผลิตภัณฑ์เครื่องดื่มสุขภาพจากข้าวกล้องงอก โดยการวิเคราะห์การเปลี่ยนแปลงคุณภาพของผลิตภัณฑ์ระหว่างการเก็บรักษาที่อุณหภูมิ 25, 35 และ 45 องศาเซลเซียส เป็นเวลา 12 สัปดาห์ พบว่า การเปลี่ยนแปลงค่าพีเอช ปริมาณของแข็งที่ละลายได้ทั้งหมด ความหนืด ความเป็นกรด และสารกาบามีค่าไม่แตกต่างกัน ($p>0.05$) ในขณะที่การเปลี่ยนแปลงค่าสีมีค่าแตกต่างกัน ($p<0.05$) ผลการทดสอบทางด้านประสาทสัมผัส พบว่า คะแนนการทดสอบมีค่าลดลง โดยเฉพาะคะแนนด้านลักษณะปรากฏ สี และความชอบรวม ($p<0.05$) แต่คะแนนด้านกลิ่นรส ความหนืด เนื้อสัมผัส และรสชาติ มีค่าลดลงไม่แตกต่างกัน ($p>0.05$) การเปลี่ยนแปลงค่าสี และผลการทดสอบความชอบรวม ถูกนำมาใช้เป็นตัวชี้วัดในการทำนายอายุการเก็บรักษาของผลิตภัณฑ์ ผลการศึกษา พบว่า สมการทำนายอายุการเก็บรักษาที่ได้มีค่าสัมประสิทธิ์สหสัมพันธ์สูง ($R^2>0.95$) เมื่อกำหนดค่าชี้วัดการเปลี่ยนแปลงค่าสี เท่ากับ 10 และคะแนนความชอบ

รวม เท่ากับ 5 อายุการเก็บรักษาของผลิตภัณฑ์ที่อุณหภูมิ 30 องศาเซลเซียส (อุณหภูมิห้อง) มีค่าเท่ากับ 40.64 สัปดาห์ (หรือ 9.5 เดือน)

Thesis Title	Bio-active compounds in germinated brown rice and its application
Author	Miss Jiraporn Banchuen
Major Program	Food Technology
Academic Year	2009

ABSTRACT

Aim of this study was to investigate bio-active compounds in germinated brown rice and develop the functional drink from germinated brown rice. Three brown rice varieties grown in the southern Thailand (var. Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung) were used as samples for investigating. Optimum germination conditions to maximize γ-aminobutyric acid (GABA) content in brown rice were determined. The brown rice was soaked in various pH solutions which are pH 7.0, 5.0, 3.0, 2.0 and distilled water at room temperature (30±2^{o}C) for 5 hours and germinated in open and closed vessel for 12, 24, 36 and 48 hours. Results indicated that the highest GABA content was obtained when the rice was soaked in solution at pH 3 and germinated in closed vessel 36 hours for Sangyod Phatthalung and Chiang Phatthalung and 48 hours for Niaw Dam Peuak Dam. The GABA content in Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung increased 11.28, 16.74 and 9.43 times when compared to un-germinated brown rice. The nutritional components (protein, fat, ash and dietary fiber) and bio-active compounds (ferulic acid, phytate and γ-oryzanol) in germinated brown rice were also analyzed. The germinated brown rice contained more protein, fat, dietary fiber and ferulic acid ($p<0.05$) than brown rice, while γ-oryzanol content was in the same level for both brown rice and germinated brown rice ($p>0.05$). On the contrary, germinated brown rice contained phytate content lower than brown rice ($p<0.05$). Germinated brown rice var. Sangyod Phatthalung which had the highest of GABA content (44.53 mg/100g) was selected for further study.

Germinated brown rice was milled and separated in fractions of germ, bran and white rice. Germinated brown rice and its fractions were extracted in various

solvents (75% ethanol, 50% ethanol and distilled water) at 30, 40, 50 and 60°C with extracting time were 3, 6, 12, and 24 hours. Results showed that the highest GABA content in germinated brown rice, germ, bran, and white rice were obtained when the sample was extracted with distilled water at 30°C for 6, 6, 24 and 12 hours, respectively. The optimal extraction which had the highest of GABA content was selected for subsequent preparation of germinated brown rice extract.

To develop the functional drink from germinated brown rice, the questionnaire, as a tool, was used to survey the consumer behaviors and opinion of functional drink from germinated brown rice. The 300 residents in University, government offices and department store in Songkhla, Thailand were the sample size. The results indicated that the respondents consumed functional drink at 3-4 times/week. The top three functional drinks were dairy product, fruit or vegetable juice and cereal product. In order to purchase the functional drink, taste, nutrition and price were the major criteria for the consumer's decision. The product profile of functional drink from germinated brown rice as ready-to-drink in glass bottle with sweet flavor was preferred. Mixture design technique was used to optimize the formulation of the functional drink from germinated brown rice. The regression model on sensory on appearance, color, viscosity, texture, flavor, taste and overall acceptability were established. With the aid of analysis software (Design-expert 7.1.6), the best formulation composed of 83.3% of germinated brown rice extracts, 10.0% of soybean, 5.0% of sugar, 1.7% of milk powder. The developed functional drink was accepted by the consumer according to the acceptance test. One serving size (240 mL) composes of energy 170 Kcal, protein 10.0 g, fat 4.5 g, carbohydrate 22.0 g, dietary fiber 1.0 g, sugar 17 g, sodium 35 mg, vitamin B1 0.14 mg, vitamin B2 0.07 mg, calcium 65 mg, iron 1.0 g and GABA 41.30 mg.

Shelf life of functional drink was investigated by determining the changes of properties during 12 weeks storage. The results showed that pH value, total soluble solid, viscosity, acidity and GABA content were not significantly different ($p>0.05$) while total color difference (ΔE) was significantly different ($p<0.05$) during storage at various temperatures. Sensory evaluation of functional drink showed a decreased in attributes of sensory especially appearance, color and

overall acceptability ($p<0.05$). However, change of flavor, viscosity, texture and taste were not significantly different ($p>0.05$) during storage at various temperatures. ΔE and overall acceptability were chosen as the parameter for determining shelf life of functional drink. The shelf life equation that has a high correlation coefficient ($R^2>0.95$) was obtain. The criteria used for estimating the shelf life of the functional drink particularly ΔE is 10 and overall acceptability score is 5. Shelf life of functional drink stored at 30°C (room temperature) was 40.64 weeks (or 9.5 months).

Acknowledgement

I would like to express my greatest and sincere gratitude to advisory committee, Assoc. Prof. Paiboon Thammarutwasik, Prof. Dr. Buncha Ooraikul, Asst. Prof. Dr. Phaisan Wuttijumnong and Asst. Prof. Dr. Piyarat Sirivongpaisal for their kindness, valuable advises and suggestions, supporting, encouragement, guidance, and criticism throughout of my study program.

I also would like to express my greatest and sincere gratitude to examining committee, Dr. Sunisa Siripongvutikorn, Assoc. Prof. Dr. Chaweewan Jansakul and Assoc. Prof. Dr. Rungnaphar Pongsawatmanit for their valuable suggestion.

Appreciation is also express to all staff and members of the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University for providing the great environment and friendship during the time of my study.

This work was supported by Agricultural Research Development Agency (Public Organization) and the grant-in-aid for dissertation from Prince of Songkla University.

Finally, I would like to express my sincere gratitude and appreciation to my dear parents and also the members of my family for their love, pushing up, cheerfulness, devoting and encouragement throughout my life.

Jiraporn Banchuen

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CHAPTER 1

INTRODUCTION

Introduction

Rice grain is the seed of the monocot plant *Oryza sativa*, of the grass family (Poaceae). As a cereal grain, it is one of the most important cereal grains in the world, apart from wheat and corn (Kainuma, 2004). The total rice paddy production area is about 157 million hectare and the annual production of rice is about 650 million tons (FAOSTAT, 2008). Rice accounts for over 22% of global energy intake, and its production and consumption is concentrated in Asia. In Thailand, rice is of special importance as it is the staple food for 64.24 million people. Thailand consumes about 55% of its rice production while the remaining 45% is exported to the world market (Vanichanont, 2004). Thailand exports many kinds of rice including white rice derived from varieties of rice, aromatic rice, parboiled rice and glutinous rice. In 2008, The Thai Office of Agriculture Economics reported that approximate total rice paddy area and production of rice were 9.19 million hectares and 23 million tons including 0.31 million hectares and 0.75 million tons of rice in the South. Rice productions areas in the South are Songkhla lake basin covering 3 provinces Nakhon Si Thammarat, Songkhla and Phatthalung.

In the past, several traditional rice cultivars could be produced in the area under different conditions. There are more than 4,000 cultivars of the South. Changes in farmers' practices result in growing modern rice cultivars instead of the traditional ones. Most traditional rice cultivars have been collected for conservation whereas some of them lost (Saeton *et al*., 2009). Consequently, the concepts of direct use of traditional rice are improving the cultivars suitable for specific planting areas and studying the nutritional of traditional rice for their utilization in food, pharmaceutical and cosmetic product in order to increased demand and added value of rice.

Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung are the traditional rice planed in the South. The brown rice grains are different in pigment color such as black, purple, or red. The black and red varieties are planted

mainly in the South East Asia, Italy Greece, United State, China, and Japan which people have been consumed pigmented rice. However, in Thailand, total consumption of pigmented rice is very low due to hard texture. In pigmented rice, there is natural colorant which called anthocyanin. A common anthocyanin found in red rice is acetylated procyanidins which was reported to posses a free radical scavenging activity (Oki *et al*., 2002), however, the information of traditional rice is limited.

Brown rice contains more nutritional components, such as dietary fibers, phytic acid, vitamin E and vitamin B than the ordinary milled rice. These bio-functional components exist mainly in the germ and bran layers most of which are removed by polishing or milling (Champagne *et al*., 2004). Since traditional rice very little attention in academic and commercials aspects, it is interesting to study its properties in more details. Unfortunately, brown rice takes longer to cook and cooked brown rice is harder to chew and not as tasty as white rice. In germinated grains, hydrolytic enzymes are activated and they decompose starch, non-starch polysaccharides and proteins, which leads to the increase of oligosaccharides, and amino acids (Manna *et al*., 1995). The decomposition of the high molecular weight polymers during germination leads to the generation of bio-functional substances and the improvement of the organoleptic qualities due to the softening of texture and the increase in the flavor. Germinated brown rice offers considerable benefits, which include the increase in γ-aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolylendopeptidase inhibitor. Additionally, the germination of brown rice frees its bound minerals, making them more absorbable by the body and the rice tendered and tastier (Kayahara, 2004).

Presently, germinated brown rice is becoming increasingly popular in Japan, Korean and Thailand. The products base on germinated brown rice e.g. porridge, vinegar and miso, are commercially available and produced in Japan. In Thailand, pasteurized drinks and instant powder are commercial product of germinated brown rice. To develop some value-added rice products is important to encourage rice consumption especially rice products of traditional rice from the South, however, the information of theirs is limited.

In this study, the nutrients and bio-active compounds of germination brown rice were investigated. The optimized extraction methods were used for extractions for the bio-active compounds from germinated brown rice and its fractions. Additionally, functional drinks from germinated brown rice were formulated.

Review of Literatures

Rice and structure

Rice (*Oryza sativa L.*) is a semi-aquatic, annual, grass plant. Rice has more than 120,000 varieties and cultivated rice is designated as either *Oryza sativa L.* the predominate species or *Oryza glaberrima Steud.*, which is grown only in Africa on a limited scale (Juliano, 1993). Nowadays, about 90% of the world's rice production occurs in Asia. Rice grown in Asia is *O. sativa*, which can be grown under a broad range of climatic and geographic conditions extending from 53°north latitude to 35°south latitude and from sea level to an altitude of 2,500 meters. In addition, the optimum temperature is 30°C to 34°C. The species *O. sativa*, can be categorized into three subspecies that are Japonica (planted in temperate area such as Japan and China), Javanica (planted only in Indonesia) and Indica (planted in tropical area such as India and the Philippines, including Thailand) (Juliano and Bechtel, 1985).

Rice is classified by amylose content into waxy (0-2%) and non-waxy: low (12-20%), intermediate (20-25%), and high (25-33%). In addition, rice is also classified into three types by grain size, namely: short-grain, medium-grain and long-grain rice. Short and medium-grain varieties have low amylose content (12-20%) and some long-grain varieties have intermediate amylose content (20-25%) and others have high amylose content (>25%) (Juliano and Bechtel, 1985).

The rice grain consists of hull, pericarp, seed coat, nucellus, embryo, aleurone layer and endosperm as shown in Figure 1.

Hull

The hull is the outer covering for the caryosis (brown rice). It serves a protective function against insect infestation and rapid changes in moisture content of the grain due to large humidity fluctuations in the external environment. It comprises 18-20% by weight of the paddy grain. Hulls are low in protein, fat and starch but high in crude fiber, crude ash and dietary fiber (Table 1).

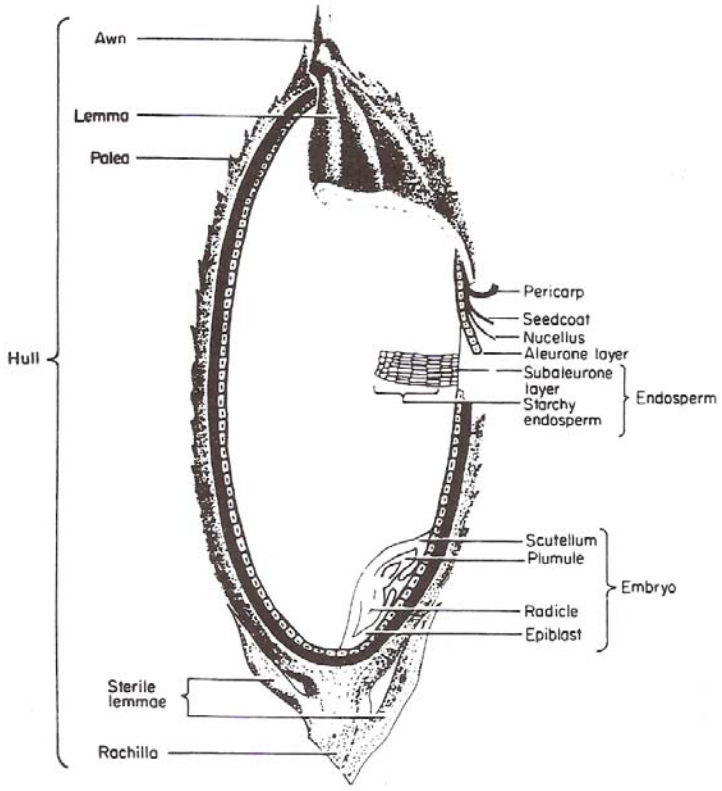


Figure 1. Structure of the mature rice grain
Source: Juliano and Bechtel (1985)

Caryopsis

Removal of the hull from paddy grain by dehulling exposes the rice caryopsis, which are the pericarp, seed coat (tegmen), nucellus and aleurone (Figure 1). Along with much of the embryo (germ), these layers comprise the bran portion of the rice grain. The bran portion accounts for 8% of the brown rice weight. The bran is the most nutritious part of the caryopsis. Bran contains of protein, fat (rice oil), carbohydrate (starch) and dietary fiber (Table 1).

Subaleurone layer and starchy endosperm

Further milling of the rice caryopsis removes the subaleurone layer and a small part of the starchy endosperm. This milling fraction is referred to as polish. The end result of bran and polish removal is milled (white) rice. The polish comprises 3-4% by weight of brown rice. The starchy endosperm is rich in starch granules; contain some protein bodies, especially in the outer endosperm layers, and almost no lipid bodies. Polish contains only slightly less protein and lipid but

considerably more starch than bran (Table 1). The polish fraction is only slightly less nutritious than the bran fraction, primarily because it contains lower levels of minerals and vitamins than the bran.

Table 1. Proximate compositions of rice grains

Rice	Protein	Fat	Fiber	Ash	Starch	Dietary fiber
Rough	6.7-8.3	2.1-2.7	8.4-12.1	3.4-6.0	62.1	19.1
Brown	8.3-9.6	2.1-3.3	0.7-1.2	1.2-1.8	77.2	4.5
Milled	7.3-8.3	0.4-0.6	0.3-0.6	0.4-0.9	90.2	2.7
Hull	2.3-3.2	0.4-0.7	40.1-53.4	15.3-24.4	1.8	77.3
Bran	13.2-17.3	17.0-22.9	9.5-13.2	9.2-11.5	16.1	27.6-33.3
Embryo	17.7-23.9	19.3-23.8	2.8-4.1	6.8-10.1	2.4	-
Polish	13.0-14.4	11.7-14.4	2.7-3.7	6.1-8.5	48.3-55.4	-

Source: Juliano and Bechtel (1985)

Germination of brown rice

Germination is the growth of an embryonic plant contained within a seed; it results in the formation of the seedling. A process involved in incorporating those events that commence with the uptake of water by a quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994). The visible sign of complete germination is the penetration of the structures surrounding the embryo by the radicle; the result is called visible germination. Subsequent events, including the mobilization of the major storage reserves, are associated with growth of the seedling (Bewley, 1997).

A mature dry seed uptake of water is shown tri-phase (Figure 2), with a rapid initial uptake (phase I). Followed by a plateau phase (phase II) which embryonic axes elongate and enter to complete germination (phase III).

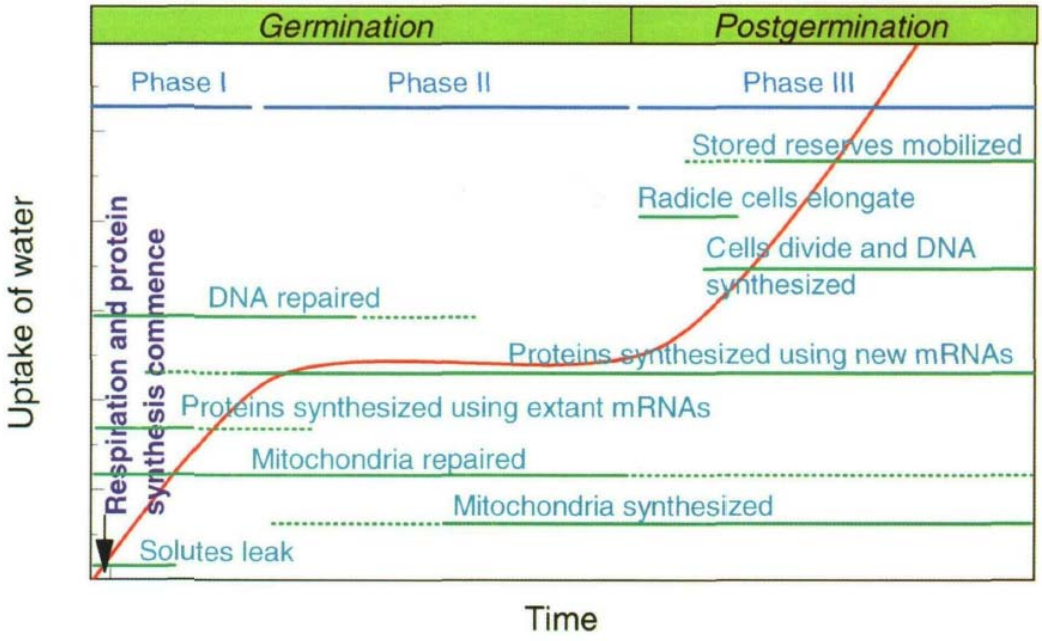


Figure 2. Time course of major events associated with germination and subsequent post-germination growth

Source: Bewley (1997)

The influx of water into the cells of dry seeds during phase I results in temporary structural perturbations, particularly to membranes, which lead to an immediate and rapid leakage of solutes and low molecular weight metabolites into the surrounding imbibition solution (Crowe and Crowe, 1992). Upon imbibition, the quiescent dry seed rapidly resumes metabolic activity. The structures and enzymes necessary for this initial resumption of metabolic activity are generally assumed to be present within the dry seed, having survived and resumption of respiratory activity. After a steep initial increase in oxygen consumption, the rate declines until the radicle penetrates the surrounding structures. At this time, another burst of respiratory activity occurs (Botha *et al.*, 1992; Bewley and Black, 1994). The glycolytic and oxidative pentose phosphate pathways both resume during phase I, and the Kreb's cycle enzymes become activated (Nicolas and Aldasoro, 1979; Salon *et al.*, 1988). Tissues of the mature dry seed contain mitochondria. They contain sufficient Kreb's cycle enzymes and terminal oxidizes to provide adequate amounts of ATP to support metabolism for several hours after imbibition (Ehrenshaft and Brambl, 1990; Attucci *et al.*, 1991).

Requirements for seed germination are dependent on internal and external condition. Different plants have seed that require distinctive variables for successful germination. Often this depends on the viability and life span of seed. The length of time for which seeds can remain viable is extremely variable and depends both on the storage conditions and the type of seed. In general, viability is retained best under conditions in which the metabolic activity of seeds is greatly reduced, i.e. low temperature and high carbon dioxide concentration. In addition, however, other factors are of great importance, particularly those which determine seed dormancy. The period for which seeds remain viable is determined genetically and by environmental factors. The latter will in fact have a decisive effect on the life span of any given seed, i.e. whether the seed will remain viable for the longest genetically possible period or whether it will lose its viability at some earlier stage (Mayer and Poljakoff-Mayber, 1975). Future germination is affecting environmental conditions during seed formation.

In order that a seed can germinate, it must be placed in environmental conditions favorable to this process. Among the conditions required are an adequate supply of water, a suitable temperature and composition of the gases in the atmosphere, as well as light for certain seeds. The requirement for these conditions varies according to the species and variety.

Water is required for germination. Mature seeds are often extremely dry and need to uptake of water and growth can resume. The uptake of water by seeds is called imbibitions, which leads to the swelling and the breaking of the seed coat. The extent to which imbibition occurs is determined by 3 factors; the composition of the seed, the permeability of the seed coat to water and the availability of water in liquid or gaseous form in the environment. When seeds are formed, most plants store a food reserve with in the seed, such as starch, protein, or oil. This food reserve provides nourishment to the growing embryo. When the seed imbibes water, hydrolytic enzymes are activated which break down these stored food resources into metabolically useful chemicals.

Germination is a process related to living cells and requires an expenditure of energy by these cells. The energy-requirement of living cells is usually sustained by processes of oxidation, in the presence or absence of oxygen, i.e.

respiration or fermentation. These involve an exchange of gases, an output of carbon dioxide in both cases and also the uptake of oxygen in the case of respiration. Consequently seed germination is markedly affected by the composition of the ambient atmosphere. Most seeds germinate in air, i.e. in an atmosphere containing 20% oxygen and 0.03% carbon dioxide (Mayer and Poljakoff-Mayber, 1975).

Different seeds have different temperature ranges within which they germinate. At very low temperatures and very high temperatures the germination of all seeds is prevented. Temperature effects cellular metabolic and growth rate. Usually, seeds germinate at temperature slightly higher than room temperature (16-24°C) while others germinate in cool (-2 to 4°C) or warm (24-32°C) temperature. For instants, the optimum temperature for germination of high amylose rice var. Doongara were 25°C for 24 hours or 35°C for 16 hours (Capanzana and Buckle, 1997).

Light or darkness can be a type of environmental trigger for germination. The seeds of most cultivated plants usually germinate equally well in the dark and in the light. For instant, the changes in the content of starch, protein, and RNA of rice (var. IR8) were more rapid in the dark than in the light rice (Palmiano and Juliano, 1972).

Bio-active compounds in brown rice

γ-aminobutyric acid (GABA)

γ-aminobutyric acid (GABA) is a four-carbon non-protein amino acid (Figure 3). It is highly soluble in water. Structurally it is a flexible molecule that can assume several conformations in solution, including a cyclic structure that is similar to proline. GABA is zwitter-ionic (carries both a positive and negative charge) at physiological pH values (pK values of 4.03 and 10.56) (Christensen, 1994).

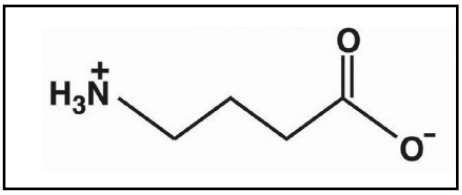


Figure 3. Structure of GABA
Source: Shelp *et al*. (1999)

GABA is mainly metabolized via a short pathway composed of three enzymes, called the GABA shunt (Figure 4). The glutamine-synthetase/glutamate-synthase (GS/GOGAT) cycle is the principal nitrogen assimilation pathway into glutamate and amino acids in plants. The glutamate dehydrogenase (GDH) is thought to function primarily in glutamate catabolism but can also function in the opposite direction. The GABA shunt is composed of three enzymes including glutamate decarboxylase, GABA transaminases and succinic semialdehyde. Glutamate decarboxylase (GAD) is a cytosolic enzyme regulated by the Ca^{2+}-calmodulin (CaM) complex, which catalyses the irreversible decarboxylation of glutamate to produce GABA. GABA is transported into the mitochondria, where it is converted into succinic semialdehyde by GABA transaminases using either α-ketoglutarate (by GABA-TK) or pyruvate (by GABA-TP) as amino acid acceptors. Succinic semialdehyde is then reduced by succinic semialdehyde dehydrogenase (SSADH) to form succinate, which enters the tricarboxylic acid (TCA) cycle (Bouche and Fromm, 2004).

Typically, GABA levels in plant tissues are low (0.03-2.00 μmol/g) (Rhodes *et al*., 1986; Fougere *et al*., 1991). Methods to increase GABA concentrations of food have been studied up to today. For example, GABA is higher in Gabaron tea, an anaerobically incubated tea (Sawai *et al*., 2001; Tsushida and Murai, 1987), in bean sprouts treated with carbon dioxide (Katagiri and Shimizu, 1989). The researchers have reported the method to increase of GABA content in rice grain as shown in Table 2. GABA accumulation in germinated brown rice was found to increase according to various conditions such as varieties of rice, germination time, gaseous treatment, high pressure treatment, soaking solution or germination temperature.

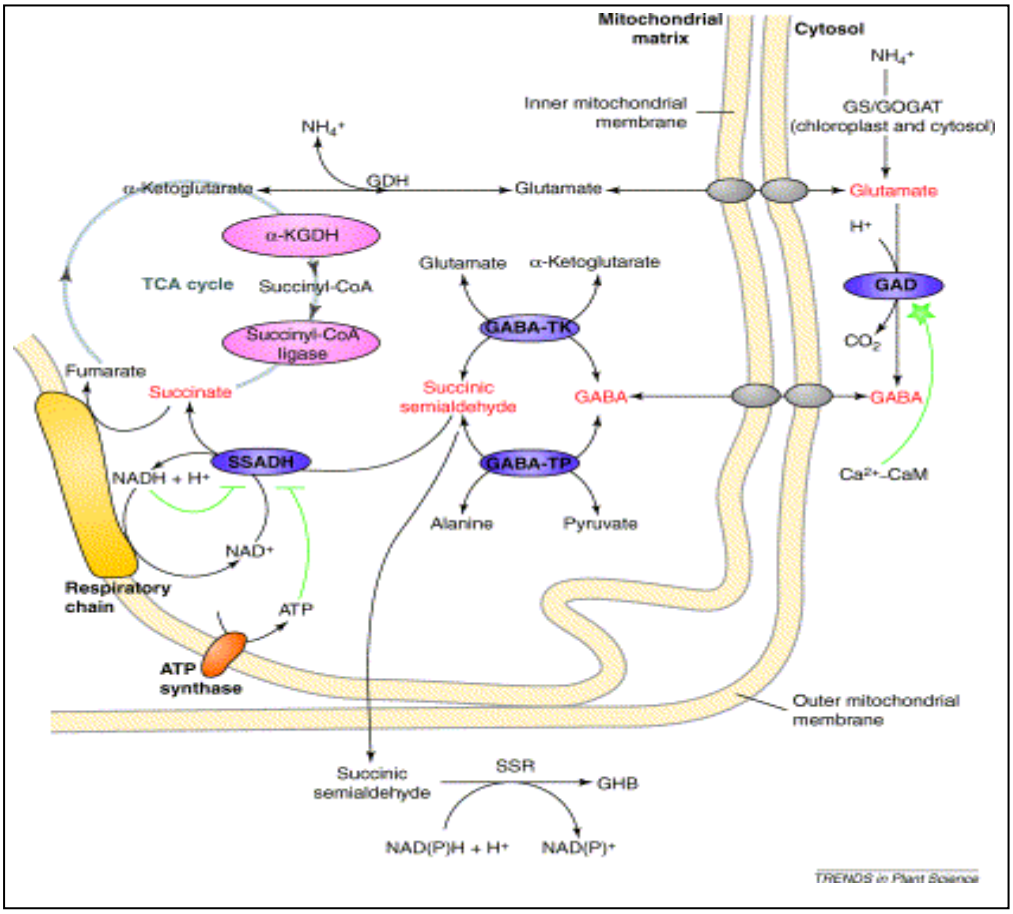


Figure 4. The GABA shunt metabolic pathway

Source: Bouche and Fromm (2004)

GABA has pharmacological functions such as, as an antihypertensive agent, as a diuretic, as well as having a tranquilizing effect and as being an inhibitory neurotransmitter in sympathetic brain functions (Su *et al*. 2003; Komatsuzaki *et al*. 2005, 2007; Huang *et al*. 2007). GABA can regulate blood pressure, heart rate, sensations of pain and anxiety (Kono and Himeno 2000; Toshio *et al*., 2000), lipid levels in serum (Miura *et al*. 2006) and assist in insulin secretion to prevent diabetes (Huang *et al*. 2007). Recently, it has been reported that germinated brown rice could effectively reduce glucose levels in diabetic rats (Hagiwara *et al*. 2004) and control of postprandial blood glucose concentration (Ito *et al*., 2005). Moreover, consumption of GABA-enriched foods can inhibit cancer cell proliferation (Park and Oh 2007; Oh and Oh, 2004) and improve memory and the learning abilities in rats (Miura *et al*. 2006).

Table 2. Method to increase of GABA content in rice grain

Sample	Method	References
Japanese rice	Soaked in distilled water at 25°C for 18 hours and subject to high pressure at 400 MPa	Miwako *et al*., 1999
	Soaking in water at 30°C for 72 hours	Ohtsubo *et al*., 2005
	Soaked in distilled water for 3 hours and subjected to gaseous treatment 35°C for 21 hours	Komatsuzaki *et al*., 2007
Korea rice	Soaked in 50 ppm chitosan in 5 mM glutamic acid at 25-26°C in the dark for 72 hours	Oh, 2003
Thai rice	Soaked in 0.1 mM $CaCl_2$, pH 5.0 at 40°C for 36 hours	Sunte *et al*., 2007
	Soaked in water at pH 6 at 35°C for 24 hours	Watchraparpaiboon *et al*., 2007
	Soaked in pH 3 for 48 hours	Charoenthaikij *et al*., 2009

γ-oryzanol

Oryzanol is a mixture of ferulic acids esterified with normal sterols or triterpene alcohols, called α, β and γ-oryzanol, of which γ-oryzanol has been the most commonly mentioned. The sterol components of γ-oryzanol are primarily campesterol and sitosterol, and the triterpene alcohol components are cycloartenol and 24-methylene cycloartanol (Dey and Harborne, 1991).

The pathway of terpenoids biosynthesis is enzymatically controlled and starts from acetyl-CoA, to mevalonate, to 2E, 6E-farnesyl pyrophophate, to squalene, and then to triterpenoids, with a final chair-boat-chair-boat configuration as shown in Figure 5. Phytosterols are further formed from triterpenoids or cycloartenol (Dey and Harborne, 1991). The initial pathway from acetyl-CoA to squalene is common to all organisms, but the sequence of the modifications of the sterol ring

system and side chain can differ from species or even in various tissues or during different developmental stages in a plant. Sterol production supposedly occurs in the cytosolic and microsomal compartments of the plant cells (Huang, 2003).

Phytosterols play a structural role in plant cells as an essential membrane constituent by analogy with cholesterol in animal cells which regulates its fluidity and enhances the mechanical stability of the membrane. Sterols are important for plant growth in two ways. One is for new membrane production by dividing cells and the other is for a 24-ethylsterol such as stigmasterol for specific stimulatory function for cell division to proceed (Goad, 1991).

Xu and Godber (1999) identified components of γ-oryzanol from rice bran oil were Δ7-stigmastenyl ferulate, stigmasteryl ferulate, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, Δ7-campestenyl ferulate, campesteryl ferulate, Δ7-sitostenyl ferulate, sitosteryl ferulate, campestanyl ferulate and sitostanyl ferulate (Figure 6). The three major components among these are cycloartenyl ferulate, 24-methylene cycloartanyl ferulate and campesteryl ferulate.

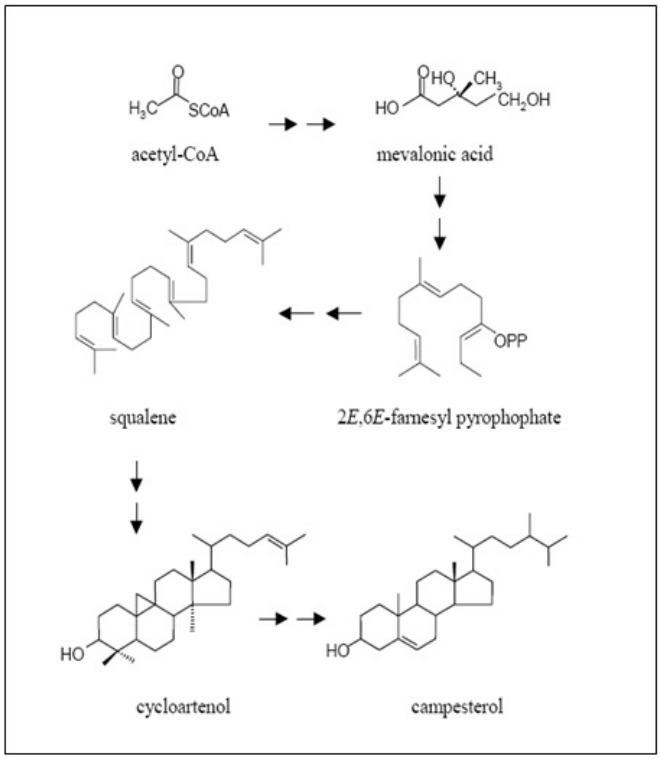


Figure 5. Pathways for biosynthesis of terpenoids in plants

Source: Huang (2003)

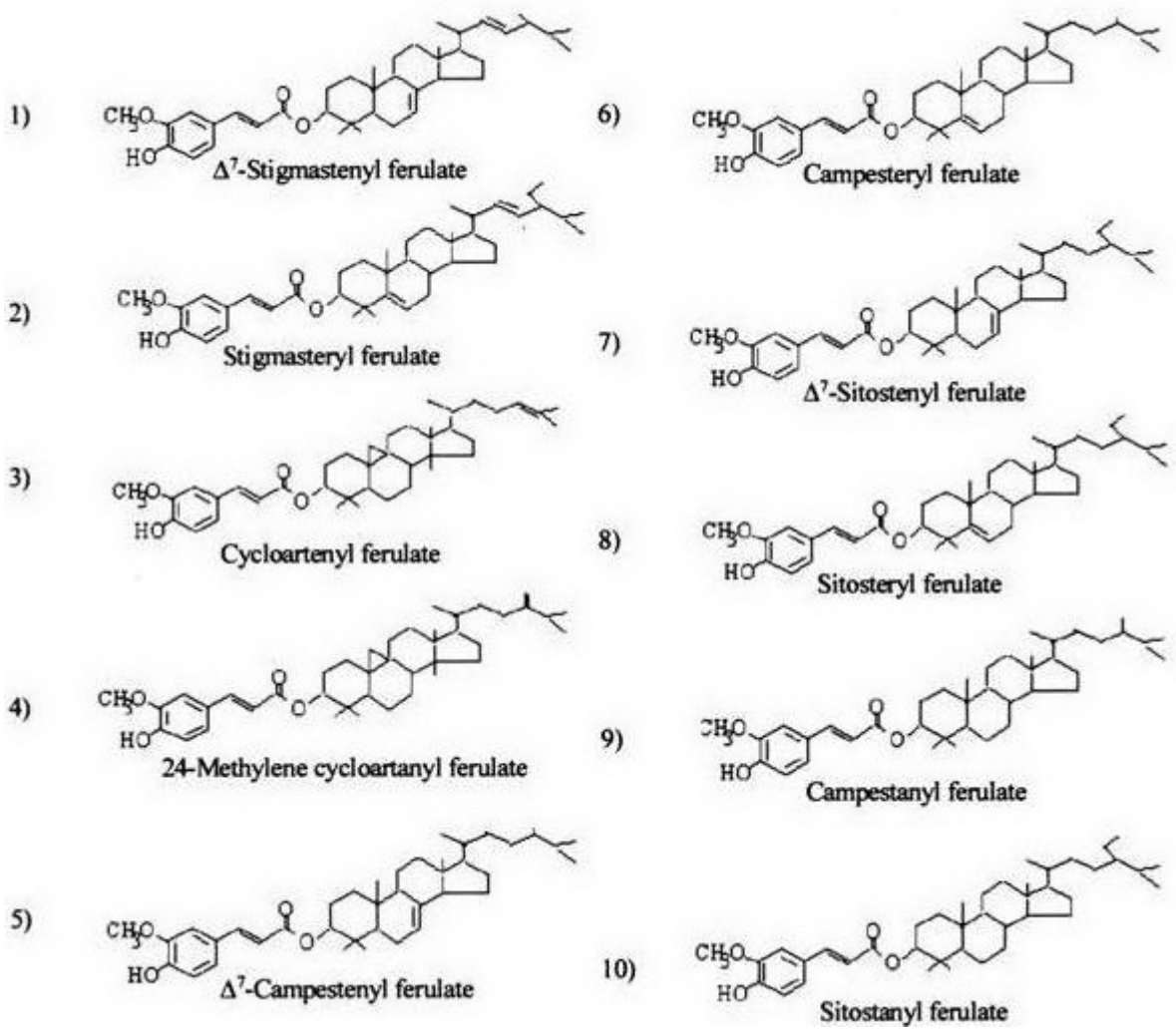


Figure 6. Molecular structures of identified components of γ-oryzanol
Source: Xu and Godber (1999)

The content of γ-oryzanol varies between different rice varieties as shown in Table 3. The highest content of γ-oryzanol were found in bran layers of the kernels and varies approximately between 20-75 mg/100g in brown rice kernels whereas the range of γ-oryzanol content in polished rice is lowest.

Table 3. γ-Oryzanol content in rice grains

Sample	Oryzanol content (mg/100g)	References
Brown rice	20.1-38.8	Aguilar-Garcia *et al*., 2007
	31.0-62.7	Miller *et al*., 2003
	36.2-62.7	Miller and Engel, 2006
	48.2	Ohtsubo *et al*.,2005
	50.0-72.0	Khatoon *et al*., 2004
Rice bran	56.0-99.0	Chotimarkorn *et al*., 2008
	155.0-272.0	Aguilar-Garcia *et al*., 2007
	251.0-686.0	Bergman and Xu, 2003
	278.76-283.96	Yu *et al*., 2007
	310.1	Shin and Godber, 1996
	440.0-474.0	Lloyd *et al*., 2000
	783.2	Devi *et al*.,2007
Polished rice	6.1	Ohtsubo *et al*., 2005
	7.0-12	Khatoon *et al*., 2004
	30.44-30.89	Boonsit *et al*., 2006

γ-oryzanol has been suggested to have potential functionality such as antioxidant activity (Xu and Godber, 2001), reduction of serum cholesterol (Sasaki *et al*., 1990), reduction of cholesterol absorption and decrease of early atherosclerosis (Rong *et al*., 1997), inhibition on platelet aggregation (Seetharamaiah *et al*., 1990) and inhibition of tumor promotion (Yasukawa *et al*., 1998). Jiamyangyuen (2006) reported that brown rice which soaked in distilled water for 6 hours and germinated for 24 hours contained more content and activity of antioxidant compared to brown rice.

Ferulic acid

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a ubiquitous phenolic compound in plant tissues. Ferulic acid is one of the metabolites of the biosynthesis of lignin from phenylalanine and tyrosine in plants as shown in Figure 7 (Zhao and Moghadasian, 2008). Ferulic acid occurs as esters in many plants, which may imply the pathway further undergoes conjugation with other molecules. The biological roles of ferulic acid has been suggested to crosslink to some cell components and lower their availability to hydrolytic degradation by endospermic enzymes and inhibit germination (Graf, 1992).

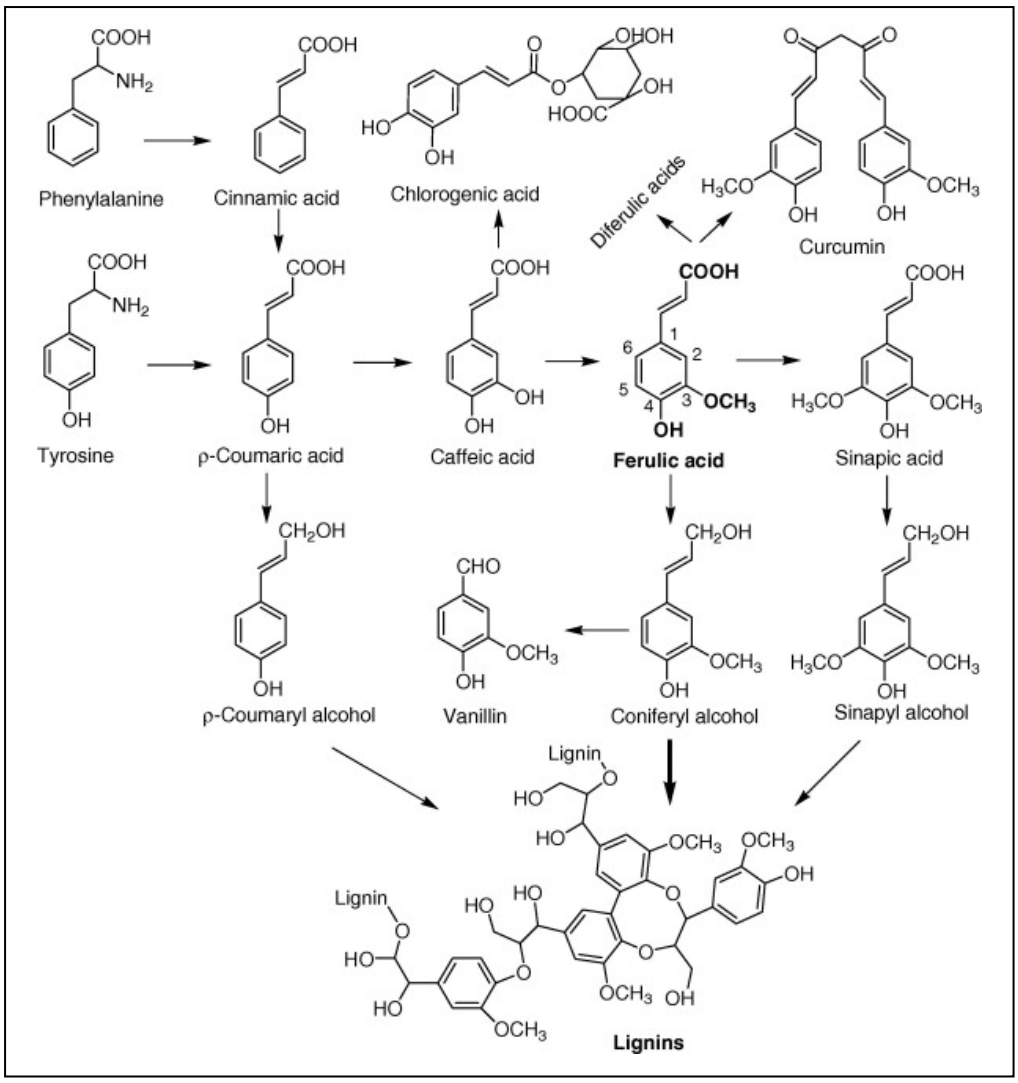


Figure 7. Metabolic pathways of ferulic acid formation in plants

Source: Zhao and Moghadasian (2008)

Ferulic acid found in plant tissues in two forms: free and conjugate; the sum of these two forms indicates total ferulic acids (Clifford, 1999; Herrmann, 1989). Cereal grains contain unique free phenolic compounds and their glycosides, which exist in solution, and a significant amount of insoluble phenolic compounds, most of which are bound to polysaccharides in the cell wall (Miller *et al*., 2000). Tian *et al*. (2004) reported amount of ferulic acid in white rice, brown rice, and germinated brown rice and demonstrated that the content of insoluble ferulic acid was significantly higher than that of soluble ferulic acid whereas ferulic acid in germinated brown rice and brown rice were more abundant than those in white rice. Total ferulic acid in white rice, brown rice and germinated brown rice were 5.13, 15.51 and 20.52 mg/100g, respectively. Adom and Liu (2002) also reported ferulic acid content in whole brown rice and found that ferulic acid was the major phenolic compound in grains, with free, soluble-conjugated, and bound ferulic acids present in the ratio 0.1:1:100.

The antioxidant properties of ferulic acid is due to its phenolic nucleus and an extended side chain, ferulic acid readily forms a resonance stabilized phenoxy radical this accounts for its free radical-scavenging effect (Graf, 1992; Palacios, 1990). This enables ferulic acid to protect DNA and lipids against oxidation through reactive oxygen species (ROS). Thus, ferulic acid may be beneficial in prevention and/or treatment of disorders linked to oxidative stress, including Alzheimer's disease, diabetes, hypertension and atherosclerosis. The strong link between inflammation and oxidative stress suggests that ferulic acid may also be effective against inflammatory diseases (Zhao and Moghadasian, 2008; Kayahara, 2004). Furthermore, the special structure of ferulic acid also endows its strong UV absorptive ability, making it an important skin protecting agent (Chan *et al*., 2004; Lin *et al*., 2005; Saija *et al*., 1999; Kayahara, 2004).

Ferulic acid is also bio-active compounds that have been studied for their cholesterol-lowering properties as well as for their antioxidant capacity. The antioxidant activity of ferulic acid is primarily based on hydrogen donation from the ferulic acid hydroxyl group (Nystrom *et al*., 2007).

Phytic acid

Phytic acid (myo-inositol 1,2,3,4,5,6 -hexakis [dihydrogen phosphate]) is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. The structure of phytic acid is a hexaphosphate of myo-inositol. The anionic nature of the six phosphate groups on inositol will be strongly negatively charged (Figure 8). It imparts a potential to bind positively charged molecules such as cations, proteins and carbohydrate. Due to its multiplicity of reactive phosphate groups, phytic acid can complex a cations within phosphate group itself, between two phosphate groups of a molecule, or between phosphate groups of different phytic acid molecules (Cheryan, 1980; Harland and Morris, 1995).

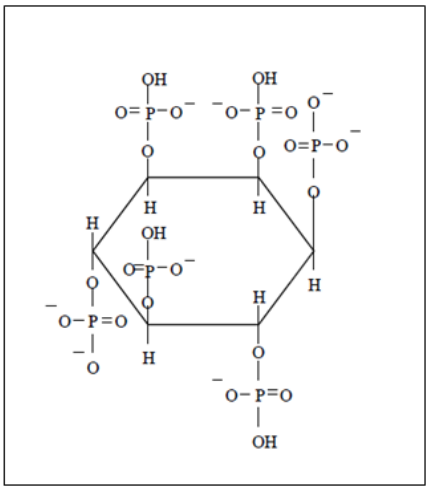


Figure 8. Structure of phytic acid
Source: Cheryan (1980)

Phytic acid is a powerful chelator, especially of polyvalent cations which are bound more strongly than monovalent cations. The salt called phytate (or phytin) commonly form one to several percent of the dry weight of many seeds and in many cases accounts for 50–80% of the total phosphorus in seeds. Phytate is as a store of inositol, phosphate, K, Mg, Ca, Mn, Fe and Zn for use by the seedling. These are released to developing seedlings by the action of phytase enzymes. A second role is the control of inorganic phosphate levels in both developing seeds and seedlings. Phytate appears to be synthesized only in the cells where it is stored, apparently in association with the endoplasmic reticulum, and small packets move to the developing

protein bodies for storage. Once inside the developing protein body the small packets, in some way, assemble into much larger and electron-dense spheres called globoids (Lott *et al*., 2000).

Phytic acid is generated by a stepwise phosphorylation of myo-inositol. The myo-inositol is the conversion of D-glucose-6-phosphate (IP6) to myo-inositol-3-phosphate (IP3) by myo-inositol-3-phosphate synthase (MIPS) or in part via phosphatidyl inositol phosphate (Ptd InsP) intermediates (Loewus and Murthy, 2000; Lott *et al*., 2000; Raboy, 2001). Therefore, the biochemical pathway of phytic acid can be summarized as consisting of two parts. The early pathway showed that glucose-6-phosphate is converted to IP3. The later pathway, phytic acid is synthesized from myoinosiol via a series of phosphorylation steps (Figure 9).

Phytic acid is hydrolyzed, enzymatically by phytases, or chemically to lower inositol phosphates such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and possibly the inositol di- and monophosphate during storage, fermentation, germination, food processing and digestion in the human gut. Only inositol hexaphosphate (IP6) and inositol pentaphosphate (IP5) have a negative effect on a bioavailability of minerals, the other hydrolytic products formed have a poor capacity to bind minerals, or the complexes formed are more soluble (Garcia-Estepa *et al*., 1999).

Phytic acid constitutes about 1-2 % by weight of many cereal seeds (Cheryan, 1980; Lott *et al*., 2000). Deposition of phytic acid found in small-grained cereals, about 90% of the seed phytic acid is found in the aleurone layer and the remaining 10% in the scutellum (Brinch-Pedersen *et al*., 2007). The phytate is important constituent of the outer layers of the rice grain. Reported of phytate contents are brown rice 0.2%; milled rice 0.04-0.06%; bran-germ 2.2-2.6%; and embryo 0.8%. Phytate represents only 0.16% of starch content in milled rice (Juliano and Bechtel, 1985).

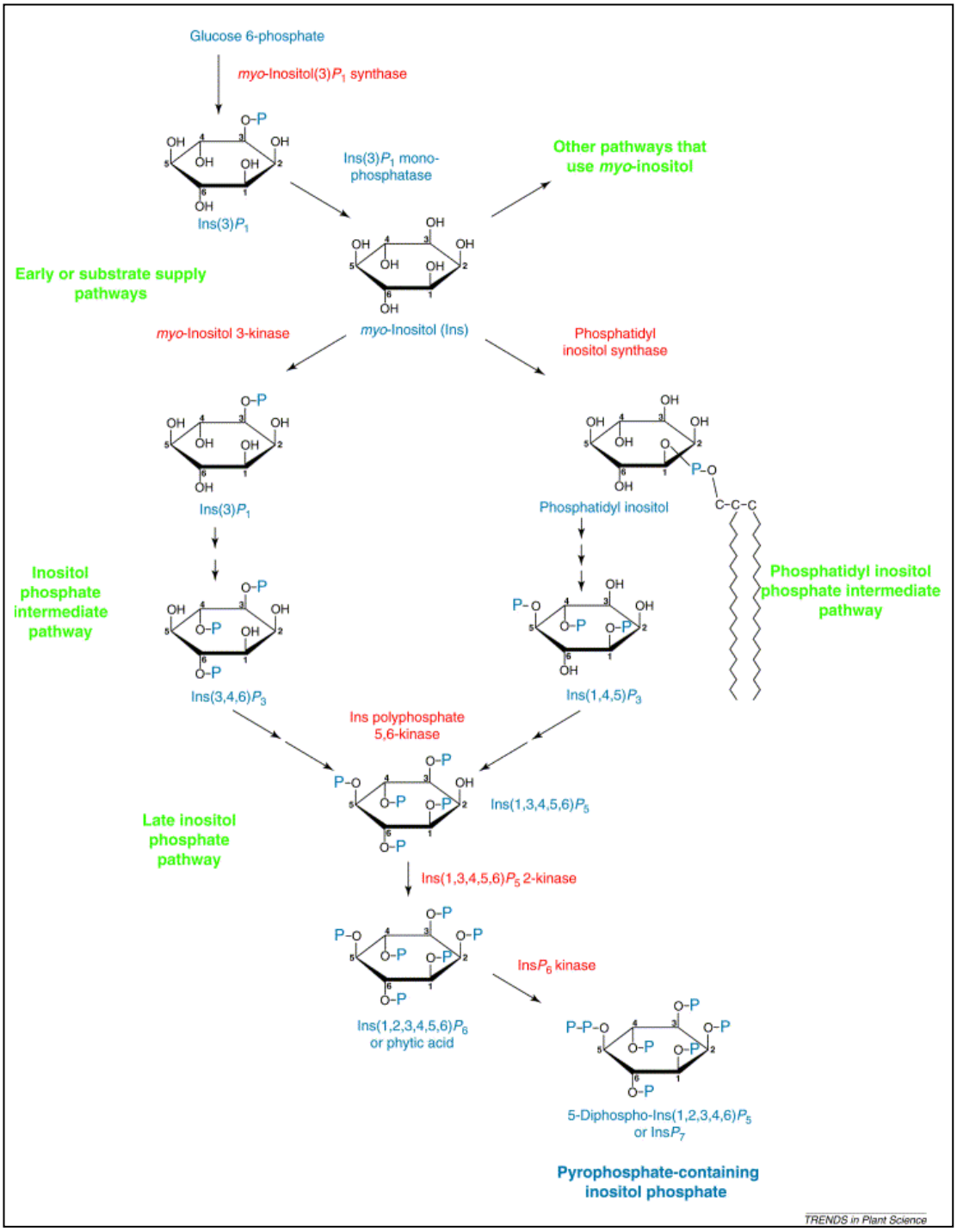


Figure 9. Biochemical pathways involving phytic acid in the eukaryotic cell
Source: Raboy *et al*. (2001)

Phytic acid has some anticancer and antioxidant functions and prevents coronary disease. It is able to prevent the build up of superoxide, as well as to boost the immune system. It recently found its way into the spotlight because of its ability to

prevent colon cancer, liver cancer, lung cancer, skin cancer, etc. It is also able to prevent control anemia and prevent cardiac infarction and diabetes (Kayahara, 2004).

Moong-ngarm (2005) studied the effect of germination on change of phytic acid content, phytase activity, and evaluates the potential of phytic acid and its degradation products as a source of antioxidant compounds. The results indicated that phytase activity of germinated rice for 4 days increase by 7.3 times compared to the un-germinated rice and the germination significantly decreased phytic acid content in germinated rice. The highest phytic content was found in rice bran whereas lowest of phytic acid was found in rice rootlet and shoot. In un-germinated rice was found IP6 and IP5. After germination, the level of IP6 was decreased where as IP5 was slightly increased. The phytic acid extract showed strong antioxidant activity which IC_{50} of the DPPH radical scavenging between 115.69-138.58 ppm and IC_{50} of BHA was 9.24 ppm.

Objectives

1. To investigate the optimum germination condition of three brown rice varieties grown in the southern Thailand.

2. To study properties of germinated brown rice.

3. To develop the functional drink from germinated brown rice or its extracts.

4. To study the properties, shelf life and consumer acceptability of functional drink.

CHAPTER 2

MATERIAL AND METHOD

Material and Equipment

Rice samples

Three indica rice cultivars (*Oryza Sativa*) with different amylose contents were obtained from the Rice Research Center located in Phatthalung, Thailand. These are Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung, which were used throughout this study (Figure 10). To obtain brown rice samples, the paddies were milled by a home-scale miller and packed under vacuum conditions in plastic bags. The samples were kept in a cold room (4°C) throughout the experiment.

Niaw Dam Peuak Dam **Sangyod Phatthalung** **Chaing Phatthalung**

Figure 10. Rice samples used in this study

Ingredients

Soybean, sugar and milk powder were purchased from supermarket in Hat Yai, Songkhla, Thailand.

Packaging

Glass bottles with twist-off cap were purchased from BTI Packaging Co., Ltd. (Bangkok, Thailand).

Chemicals

1) HPLC reagents: acetonitrite, methanol, dichloromethane (HPLC grade, Merck, Germany)

2) Extract reagents: ethanol (analytical grade, Merck, Germany).

3) The chemicals used for chemical analysis were analytical grade. The major chemicals were 4-aminobutyric acid and ferulic (Fluka, Switzerland), γ-oryzanol (Restek, USA.), D-(-) fructose, D-(+) glucose, D-(+) maltose monohydrate and sucrose (Sigma, USA).

Equipments

1) Balance (Sartorius, Germany)
2) Brookfield viscometer (DV-II+Pro, USA)
3) Differential scanning calorimeter (DSC7 Perkin Elmer, USA).
4) Hand refractometer (Atago PR-101, Japan)
5) High performance liquid chromatography (Agilent 1200 Series, Japan),
6) Hot air oven (Memmert, Germany)
7) Hunter Lab spectrophotometer (Reston, USA)
8) pH meter (Schott, Germany)
9) Rotational Rheometer (Haake, RheoStressRS75, Germany)
10) Vortex mixer (IKA, Germany)
11) Water bath (Memmert, Germany)

Methods

1. Optimization of germination conditions and properties of germinated brown rice

Chemical properties of brown rice

All samples of brown rice were analyzed for moisture, protein, lipid, ash and dietary fiber (AOAC, 2000), and soluble amylose (Shanthy *et al.*, 1980; Sombhagya and Bhattacharya, 1979).

Hydration characteristics of brown rice during soaking

Brown rice was washed with distilled water to rinse out any contaminants. Each of the rice samples was soaked in distilled water at room temperature (30±2°C) for 24 hours. At various time intervals during soaking, the rice were sampled and analyzed for moisture (AOAC, 2000) and GABA contents (Cohen and Michaud, 1993)

Optimum germination conditions for brown rice

To determine soaking solution

Washed brown rice was steeped in soaking solutions of various buffer solutions: 0.1 M glycine-hydrochloric acid buffer pH 2.0; 0.1 M citrate buffer pH 3.0 and pH 5; 0.1 M phosphate buffer pH 7.0 and distilled water. A grain-to-solution ratio of 1:2 w/v was used for 5 hours at room temperature (30±2°C, RH 90±5%). After 5 hours the soaking solutions were drained off and the rice grains were wrapped with cheesecloth to maintain moisture. They were then left in the incubator (30±2°C, RH 90±5%) for 24 hours to germinate. The germinated brown rice was dried to <13% moisture content using a tray dryer at 50°C and analyzed for GABA content. The soaking solution which gave the highest concentration of GABA was selected for further study.

To determine germination time and condition

To determine optimum germination times, the brown rice samples were steeped in the selected soaking solution as described above. The steeped rice grains were then wrapped with cheesecloth and left in either an open or closed vessel. In the open vessel, the rice grains were left in a plastic box and covered with punctured lids which allowed the air to circulate within. Thus, the amount of oxygen in the system was constant. In the closed vessel, the rice grains were left in a plastic box with an air-tight lid which excluded the air. Thus, the amount of oxygen in the system declined. The rice grains were germinated for 12, 24, 36 or 48 hours. After germination, the brown rice samples were taken out and dried to <13% moisture content using a tray dryer at 50°C. The dried germinated brown rice samples were analyzed for GABA content. The germination conditions that gave the highest concentration of GABA were selected for subsequent studies.

Properties of germinated brown rice

Germinated brown rice samples were analyzed for their chemical properties, physico-chemical properties and bio-active compounds as follow;

Chemical properties

All the samples were analyzed for moisture, protein, lipid, ash and dietary fiber contents (AOAC, 2000), and sugar content (Ohtsubo *et al.*, 2005).

Physico-chemical properties

Germinated brown rice samples were ground in an electric miller and the flour was sieved through 250 μm of wire mesh sieve (60 mesh) and analyzed for the physico-chemical properties as follows;

Flow behavior

Rice flour slurries of 4% (w/w) were prepared by stirring the flour samples in distilled water until they disperse thoroughly. The slurries were heated to 95°C in boiling water for 15 min while stirring continuously to obtain complete gelatinization. The pastes were removed from the water bath and left to cool to 60°C. The flow behaviors of the gelatinized flour were determined using a Rotational Rheometer (Haake, RheoStressRS75, Germany) equipped with coaxial cylinder geometry (Z41). Flow behaviors were determined by increasing the shear rate in the range of 0-1000 s^{-1} at 60°C.

Thermal properties

The slurries of 1:4 weight ratio of flour to water were hermetically sealed in aluminum pan and reweighed in the microbalance. After sealing the pan and leaving to equilibrate for about 12 hours, the slurries were heated from 25 to 140°C at the rate of 10°C/min using Differential Scanning Calorimeter (DSC7 Perkin Elmer, USA). An empty pan was use as reference. The transition temperatures reported were the onset (To), peak (Tp) and conclusion (Tc). The enthalpy of gelatinization (ΔH) was calculated in terms of J/g of dry flour.

Bio-active compounds

Germinated brown rice samples were ground in an electric miller and the flour was sieved through 250 μm of wire mesh sieve and analyzed for bio-active compounds as follows;

γ-aminobutyric acid (GABA)

The GABA content was determined by the method of Cohen and Michaud (1993) with slight modifications. One-fifth to one-half gram (0.2-0.5 g) of ground germinated brown rice samples were weighed in plastic tubes. Then 1.8 mL of deionized water was added and the slurries were shaken at room temperature for 1.5 hours. Thereafter, 200 μL of 3% (by volume) sulfosalicylic acid was added and the

mixtures were centrifuged at 4500xg for 10 min. Next 50 μL of the supernatants were added to 50 μL of 100 mM $NaHCO_3$ and 50 μL of 4 mM 4-dimethyl aminoazobenzene-4-sulfonyl chloride acetonitrile solutions. The mixtures were heated to 70^{o}C for 10 min to effect derivatization. After the derivatization, the samples were added to 250 μL of absolute ethanol and 250 μL of 25 mM phosphate buffer (pH 6.8). The samples were then filtered and 5 μL of the filtrate were injected into Agilent HPLC (1200 Series, Japan), with Supelcosil LC-DABS column, 4.6x150 mm, 3 μm (Supelco, Bellefonte, PA). The HPLC was equipped with a UV-Vis photodiode array detector set at 465 nm wavelength. The mobile phases were 25 mM acetate buffer and acetonitrile (65:35) operated at a flow rate of 0.5 mL/min, and 55^{o}C. Pure GABA was used as the standard for calibration.

γ-oryzanol

γ-oryzanol content was determined by the method of Chen and Bergman (2005) with slight modifications. Rice samples powder (0.05 g) were extracted in 3 mL of methanol HPLC grade. The mixtures were shaken using a vortex for 1 minute. After the extraction, the samples were centrifuged for 10 min at 825xg. The supernatants were collected by filtering and the residues were extracted twice more and 50 μL of the samples were injected into the Agilent HPLC (1200 Series, Japan), with Alltech Econosphere C18 column, 4.6x250 mm, 5 μm. The HPLC was equipped with a UV-Vis photodiode array detector set at 330 nm wavelength. The mobile phases were methanol: acetonitile: dichloromethane: acetic acid (50:44:3:3) operated at ambient temperature, and with a flow rate of 1 mL/min. γ-oryzanol was used as a standard for calibration.

Ferulic acid

Total ferulic acid content was determined using the method of Ohtsubo *et al*. (2005). A 0.5 g amount of rice was extracted with 50 mL of 1 M NaOH for 3 hours at 40^{o}C and neutralized by 26 mL of 2 M HCl. The sample was extracted three times with 50 mL of ethyl acetate, each time for 5 min. Thereafter, the ethyl acetate layer was evaporated and the sample was re-dissolved in methanol and H_2O (1:1). All

samples were filtered though a 0.45 μm pore size syringe-driven filter before injection. A 5 μL aliquot of the sample solution was injected into the Agilent HPLC (1200 Series, Japan) system equipped with a diode array detector on a 4.6x150 mm, 5 μm, and Agilent Eclipse XDB-C18 analytical column. The mobile phases were acetic acid (2.5% by volume) and acetonitrile (88:12) at a flow rate of 0.5 mL/min. The column temperature was set at 40^{o}C and ferulic acid was detected at a wavelength of 320 nm. Pure ferulic acid was used as the standard for calibration.

Phytate

Phytate was analyzed by the standard method of AOAC (2000). Rice (2.0 g) was extracted with 40 mL of 2.4% (by volume) HCl by shaking vigorously for 3 hours at room temperature before filtering. The filtrate was mixed with 1 mL Na_2EDTA/NaOH solution and diluted to 25 mL with deionized water, then poured into an anion-exchange column (Dowex 1x8, 200-400 mesh, chloride form, Fluka, Germany). The Phytate solution was eluted with 0.7 M NaCl solutions and wet-digested with a mixture of concentrated HNO_3-H_2SO_4 to release phosphorus. This was measured colorimetrically with a spectrophotometer at the wavelength 640 nm. The amount of phytate in the original sample was calculated as hexaphosphate equivalent.

Statistical analysis

All the experiments were carried out using three freshly prepared germinated samples and three replicates of each sample were analyzed. The results were statistically analyzed using one way analysis of variance (ANOVA). Means were compared using the Duncan multiple range test with mean square error at 5% probability. The differences between un-germinated and germinated brown rice were assessed by paired t-test with a level of significance of 0.05.

2. Optimization method for extract GABA from germinated brown rice and its fractions

Milling and fractionation of germinated brown rice

Germinated brown rice sample which gave the highest of the GABA content in part 1 was selected to use as the sample. Whole germinated brown rice was milled to remove germ and bran in a polishing machine. Rice germ were obtained from germinated brown rice and separated from broken rice by hand. Germinated brown rice and its fractions (germ, bran, and white rice) were ground in an electric mill and sieved through 250 μm wire mesh sieve. Germinated brown rice and its fractions were analyzed for GABA content (Cohen and Michaud, 1993)

Extraction of GABA from germinated brown rice and its fractions

Germinated brown rice flour and its fractions were extracted with various solvents, using flour-to-solvent ratio of 1:3 w/v at 30, 40, 50 and 60°C. Water and ethanol were selected as the extraction solvents since both are commonly used in the food industry, and are more highly stable in the human body than any other solvents. The germinated brown rice flour and its fractions were extracted with 75% ethanol, 50% ethanol and distilled water for 6 hours after which the extracts was filtered through Whatman No.1 to remove any debris. The filtrate was analyzed for GABA content (Cohen and Michaud, 1993). The solvent and extraction temperature, which gave the highest concentration of GABA content, was selected for further study.

To determine optimum extraction times, the germinated brown rice flour and its fractions were extracted with solvents as described above. The extraction times were 3, 6, 12 and 24 hours. The extract was filtered through Whatman No.1 to remove any debris. The filtrate was analyzed for GABA content (Cohen and Michaud, 1993).

Statistical analysis

All experiments were carried out using three replicates and the all results were expressed as replicate of means. The results were statistically analyzed using one way analysis of variance (ANOVA). Means were compared by Duncan multiple range test with mean square error at 5% probability.

3. Development of functional drink from germinated brown rice

Consumer survey of functional drink

The objectives of this survey were 1) to study the behavior, attitude, and perception of consumers to functional drink 2) to study the factors that affect behavior and 3) to understand the consumer requirement for developing functional drink from germinated brown rice. A questionnaire was developed from the focus group. The questionnaire consisted of 3 parts; the consumer attitude and behavior toward functional drink, the development functional drink from germinated brown rice, and the personal data. The questionnaire was pre-tested by 15 consumers and then it was corrected before being given to a further 15 consumers to make sure that any defects in the questionnaire had been corrected. The corrected questionnaire can be found in Appendix A. The consumer survey was conducted at university, government offices and department stores in Songkhla, Thailand. This survey focused on 3 consumer age group; 15-25, 26-45 and over 45 years old. The profile of an ideal functional drink from germinated brown rice was used in further study.

Mock-up of functional drink

Mock-up of functional drink was selected from commercial functional drink which gave the highest of sensory score. Three commercial functional drinks were evaluated by nine-point hedonic scale. Panelists (n = 30) were recruited from the staffs and students of the Faculty of Agro-Industry, Prince of Songkla University. The panelists evaluate the overall acceptability, appearance, color, viscosity, texture, flavor and taste. A nine-point hedonic scale was used where 1= dislike extremely and

9=like extremely (Peryam and Pilgrim, 1957). The commercial functional drink that gave the highest of sensory score was selected for formulation of subsequent studies.

Optimization formula of functional drink from germinated brown rice

Experimental design

The D-optimal method in the mixture design, provided by the software Design-expert (version 7.1.6, Stat-Ease Int. Co., Minneapolis, MN, USA), was used to optimize the functional drink formulation. Generally the mixture design is used to study the relationships between the proportion of different variables and responses. A four-component was developed using germinated brown rice extracts (X_1:76–83%), soybean (X_2:8–15%), sugar (X_3:0–6%) and milk powder (X_4:0–2%). The experiment design gave fifteen points with three point replications as shown in Table 4. The predictive regression models were generated by Design-expert software. All graphs were optimized to obtain an optimum formula by using maximum sensory responses as criteria.

Preparation of functional drink from germinated brown rice

The experiments were conducted according to the ratio given by the experiment designs which were composed of germinated brown rice, sugar and the other ingredients. The germinated brown rice was extracted with solvent which selected in part 2. After that the extracts were adjusted to pH 6.8-7.0. Soybean were washed and soaked in water with a soybean to water ratio of 1:3 at 4^{o}C for 16-18 hours. The hydrated beans were drained, rinsed, and blended with the extracts from germinated brown rice, using a blender. The slurry was filtered through 4 layers of cheesecloth and 75 μm of wire mesh sieve, respectively. Sugar or milk powder was added to the mixed solution and heated at 80°C to dissolve the ingredient completely. The final functional drink was pored into glass bottles and sterilized at 118°C.

Table 4. The experiment design

Formula	Germinated brown rice extract (%)	Soybean (%)	Sugar (%)	Milk powder (%)
1	76.3	15.3	6.1	2.3
2	82.1	10.7	5.3	2.0
3	83.0	15.8	0.0	1.2
4	78.1	15.6	6.3	0.0
5	83.3	10.0	6.7	0.0
6	79.7	15.9	3.2	1.2
7	80.6	12.9	6.5	0.0
8*	83.0	15.8	0.0	1.2
9	81.3	14.6	2.1	2.0
10*	76.3	15.3	6.1	2.3
11	83.3	16.7	0.0	0.0
12	82.6	8.3	6.6	2.5
13	81.3	16.3	0.0	2.4
14*	83.3	16.7	0.0	0.0
15	79.2	14.3	5.2	1.3
16	79.4	11.9	6.3	2.4
17	81.8	13.1	4.3	0.7
18	83.0	11.6	2.9	2.5

* 8, 10 and 14 were replicated design point of 3, 1 and 11, respectively.

Sensory evaluations

Panelists (n = 30) were recruited from the staff and students of the Faculty of Agro-Industry, Prince of Songkla University. The panelists noted the appearance and smell and then drank each sample to evaluate the overall acceptability, appearance, color, viscosity, texture, flavor and taste. A nine-point hedonic scale was used ranging from 1 (dislike extremely) to 9 (like extremely) (Peryam and Pilgrim, 1957).

Statistical analysis

The questionnaire was coded to facilitate data entry. The percentages of responses of the personal data and attitudinal data were calculated. The mixture design experiment was provided by Design-expert (version 7.1.6, Stat-Ease Int. Co., USA). The results were statistically analyzed using one way analysis of variance (ANOVA). Means were compared by Duncan multiple range test with mean square error at 5% probability.

4. Study on the properties of functional drink from germinated brown rice

Functional drink from germinated brown rice which selected from part 3 was conducted heat penetration test and sterility test by Agro-Industry Development Center for Export (ADCET). Sterilized functional drink product were analyzed for their physical properties, nutrition composition and bio-active compounds as the follow;

Physical properties

Total soluble solid: The total soluble solid of the functional drink was analyzed by hand refractometer (Atgo, Japan) at 20°C.

pH: A pH meter (Schott, Germany) was used to measure the pH of the samples.

Acidity: Samples were diluted (1:4) with distilled water and titrated to a phenolphthalein endpoint of pH 8.1 with 0.1 N NaOH. Titratable acidity was calculated as g citric acid per 100 mL (AOAC, 2000).

Color: The color of the drinks was evaluated using a Hunter Lab CIE 10°/D65 spectrophotometer (Reston, USA). The samples were placed in 6 cm-diameter cups fitted with a 1-mm black rubber ring. Each sample cup was filled up to the level of the ring at room temperature. A black cover was placed over the cup prior to measure. The measurements were recorded as: L*, a* and b* values which

represent the light-dark spectrum with a range from 0 (black) to 100 (white): a green-red spectrum.

Viscosity: The viscosity of the samples was measured in triplicate using a Brookfield viscometer (DV-II+Pro, USA) at 20°C.

Nutrition compositions

Nutritional compositions were obtained by analyzing the functional drink for total fat, saturated fat, cholesterol, protein, total carbohydrate, dietary fiber, sugar, sodium, vitamin A, vitamin B1, vitamin B2, calcium, iron, ash and moisture (AOAC, 2000). The total energy was calculated as amount per serving (240 mL).

Bio-active compounds

GABA, γ-oryzanol, ferulic acid and phytate were determined.

Consumer acceptability of functional drink from germinated brown rice

Two hundred consumers participated to evaluate the acceptability of functional drink from germinated brown rice. The surveys conducted at the university, government offices and National Agricultural Exhibition. The questionnaire consisted of 4 parts, the demographic information, the acceptability test and the product information parts and the personal data (Appendix B). Panelists were evaluated the developed functional drinks from germinated brown rice using a nine-point hedonic scale (Resurreccion, 1998; Meilgaard *et al*., 1999), with 1= dislike extremely and 9=like extremely (Peryam and Pilgrim, 1957) for overall acceptability, appearance, color, texture, flavor and taste.

Statistical analysis

All experiments were carried out using three replicates and the all results were expressed as replicate of means. The results were statistically analyzed using one way analysis of variance (ANOVA). Means were compared by Duncan multiple range test with mean square error at 5% probability.

5. Study on the shelf life of functional drink from germinated brown rice

Functional drink samples and storage conditions

Functional drink from germinated brown rice was prepared and packed in glass bottle and sterilized at 118^{o}C for 24 min. The products were stored at 25, 35 and 45°C for 12 weeks.

Quality assessment

The following quality characteristics of functional drink were weekly analyzed.

Total soluble solid, pH, acidity, color, viscosity and GABA content were analyzed.

Total color difference (ΔE) was calculated using following equation, where subscript “0” refers to the color reading of initial functional drink. Initial functional drink (after processing, time 0) was used as the reference and a larger ΔE denotes greater color change from the reference material.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

Sensory evaluations were evaluated by 30 trained panelists. The panelists observed appearance and smell then drank of each sample to evaluate the overall acceptability, appearance, color, viscosity, texture, flavor and taste. A nine-point hedonic scale was used where, 1= dislike extremely and 9=like extremely (Peryam and Pilgrim, 1957).

Statistical analysis

All experiments were carried out using three replicates and the all results were expressed as replicate of means. The results were statistically analyzed using one way analysis of variance (ANOVA). Means were compared by Duncan multiple range test with mean square error at 5% probability.

CHAPTER 3

RESULTS AND DISCUSSION

1. Optimization of germination conditions and properties of germinated brown rice

Chemical properties of brown rice

Chemical properties of three rice varieties are shown in Table 5. Protein content varied from 7.37-8.93%, with Sangyod Phatthalung showing the highest amount at 8.93%. Fat content varied from 2.24-2.94%. Total dietary fiber varied from 2.61-4.13%, with Sangyod Phatthalung having the highest amount at 4.13%. Sawaddiwong (2008) reported Chiang Phatthalung contained 6.12% of protein, 2.58% of fat, 1.30% of ash and 2.60% of dietary fiber. Pongsawatmanit *et al*. (2003) also reported Sangyod Phatthalung contained 10.09 % of protein, 0.29% of fat and 0.51% of ash. The difference rice varieties contained chemical properties in difference amount.

Table 5. Chemical properties of three varieties of brown rice

Composition (%db)	Niaw Dam Peuak Dam	Sangyod Phatthalung	Chiang Phatthalung
Protein	8.06±0.03^{b}	8.93±0.04^{a}	7.37±0.03^{c}
Fat	2.94±0.02^{a}	2.24±0.02^{c}	2.67±0.06^{b}
Ash	1.67±0.01^{a}	1.20±0.01^{c}	1.48±0.02^{b}
Total dietary fiber	3.34±0.01^{b}	4.13±0.28^{a}	2.61±0.01^{c}
Amylose content	2.17±0.08^{c}	14.69±0.30^{b}	21.72±0.34^{a}

$^{a-c}$ The different superscripts letters under the same row were significantly different ($p<0.05$).

Amylose and amylopectin are the main components of starch, affecting its physical properties such as gelatinization, retrogradation, viscosity and viscoelasticity. In this study, soluble amylose content in rice starch was determined by interaction with iodine as the result shown in Table 5. As the result, southern Thai rice varieties were classified into 3 classes. Niaw Dam Peuak Dam is low amylose content, Sangyod Phatthalung is intermediate amylose content and Chiang Phatthalung is high amylose content. These results agree with result were report by Khunae (2007) and Pongsawatmanit *et al.* (2003) found that Sangyod Phatthalung and Chiang Phatthalung are intermediate amylose content and high amylose content, respectively.

Hydration characteristics of brown rice during soaking

Changes in moisture content in three varieties of rice during soaking in distilled water at room temperature are shown in Figure 11. All three rice varieties exhibited similar water uptake behavior. At the early stage of soaking (at 0-2 hour), water uptake rapidly increased due to the absorption into the embryo of the kernel (Bello *et al*., 2004; Wijngaard *et al*., 2005). Subsequently (at 2-5 hour), rice kernels absorbed water slowly and came to an equilibrium or saturation point. The moisture content after 5 hours changed non-significant ($p>0.05$) and the uptake rate was extremely slow. After soaking for 24 hours moisture content of Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung reached 42.88, 37.64 and 32.50%, respectively (Figure 11). A similar trend of result was reported by Chung *et al*. (2009) found that the water content of waxy hull-less barley soaked in water was significant increased at the early stage of steeping (8 hours).

Normally, germinated brown rice contained 30–35% of moisture and high moisture levels (35–50%) promoted microbial growth (Komatsuzaki *et al*., 2007). With rice grains, soaking in warm water (30°C) for several hours caused microorganisms to grow rapidly (Bandara *et al*., 1991). Similar result has also been reported by Dewar *et al*. (1997) claimed that the steeping for an extended period with excess water might lead to anoxic conditions, which might be compounded by microbial proliferation because the oxygen requirements by the grains could not be satisfied. Adequate hydration was achieved when the brown rice was soaked for a

period sufficient to ensure proper germination (until the germ swelled) and to attain a desired moisture level for subsequent gelatinization of the starch. The water content after soaking is important for controlling the development of enzymes needed for germination because the grains absorbed water during steeping (Rimsten, 2003). The optimum water content for germination in barley grains has been reported to be from 39-44% (Haraldsson *et al*., 2004). Therefore, soaking three varieties of brown rice grains for 5 hours to attain the moisture of 30-40% is considered an optimal soaking time and used for further study. Similarly, Benjamasuttikul and Naivikul (2007) found that 5 hours was the optimal soaking time of Kor-Khor 6 and Khao Dawk Mali 105.

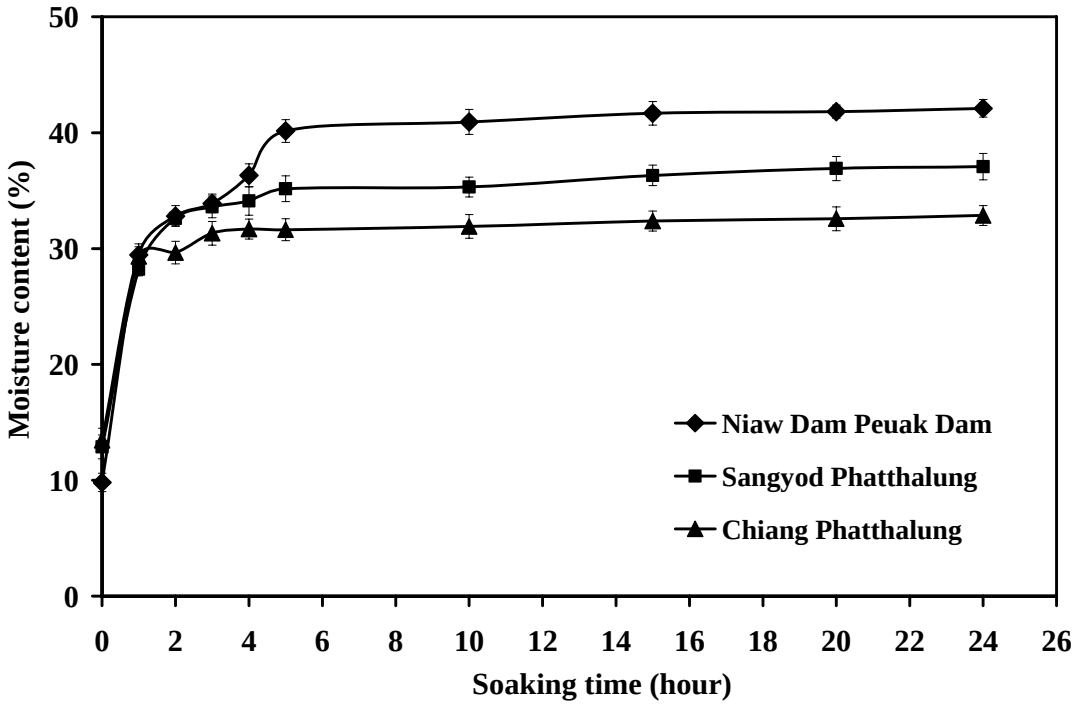


Figure 11. Changes in moisture content in three varieties of rice during soaking in distilled water at room temperature for 24 hours

Amylose and amylopectic contents in the rice affected the amount of water uptake. Niaw Dam Peuak Dam was found to have greater amount of water uptake than Sangyod Phatthalung and Chiang Phatthalung due to the greater amount of amylopectin. Normally, glutinous rice (Niaw Dam Peuak Dam) had amount of amylopectin content higher than non-glutinous rice (Sangyod Phatthalung and Chiang

Phatthalung). Benjamasuttikul and Naivikul (2007) reported Kor-Khor 6 (6% amylose) have the water uptake higher than Khao Dawk Mali 105 (14% amylose).

Changes in the GABA content of brown rice during soaking in distilled water are shown in Figure 12. Initial value of GABA content in Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung were 3.61, 2.64, 3.09 mg/100g, respectively. As the soaking time increased, the GABA content also increased. After 24 hours, the GABA contents of Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung were 18.70, 10.67 and 10.66 mg/100g, respectively. Results indicating that GABA content in brown rice greatly increased during soaking in the water. A similar result was reported by Saikusa *et al*. (1994) found that the GABA content of Japonica rice germ var. Koshihikari greatly increased during soaking in the water. Varanyanon *et al*. (2005) also reported that the GABA content in Thai rice germ var. Pathum Thani 1, Khao Dawk Mali 105, Chai Nat 1 and Suphan Buri increased during soaking in water at 40°C for 4 hours which Pathum Thani 1 showing the highest amount at 55.51 mg/100g.

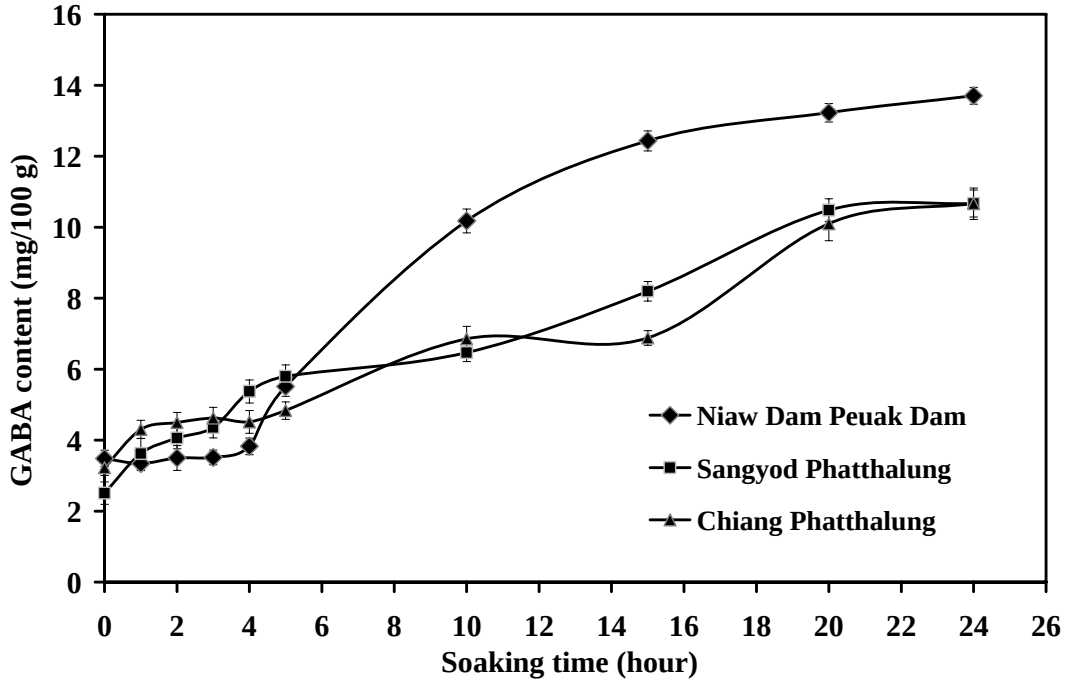


Figure 12. Changes in GABA content in three varieties of rice during soaking in distilled water at room temperature for 24 hours

Increasing GABA content in brown rice during soaking in water was due to the synthesis of glutamic acid by glutamate decarboxylase (GAD). In addition, the amino acids in brown rice being used as storage protein, these are decomposed by water absorption, changed into transportable amides and supplied to the growing parts of the rice seedling (Lea *et al.,* 1990).

Optimum germination conditions for brown rice

The GABA content of germinated brown rice in various soaking solutions are shown in Table 6. The highest value was found in samples soaked in citrate buffer at pH 3 for 5 hours, with Niaw Dam Peuak Dam having the highest amount at 14.48 mg/100g. A similar result was reported by Charoenthaikij *et al*., (2009) found that the highest amount of GABA could be accumulated when soaking brown rice var. Khao Dawk Mali 105 in citrate buffer pH 3 for 48 hours (67 mg/100g flour) and soaked brown rice var. Kor-Khor 6 in citrate buffer pH 3 for 24 hours (30.69 mg/100g flour). However, Sunte *et al*. (2007) found that brown rice var. Khao Dawk Mali 105 soaked in citrate buffer solution pH 5 had the highest of GABA content while Watchraparpaiboon *et al*. (2007) found brown rice var. Khao Dawk Mali 105 soaked in water at pH 6 had the highest.

Table 6. GABA content in three varieties of germinated brown rice at various soaking solutions at room temperature for 5 hours

Soaking solution	GABA content (mg/100g db)		
	Niaw Dam Peuak Dam	Sangyod Phatthalung	Chiang Phatthalung
Glycine-HCl buffer, pH 2.0	11.28±0.08^{b}	7.02±0.05^{b}	8.79±0.16^{b}
Citrate buffer, pH 3.0	14.48±0.23^{a}	8.36±0.06^{a}	14.01±0.11^{a}
Citrate buffer , pH 5.0	9.13±0.10^{c}	5.79±0.10^{d}	6.78±0.07^{c}
Phosphate buffer, pH 7.0	12.13±0.09^{b}	4.58±0.31^{e}	8.510.34b^{b}
Distilled water	9.47±0.38^{c}	6.36±0.06^{c}	8.73±0.15^{b}

$^{a-e}$ The different superscripts letters under the same column were significantly different ($p<0.05$).

It is apparent that GABA increased in germinated brown rice when the rice was soaked in an acid solution. This was perhaps due to the fact that GABA synthesis increases rapidly in response to a variety of environmental signals, including acidosis condition (Scott-Taggart *et al*., 1999). Similarly, the synthesis of GABA through glutamate decarboxylase in a reduced oxygen supply occurred through the effect of decreasing cytoplasmic pH in carrot cell suspension (Carroll *et al*., 1994). Therefore, citrate buffer pH 3, which gave the highest concentration of GABA, was selected for the study.

Feature of germinated brown rice preparing different germination times are shown in Figure 13. Immediately after soaking (0 hour), the embryonic axis did not visibly emerge and became more obvious as the germination time was extended (after 12 hour). The embryonic axes approximately 1 millimeter long after 24 hours. As can be seen that the longer germination time, the longer the embryonic axis. After 48 hours of germination, the embryonic axes approximately 1 centimeters long.

Figure 13. Feature of germinated brown rice using difference germination times

The GABA content of brown rice germinated for different lengths of time are shown in Table 7. The highest GABA content was obtained when germinated for 36, 36 and 48 hours in a closed vessel for Sangyod Phatthalung, Chiang Phatthalung and Niaw Dam Peuak Dam, respectively. The longer of germination time (>48 hours) was not carried out in this study due to the occurrence of fermented odor and mold grown. The GABA content increased 11.28, 16.74 and 9.43 times for Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung, respectively, as compared to the un-germinated brown rice. A similar result was reported by Komatsuzaki *et al*. (2007) found that GABA content in brown rice var. Haiinori germinated in gaseous treatment (air-tight conditions) at 35°C for 21 hours was higher than soaking in water for 24 hours. Chung *et al*. (2009) also reported that GABA content in barley grain germinated in anaerobic treatment for 12 hours increased 4 times as compared with un-germinated barley.

Table 7. GABA content in three brown rice varieties germinated in either open or closed vessel at room temperature for various times

Rice	Germination method	GABA content (mg/100g db)			
		12 hours	24 hours	36 hours	48 hours
Niaw Dam Peuak Dam	Open	10.34 ± 0.29^{Ad}	14.42 ± 0.15^{Ac}	20.92 ± 0.86^{Ab}	24.66 ± 1.10^{Ba}
	Close	8.49 ± 1.02^{Ad}	15.32 ± 0.41^{Ac}	18.82 ± 0.74^{Bb}	40.72 ± 0.29^{Aa}
Sangyod Phatthalung	Open	6.86 ± 0.24^{Bd}	8.34 ± 0.04^{Bc}	26.88 ± 0.71^{Ba}	22.82 ± 1.01^{Bb}
	Close	9.12 ± 0.42^{Ad}	17.51 ± 0.77^{Ac}	44.53 ± 1.93^{Aa}	39.04 ± 1.54^{Ab}
Chiang Phatthalung	Open	8.36 ± 0.36^{Bd}	14.05 ± 0.10^{Bc}	22.98 ± 0.51^{Bb}	25.81 ± 1.00^{Aa}
	Close	16.91 ± 0.78^{Ad}	24.88 ± 1.21^{Ab}	29.25 ± 0.77^{Aa}	20.70 ± 0.14^{Bc}

$^{A-B}$ The different superscripts letters under the same column for each rice variety were significantly different using a paired t-test ($p<0.05$).

$^{a-d}$ The different superscripts under the same row for each rice variety with different germination time were significantly different (($p<0.05$).

The synthesis of GABA is known as an adaptive response of plant tissues to stress-induced cellular acidosis. The process of GABA synthesis would accompany the H^+ consumption during decarboxylation, which improves cytosolic acidification (Crawford *et al*., 1994; Shelp *et al*., 1999). Anaerobic conditions have been reported to reduce cytosolic pH by 0.4–0.8 due to oxygen deficit stress (Crawford *et al*., 1994). Thus, a reduction in intracellular pH from normal physiological value under anoxia elevates GABA levels by stimulating GAD activity. The cytosolic acidification could initiate a rapid accumulation of GABA, but excess pH reduction in extracellular due to long anoxia treatment could preclude the cytosolic buffer capacity (recovery of the cytosolic pH) based on H^+ consuming decarboxylation.

Therefore, the optimum conditions to produce the highest GABA content in three varieties of brown rice were: soaking in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

Properties of germinated brown rice

Flow behavior

The flow behavior index, *n*, and the consistency index, *k*, are useful parameters for describing the flow behavior of flour suspensions. The results from this study is shown in Table 8. The relationship between shear rate and shear stress for brown rice flour at concentration of 4% db, in the shear rate range 100-800 s^{-1} followed the power law equation.

$$\sigma = k\dot{\gamma}^{n}$$

when σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), *k* is the consistency index ($Pa.s^n$) and *n* is the flow behavior index.

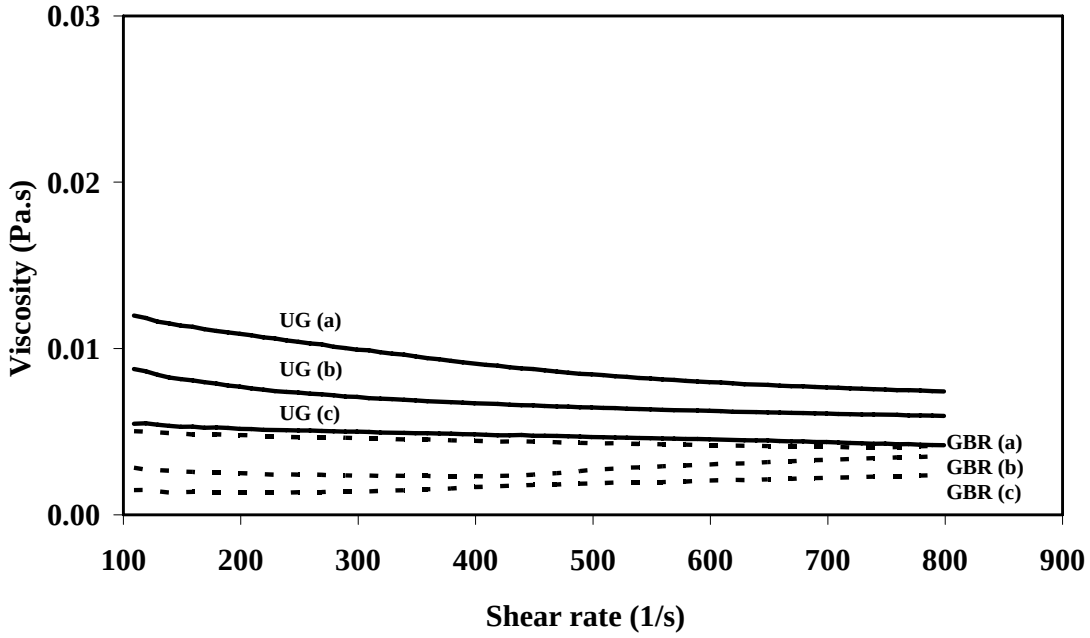
Table 8. Consistency coefficient (*k*) and flow behavior index (*n*) of un-germinated (UG) and germinated brown rice (GBR) flours

Rice		*k* (Pa.s)	*n*
Niaw Dam Peuak Dam	UG[1]	0.031±0.007[a]	0.796±0.007[b]
	GBR[2]	0.008±0.001[b]	0.976±0.020[a]
Sangyod Phattalung	UG	0.023±0.001[a]	0.829±0.009[b]
	GBR	0.006±0.000[b]	0.924±0.011[a]
Chiang Phattalung	UG	0.015±0.008[a]	0.896±0.041[b]
	GBR	0.001±0.001[b]	0.910±0.002[a]

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

[a-b] The different superscripts letters under the same column for each rice variety were significantly different ($p<0.05$).

All of un-germinated brown rice flour exhibited shear thinning behavior (pseudoplastic) with value of *n* less than 1.0 (Figure 14). The viscosity decreased with shear rate. The consistency index (*k*) was found to be amylose content dependent. The lower hydrodynamic volume for the high amylose content indicated the higher rigidity of polymer chains in the granule which corresponded to a low swelling powder (Launay *et al*., 1986) whereas low amylose content, the granules were more fragile and swelled more easily giving a high viscosity and more shear thinning behavior. After germination, brown rice flour exhibited like Newtonian behavior with value of *n* considerably nearly 1.0 (Figure 14). This was due to the granules of flour were more rigid and swell less.

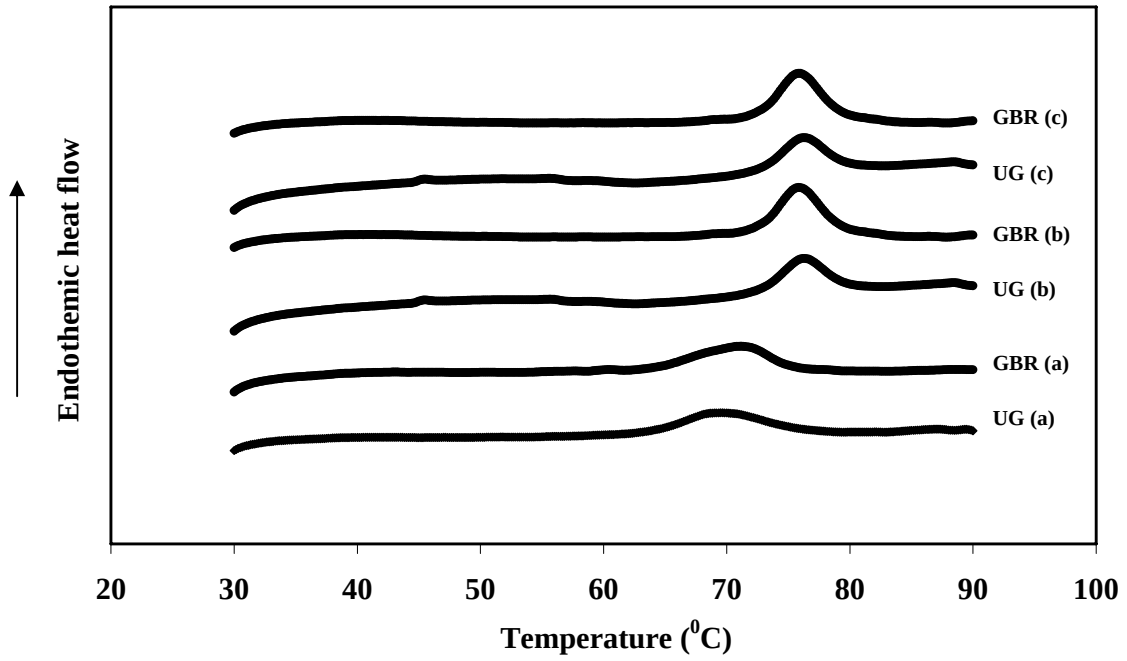


UG: Un-germinated brown rice, GBR: Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam (a) and 36 hours for Sangyod Phatthalung (b) and Chiang Phatthalung (c).

Figure 14. Relationship of apparent viscosity and shear rate of un-germinated (UG) and germinated brown rice flours (GBR) using a Rotational Rheometer

Thermal properties

Gelatinization behavior of brown rice flour in excess water was measured by Differential Scanning Calorimeter. It was found that all brown rice flour gave the typical endotherm enthalpy peak showing a melting transition of the crystalline region (Figure 15). The gelatinization parameters for this rice flour: onset temperature (To), peak temperature (Tp), end temperature (Tc) and enthalpy of gelatinization (ΔH) are shown in Table 9.



UG: Un-germinated brown rice, GBR: Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam (a) and 36 hours for Sangyod Phatthalung (b) and Chiang Phatthalung (c).

Figure 15. Thermograms of un-germinated (UG) and germinated brown rice (GBR) flour using a Differential Scanning Calorimeter

The gelatinization temperature (To) of brown rice flour var. Niaw Dam Peuak Dam, Sangyod Phattalung and Chiang Phattalung were 63.62, 72.63 and 76.46°C, respectively. Pongsawatmanit *et al*. (2003) found that the onset temperature of rice flour var. Sangyod Phatthalung and Chiang Phatthalung were 77.04 and 76.78°C, respectively. As the result, the rice flour could be classified into three groups according to To values, i.e. low amylose (Niaw Dam Peuak Dam) with To values of 63.62°C and intermediate (Sangyod Phattalung) and high amylose (Chiang Phattalung) with To values of 72.63 and 76.46°C, respectively. The difference in To values for these rice flours was influenced by amylopectin structure which has more long chains and less tendency to disperse (Han and Hamaker, 2001). This study also indicated that amylose content of rice may affect the gelatinization temperature. Thermal characteristic of germinated brown rice showed similar pattern with brown rice. The gelatinization temperature of germinated brown rice flour of Niaw Dam

Peuak Dam, Sangyod Phattalung and Chiang Phattalung were 64.03, 72.43 and 75.69°C, respectively.

Table 9. Gelatinization parameters of un-germinated (UG) and germinated brown rice (GBR) flours

Rice		Thermal parameter			
		To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Niaw Dam	UG[1]	63.62±0.08^{ns}	69.39±0.08^{ns}	76.43±0.12^{ns}	7.94±0.07^{ns}
Peuak Dam	GBR[2]	64.07±0.05^{ns}	70.60±0.81^{ns}	75.39±0.06^{ns}	7.88±0.03^{ns}
Sangyod	UG	72.40±0.05^{ns}	76.23±0.14^{ns}	79.76±0.12^{ns}	7.01±0.04^{ns}
Phattalung	GBR	72.23±0.08^{ns}	75.73±0.14^{ns}	79.57±0.05^{ns}	7.14±0.04^{ns}
Chiang	UG	76.38±0.05^{ns}	80.26±0.12^{ns}	84.22±0.04^{ns}	8.83±0.14^{ns}
Phattalung	GBR	75.57±0.17^{ns}	80.19±0.03^{ns}	83.61±0.21^{ns}	9.27±0.68^{ns}

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

^{ns} denote not significant differences which comparing UG *vs* GBR among same parameter for each rice variety ($p>0.05$).

As the result of physical-chemical properties, the germinated brown rice trends to be cooked similar to conventional rice. Applications of germinated brown rice comprises many categories of food products, beginning with functional cereals used for preparing staple foods (e.g. rice porridge, rice noodle, soup, crispy rice flour), functional drinks (e.g. non-alcoholic beverages fortified with GABA), fermented beverages (e.g. drinking yogurt), snacks (e.g. rice cracker), desserts (e.g. functional ice cream) and confectionery.

Chemical properties

Chemical properties of germinated brown rice are shown in Table 10. Protein content of three rice varieties ranged from 9.20-9.73%; fat content from 2.75-3.18%; and total dietary fiber from 4.64-5.27%. The protein and fat content increased significantly after germination ($p<0.05$), probably because of the biosynthesis of new compounds during germination. This agrees with other reported increasing of proximate composition in germinated brown rice (Jung *et al*., 2005; Lee *et al*., 2007, Sawaddiwong, 2009). The total dietary fiber content of germinated brown rice increased 1.39, 1.28 and 2.01 times for Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung, respectively as compared to the un-germinated brown rice. This tendency for an increase in dietary fiber after germination process agrees with result was report by Lee *et al*. (2007) found that total dietary fiber of rough rice var. Ilpum, Goami 2, Keunnun, and Heugkwang increased 1.1-1.9 times after germination at 15°C for 3 days. Ohtsubo *et al*. (2005) also reported that germinated brown rice contained more total dietary fiber, soluble dietary fiber and insoluble dietary fiber which increased 1.45, 1.20 and 1.50 times, as compared to the brown rice. Increase of dietary fiber results from the formation of primary cell walls, through an increase in pectic substance in the middle lamella (Lee *et al*., 2007).

Free sugar content in un-germinated and germinated brown rice are shown in Table 11. Monosaccharide such as glucose and fructose, and disaccharide such as maltose and sucrose, was found in un-germinated and germinated brown rice. Un-germinated brown rice var. Niaw Dam Peuak Dam contained glucose and sucrose, while un-germinated Sangyod Phatthalung and Chiang Phatthalung contained fructose and sucrose.

Table 10. Chemical compositions in un-germinated (UG) and germinated brown rice (GBR)

Rice		Protein (% db)	Fat (% db)	Ash (% db)	Total dietary fiber (% db)
Niaw Dam Peuak Dam	UG[1]	8.06±0.03[b]	2.94±0.02[b]	1.67±0.01[b]	3.34±0.01[b]
	GBR[2]	9.65±0.24[a]	3.18±0.01[a]	1.75±0.01[a]	4.64±0.14[a]
Sangyod Phatthalung	UG	8.93±0.04[b]	2.24±0.02[b]	1.20±0.01[b]	4.13±0.28[b]
	GBR	9.73±0.14[a]	2.75±0.03[a]	1.50±0.07[a]	5.27±0.18[a]
Chiang Phatthalung	UG	7.37±0.03[b]	2.67±0.06[b]	1.48±0.02[b]	2.61±0.01[b]
	GBR	9.20±0.04[a]	2.83±0.01[a]	1.54±0.04[a]	5.26±0.28[a]

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

[a-b] The different superscripts letters under the same column for each rice variety were significantly different ($p<0.05$).

The free sugar content of the brown rice increased after germination. Total free sugar content increased 1.78, 2.00 and 2.57 times of Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung, respectively, as compared to the un-germinated brown rice. A similar result was reported by Lee *et al*. (2007) found that total free sugars increased 4-9 times of rough rice after germination. There was due to starch hydrolysis. Ayernor and Ocloo (2007) also reported that, the main sugar of rice embryo and endosperm is glucose, together with small amounts of raffinose, and fructose. Reducing and non-reducing sugars also increased during germination at 32°C for 9 days due to the action of hydrolytic enzymes such as α- and β-amylases, which hydrolyze starch into low molecular weight carbohydrates such as maltose, glucose and dextrin.

Table 11. Free sugar content of un-germinated (UG) and germinated brown rice (GBR)

Rice		Free sugar content (% db)				
		Fructose	Glucose	Sucrose	Maltose	Total
Niaw Dam Peuak Dam	UG[1]	ND	0.38±0.01	3.20±0.01	ND[3]	3.58±0.01[b]
	GBR[2]	0.50±0.01	0.47±0.01	4.06±0.02	1.34±0.04	6.36±0.05[a]
Sangyod Phatthalung	UG	0.26±0.01	ND	0.24±0.01	ND	0.50±0.02[b]
	GBR	0.36±0.01	0.12±0.00	0.29±0.00	0.22±0.01	1.00±0.01[a]
Chiang Phatthalung	UG	0.09±0.00	ND	0.41±0.01	ND	0.49±0.01[b]
	GBR	0.56±0.03	0.44±0.00	0.26±0.01	ND	1.26±0.03[a]

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

[3] Not detected

[a-b] The different superscripts letters under the same column for each rice variety were significantly different ($p<0.05$).

It can be seen that total free sugar content of Niaw Dam Peuak Dam was higher than Sangyod Phatthalung and Chiang Phatthalung. Wijngaard *et al*. (2005) reported that buckwheat starch (low amylose content) degraded easier than barley starch (high amylose content) that would produce more total free sugar.

GABA

The GABA content in un-germinated and germinated brown rice is shown in Table 12. Among the un-germinated brown rice samples, Niaw Dam Peuak Dam had the highest GABA content. After germination, GABA content in Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung increased 11.28, 16.74 and 9.47 times, respectively. In particular, GABA increased greatly in the Sangyod Phatthalung. This indicates that introducing a germination process was successful in terms of increasing the GABA content in brown rice. A similar result was reported by Sunte *et al*. (2007) found that GABA content in germinated brown rice (Khao Dawk Mali 105) increased 9.2 times after soaked in the 0.1 mM $CaCl_2$, pH 5 at 40°C for 36

hours, as compared with brown rice. Charoenthaikij *et al*. (2009) also reported GABA content in germinated brown rice (Khao Dawk Mali 105) increased 31.75 times, as compared with brown rice, after soaked in buffer solution at pH 3 for 72 hours. Watchraparpaiboon *et al*. (2007) also reported that GABA content in germinated brown rice (Khao Dawk Mali 105) soaked in water at pH 6 at 35°C for 24 hours, had higher than soaked in water pH 3, 4 and 8.

Table 12. GABA content in un-germinated (UG) and germinated brown rice (GBR)

Rice	GABA content (mg/100g db)	
	UG[1]	GBR[2]
Niaw Dam Peuak Dam	3.61±0.06[b]	40.72±0.29[a] (11.28)
Sangyod Phatthalung	2.66±0.11[b]	44.53±1.93[a] (16.74)
Chiang Phatthalung	3.09±0.05[b]	29.25±0.77[a] (9.47)

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

[a-b] The different superscripts letters under the same row were significantly different ($p<0.05$).

Figure in parentheses indicate time increased over un-germinated brown rice

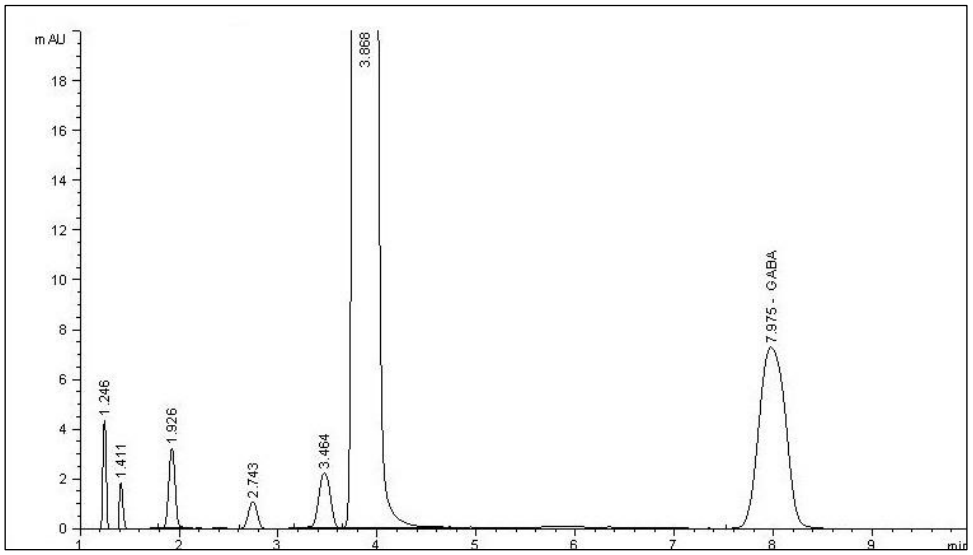
Furthermore, Varanyanond *et al*. (2005) found that the highest of GABA content was found in germ rice var. Khao Dawk Mali 105 (18.62 mg/ 100g) Pathum Thani 1 (15.46 mg/100g) and Chai Nat 1 (14.45 mg/100g) which soaked in water at 40°C for 4 hours. Lee *et al*. (2007) reported that GABA content in Korean rough rice var. Ilpum, Goami2, Keunnun and Heugkwang increased 2.4, 2.5, 6.1 and 3.4 times, respectively, after germinated at 15°C for 24 hours. Ohtsubo *et al*. (2005) found that GABA content in Japonica rice var. Koshihikari which soaking in water for 72 hours at 30°C increased 40 times as much as that of polished rice and 11.5 times as much as that of brown rice. Komatsuzaki *et al*. (2007) reported GABA content in Japonica rice var. Haiminori after soaking for 3 hours and gaseous treatment for 21 hours at 35°C had higher than soaking in water for 24 hours for 2.46 times.

In germinated cereal grains, hydrolytic enzymes are activated and this decomposes starch, non-starch polysaccharides, and amino acids. The decomposition

of high molecular weight polymers during germination leads to the generation of bio-active compounds. This leads to improvements in the organoleptic qualities due to the softening of the texture and increase of the flavor in cereal grains (Kayahara, 2004).

To confirm GABA composition in brown rice from HPLC analysis (Figure 16), mass spectrometry was use to detect GABA based on the molecular weight of derivative formed (GABA-HN) as shown in Figure 17.

(a)



(b)

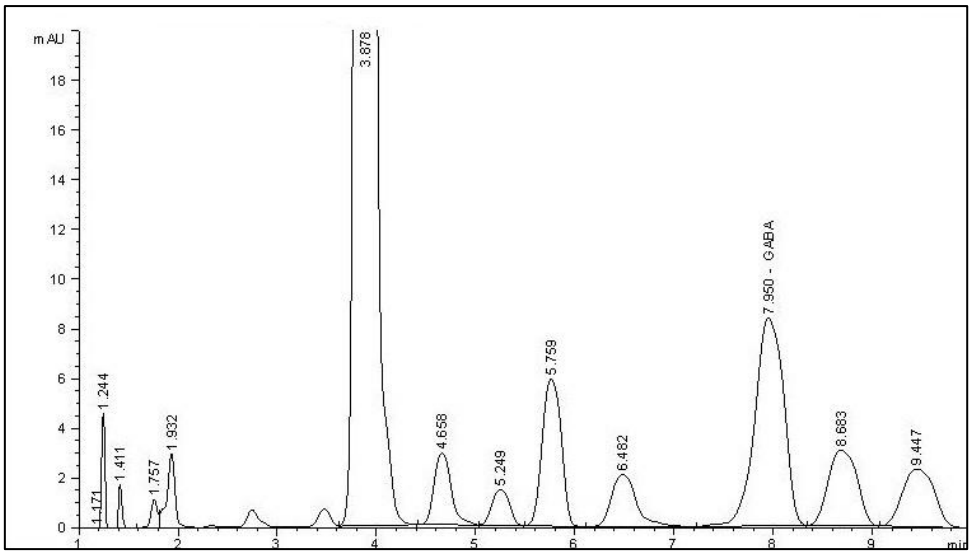


Figure 16. HPLC chromatograms of GABA standard (a) and sample extraction containing GABA (b)

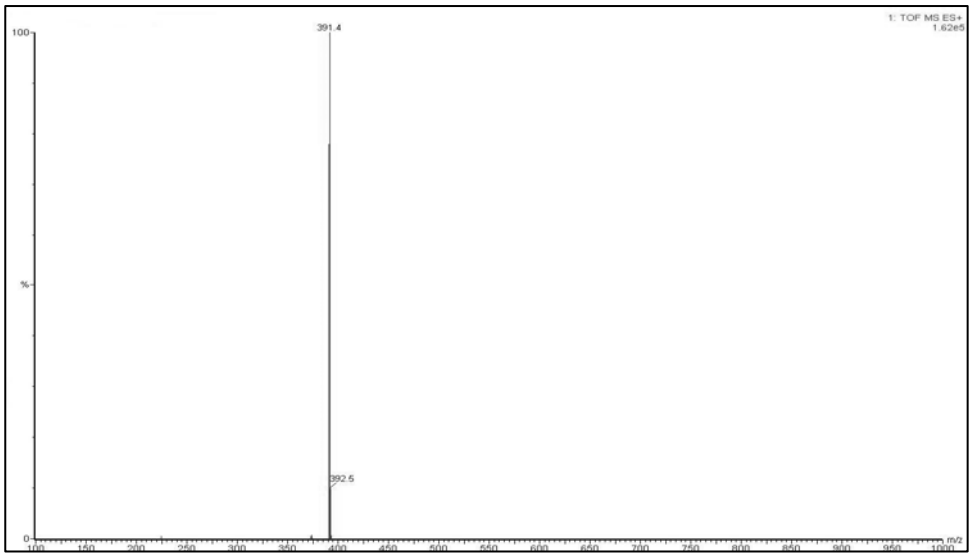


Figure 17. Mass spectrum of GABA derivative

γ-oryzanol

Four major components of γ-oryzanol content in un-germinated and germinate brown rice were identified as cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and sitosteryl ferulate (Xu and Godber, 1999) and the result are shown in Table 13. The results indicated that Niaw Dam Peuak Dam had the highest γ-oryzanol content compared with Sangyod Phatthalung and Chiang Phatthalung as also reported by Manuswarakul *et al*. (2003) showed that the black-glutinous rice have the highest γ-oryzanol content compared with the white-glutinous rice and non-glutinous rice. The γ-oryzanol content was in the same level for the brown rice and the germinated brown rice. A similar with research report of Japonica rice var. Koshihikari soaked in water at 30°C for 72 hours (Ohtsubo *et al*., 2005). Kiing *et al*. (2009) was also investigated the effect of germination process on γ-oryzanol levels in Sarawak local rice (Malaysia rice) and found that after germination of 24 hours, brown rice var. Sabak, Silah and Hitam showed slight increases in γ-oryzanol content. On the other hand, brown rice var. Chelum, Biris, Boria, Udang Halus and Mamut showed reduction in γ-oryzanol content compared to brown rice levels. The highest γ-oryzanol content after 24 hours was Silah (26.4 mg/100g) meanwhile the lowest γ-oryzanol content was Udang Halus (7.7 mg/100g).

However, a reverse trend was observed by Sungsopha *et al*. (2009) who reported that γ- oryzanol in germinated brown rice increase 29.31% compared with un-germinated brown rice. Jiamyangyeun (2006) also found that the red brown rice and brown rice var. Khao Dawk Mali 105 soaked in water for 6 hours and germinated in the dark for 24 hours showed increased of the γ-oryzanol content 1.3-1.5 times, compared to un-germinated brown rice. In addition, brown rice soaked in pandanus solution for 6 hours and germinated in the dark for 24 hours, the γ-oryzanol content increased. On the other hand, when brown rice soaked in lemon grass solution for 6 hours and germinated in the dark for 24 hours, the γ-oryzanol content decreased (Chutipanya, 2006).

Table 13. γ-oryzanol content in un-germinated (UG) and germinated brown rice (GBR)

Rice		γ-oryzanol content (mg/100g db)			
		Cycloartenyl ferulate	24-Methylene cycloartanyl ferulate	Campesteryl ferulate	Sitosteryl ferulate
Niaw Dam Peuak Dam	UG[1]	77.79±0.83^{ns}	92.39±0.42^{ns}	133.02±1.90^{ns}	204.08±2.66^{ns}
	GBR[2]	73.03±3.04^{ns}	91.82±1.11^{ns}	130.69±1.49^{ns}	201.57±4.64^{ns}
Sangyod Phatthalung	UG	26.25±1.12^{ns}	64.16±1.10^{ns}	101.41±1.89^{ns}	135.09±3.16^{ns}
	GBR	22.46±0.45^{ns}	63.61±2.40^{ns}	96.17±3.54^{ns}	129.07±2.84^{ns}
Chiang Phatthalung	UG	31.67±1.49^{ns}	71.27±0.65^{ns}	81.32±1.13^{ns}	81.59±0.69^{ns}
	GBR	26.58±2.44^{ns}	67.84±1.40^{ns}	77.21±1.49^{ns}	75.86±3.51^{ns}

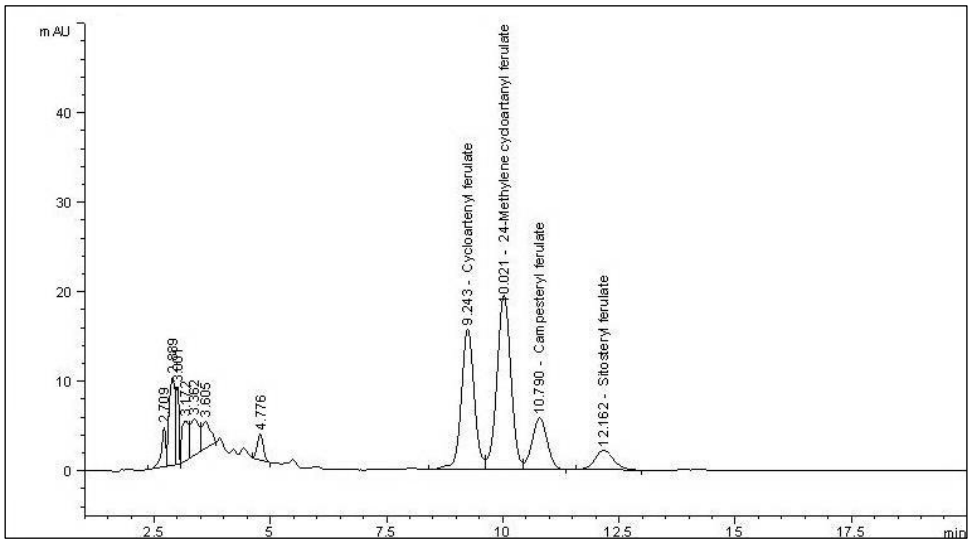
[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

^{ns} denote not significant differences which comparing UG *vs* GBR among same compound for each rice variety ($p>0.05$).

Concentrations of γ-oryzanol in germinated brown rice depend on varieties and germination duration. This may be attributed to genetic and environmental factors. The reportes of Bergman and Xu (2003) and Miller and Engel (2006) found enormous variation in oryzanol content among genotypes. Furthermore, the trends observed in different varieties may also be due to different water uptake rates by the different rice seeds (Alam *et al*., 2003).

To confirm γ-oryzanol composition in brown rice from HPLC analysis (Figure 18), mass spectrometry was use to detect γ-oryzanol based on the molecular weight as shown in Figure 19.

(a)



(b)

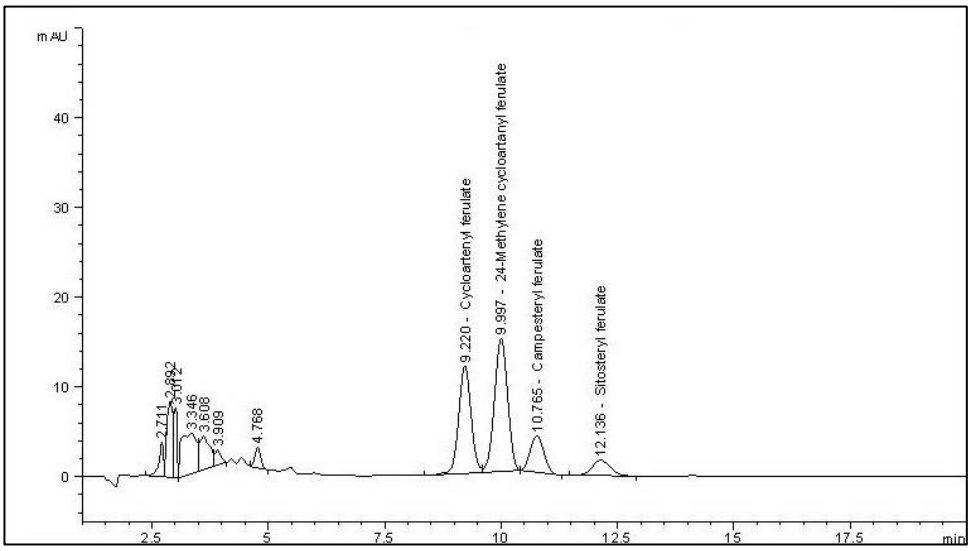


Figure 18. HPLC chromatograms of γ-oryzanol standard (a) and sample extraction containing γ-oryzanol (b)

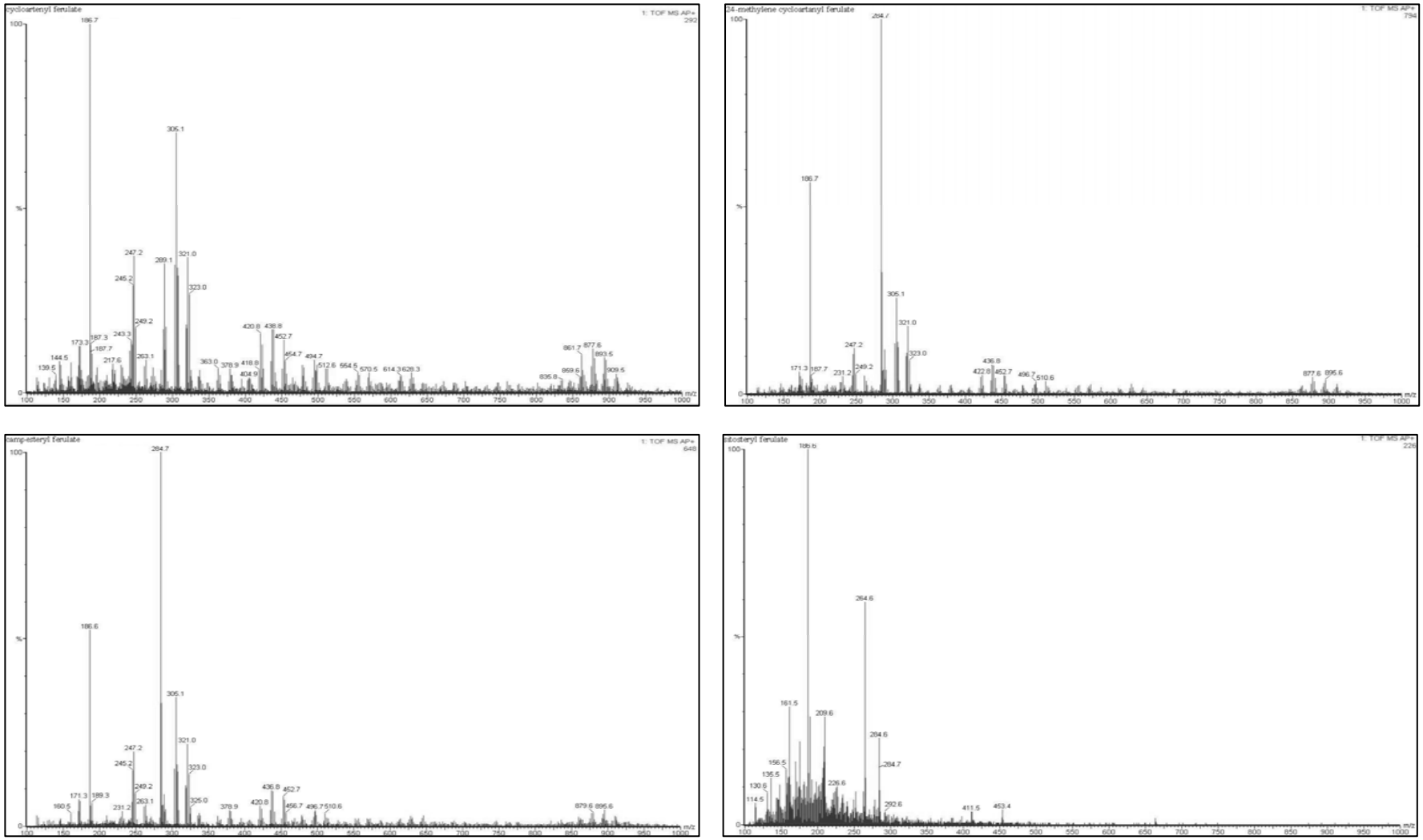


Figure 19. Mass spectrum of γ-oryzanol

Ferulic acid

The ferulic acid content in un-germinated and germinated brown rice were analyzed by HPLC (Figure 20) and the results are shown in Table 14. The ferulic acid content of Niaw Dam Peuak Dam, Sangyod Phatthalung, and Chiang Phatthalung increased 1.12, 1.43 and 1.37 times, respectively after germination. The results indicated that germination induced increased ferulic acid content. This is similar to the reported by Ohtsubo *et al*. (2005) found that total ferulic acid in germinated brown rice var. Koshihikari increased 1.26 and 3.93 times, compared to brown rice and polished rice, respectively.

Table 14. Ferulic acid content in un-germinated (UG) and germinated brown rice (GBR)

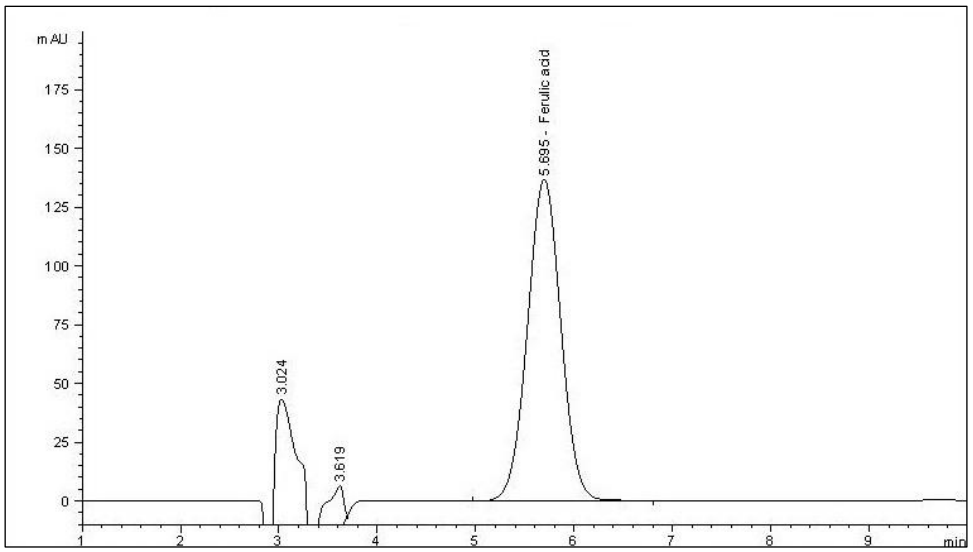
Rice	Ferulic acid content (mg/100g db)	
	UG[1]	GBR[2]
Niaw Dam Peuak Dam	26.03±1.08[b]	29.23±1.15[a] (1.12)
Sangyod Phatthalung	21.75±0.64[b]	31.02±1.02[a] (1.43)
Chiang Phatthalung	23.02±0.67[b]	31.50±0.43[a] (1.37)

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

[a-b] The different superscripts letters under the same row were significantly different ($p<0.05$).

Figure in parentheses indicate time increased over un-germinated brown rice

(a)



(b)

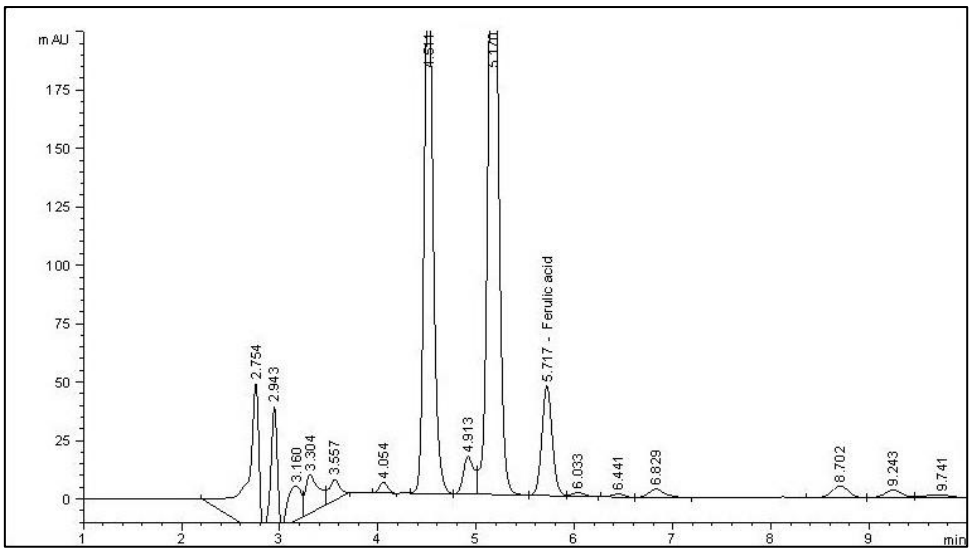


Figure 20. HPLC chromatograms of ferulic acid standard (a) and sample extraction containing ferulic acid (b)

Phytate

The phytate content in un-germinated and germinated brown rice are shown in Table 15. The phytate content of brown rice varies from 860.77 to 883.91 mg/100g. Chansuwan (2005) reported the phytate content of brown rice (Chai Nat 1, Pathum Thani 1 and Kor-Khor 23) ranged 796.24-868.84 mg/100g and colored rice (Cha Hom Nil) ranged 543.11-864.19 mg/100g. Ravindean *et al*. (1994) suggested that phytate content remains higher or lower in rice grain depending on polishing process. Phytate content in germinated brown rice decreased 0.270.29%, as compared with un-germinated brown rice. A similar result was reported by Liang *et al*. (2008) found that phytic acid in brown rice var. Kenjian 90-31 (China rice) which germinated at 30°C for 72 hours decreased 60%, as compared with un-germinated brown rice. Khampang *et al*. (2009) also found that, the phytic acid in brown rice var. Khao Dawk Mali 105 soaked in water at 25°C, pH 5.5 and pH 6.5 for 48 hours decreased 3.47 and 5.27%, respectively, as compared with brown rice. Moong-ngarm (2005) also found that, the phytic acid in paddy rice var. Kor-Khor 6 which germinated at 28°C for 4 days decreased 63% and the phytase activity increased as high as 936%, as compared with un-germinated rice. In un-germinated rice only inositol hexaphosphate (IP6) and inositol pentaphosphate (IP5) were detected and the IP6 was the major component, up to 90% of the total phytic acid. After germination, the level of IP6 decreased whereas IP5 slightly increased on the first and second germinating day.

Decreases caused by germination are mainly based on the action of enzymes while, in soaking, a combination of diffusion and enzymatic action is expected (Henderson and Ankrah, 1985; Mahgoub and Elhag, 1998). Soaking of intact grains, as a first step of germination, was decreased of 14–28% of phytic acid due to the activity of endogenous phytase and diffusion of phytic acid into the soaking medium (Liang *et al*., 2008). Diffusion of phytic acid was reportedly influenced by the nature of the phytate, which may be in the form of salts with different minerals, such as potassium, calcium or magnesium, and the pH of the medium (Henderson and Ankrah, 1985; Mahgoub and Elhag, 1998). Liang *et al*. (2009) also observed that soaking in acidic buffer was more effective to remove phytic from brown rice and rice

bran than in de-mineralized water, presumably because of the higher solubility of phytate in acidic conditions.

Table 15. Phytate content in un-germinated (UG) and germinated brown rice (GBR)

Rice	Phytate content (mg/100g db)	
	UG[1]	GBR[2]
Niaw Dam Peuak Dam	862.86±20.85[a]	629.98±23.13[b] (0.27)
Sangyod Phatthalung	860.77±7.55[a]	609.17±4.48[b] (0.29)
Chiang Phatthalung	883.91±29.89[a]	633.25±41.02[b] (0.28)

[1] Un-germinated brown rice, [2] Germinated brown rice which brown rice soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel 48 hours for Niaw Dam Peuak Dam and 36 hours for Sangyod Phatthalung and Chiang Phatthalung.

[a-b] The different superscripts letters under the same row were significantly different ($p<0.05$).

Figure in parentheses indicate time of decreased over un-germinated brown rice

Furthermore, the reduction of phytic acid increased with germination time, which agrees with previous studies reporting that the activity and/or production of phytase increased during germination (Henderson and Ankrah, 1985; Larsson and Sandberg, 1995; Liang *et al*., 2008; Moong-ngarm, 2005). The stepwise hydrolysis of phytate to phosphate and inositol occurs by the action of phytase. Phosphatase hydrolyzes a broad spectrum of phosphate esters, while phytase is a phytate-specific phosphatase (Greiner *et al*., 1998). The phosphatase is not capable of degrading phytate. Two types of phytase have been identified which initiate the hydrolysis of phytate at either the 3- or 6-position of the inositol ring (Konietzny *et al*., 1995; Greiner *et al*., 2001). The phytases from microorganism such as *Aspergillus niger* is considered as 3-phytases (EC 3.1.3.8), while plant phytases as 6-phytases (EC 3.1.3.26) (Turk *et al*., 1996; Greiner *et al*., 1998, 2000).

The principal function of phytase in seeds or grains is to produce inorganic phosphate from phytate during germination. The inorganic phosphate thus produced is then utilized for the purpose of plant growth. Previous authors have studied various plant phytases from maize, barley, spelt, canola seed and rye (Houde *et al*., 1990; Laboure *et al*., 1993; Konietzny *et al*., 1995; Greiner *et al*., 1998, 2000).

It appears that phytase activity usually increases on germination and the types of phytase may be plant-dependent.

2. Optimization method for extract GABA from germinated brown rice and its fractions

Fraction of germinated brown rice

Brown rice var. Sangyod Phatthalung was soaked in citrated buffer pH 3.0 for 5 hours and germinated in closed vessel for 36 hours. The germinated brown rice was dried to <13% of moisture content. After that, the germinated brown rice was milled to remove germ and bran in a polishing machine. The germinated brown rice contained 10.64% of germ, 7.45% of bran and 81.91% of white rice (Figure 21). This is similar to the result was report by Juliano and Bechtel (1985) found that the bran portion account for 8% of the brown rice weight. Varanyanond *et al*. (2005) reported that germ portion of un-germinated brown rice var. Khao Dawk Mali 105 had 3.61% of brown rice.

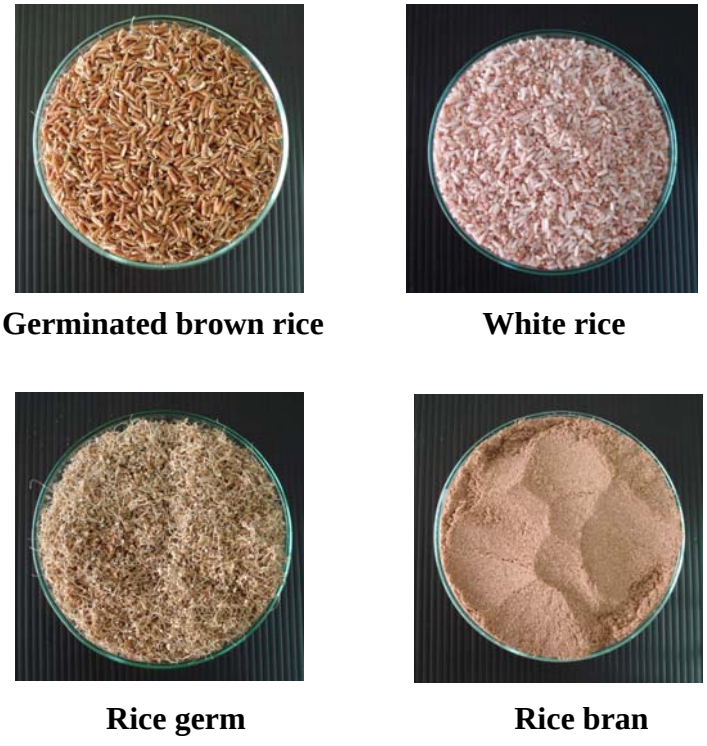


Figure 21. Germinated brown rice and its fractions

The GABA content of germinated brown rice and its fractions varied from 11.74-272.15 mg/100g, with germ showing the highest amount at 272.15 mg/100g (Table 16). Varanyanond *et al*. (2005) reported that the GABA content in germ of Thai rice which germinated in distilled water and incubated at 40°C ranged 29-55 mg/100g of germ.

Table 16. GABA content in germinated brown rice and its fraction**s**

Fraction	GABA content (mg/100g db)
Germinated brown rice[1]	41.44±1.93[c]
Germ	272.15±2.59[a]
Bran	105.50±3.13[b]
White rice	11.74±0.45[d]

[1] Brown rice var. Sangyod Phatthalung soaked in citrate buffer at pH 3 for 5 hours and germinated in closed vessel for 36 hours.

[a-d] The different superscripts letters under the same column were significantly different ($p<0.05$).

White rice had the lowest of the GABA content. It contained only slightly protein and lipid but considerably more starch (Juliano and Bechtel, 1985). By removing rice bran and germ, more than 75% of GABA content has been lost once it becomes white rice.

Optimum extraction method for extract GABA for germinated brown rice and its fractions

GABA content of the extracts from germinated brown rice and its fractions using various solutions and extracting temperature are shown in Table 17. All fractions, the highest GABA content was obtained when extracted in distilled water at 30°C. Germ showed highest amount of GABA content, followed by rice bran and white rice, respectively. The higher of ethanol concentration is, the lower the GABA content ($p<0.05$). A similar result was report by Takayo *et al*. (2001) found that ethanol decreased the accumulated GABA content of defatted rice germ. GABA is a four-carbon non-protein amino acid which is highly soluble in water (Shelp *et al*.,

1999). Therefore, distilled water is more suitable for the extraction of GABA than aqueous ethanol solutions.

Table 17. GABA content in the extracts from germinated brown rice and its fractions using different extraction solvent for various temperatures

Fraction	Temperature (°C)	GABA content (mg/100g db)		
		Distilled water	50% Ethanol	75% Ethanol
Germinated brown rice extracts	30	40.87±1.35^{Aa}	32.55±0.92^{Ab}	28.74±0.95^{Ac}
	40	38.25±0.58^{Ba}	31.02±0.87^{Bb}	26.92±0.31^{Bc}
	50	37.21±0.62^{Ba}	31.33±0.43^{ABb}	25.78±0.46^{Bc}
	60	37.95±1.10^{Ba}	28.83±0.34^{Cb}	26.50±0.97^{Bc}
Germ extracts	30	271.45±0.45^{Aa}	245.72±1.73^{Ab}	223.79±0.46^{Ac}
	40	269.44±1.58^{Aa}	228.35±1.54^{Bb}	218.47±1.77^{Bc}
	50	261.27±3.21^{Ba}	227.83±1.60^{Bb}	218.48±1.00^{Bc}
	60	262.10±3.37^{Ba}	228.89±0.69^{Bb}	218.05±1.39^{Bc}
Bran extracts	30	55.73±0.21^{Aa}	41.48±1.02^{Ab}	38.43±1.24^{Ab}
	40	54.54±0.40^{Ba}	42.58±1.17^{Ab}	34.01±1.04^{Bc}
	50	53.95±0.76^{Ba}	40.30±1.78^{Ab}	34.12±0.35^{Bc}
	60	53.95±0.81^{Ba}	41.21±1.05^{Ab}	34.54±0.66^{Bc}
White rice extracts	30	5.73±0.23^{Aa}	4.48±0.16^{Ab}	3.72±0.16^{Ac}
	40	5.65±0.14^{Aa}	4.43±0.17^{Ab}	3.56±0.17^{Ac}
	50	5.22±0.09^{Ba}	4.06±0.18^{Bb}	3.65±0.13^{Ac}
	60	5.46±0.23^{Ba}	4.11±0.14^{Bb}	3.61±0.06^{Ac}

$^{A-B}$ The different superscripts letters under the same column for each fraction with different temperature were significantly different ($p<0.05$).

$^{a-c}$ The different superscripts letters under the same row for each fraction with different solvent were significantly different ($p<0.05$).

The effect of extraction temperature on the GABA content in germinated brown rice and its fraction (Table 17) showed that extraction temperature of 40-60°C affect the GABA content with a slightly decreased. However, a reverse trend was observed by Monsoor *et al*. (2003) who studied the effect of temperature

(20–60°C) on the aqueous extraction of emulsified rice bran oil from commercial rice bran and found that higher temperatures more solids were extracted compare to lower temperatures due to the greater extractability and solubility of the components at higher temperatures.

GABA content of the extracts from germinated brown rice and its fractions for different times of extraction are shown in Table 18. The GABA content accumulation differed among rice fractions. The highest GABA content in germinated brown, germ, bran, and white rice were obtained when the sample was extracted with distilled water for 6, 6, 24 and 12 hours, respectively.

Table 18. GABA content in the extracts from germinated brown rice and its fractions using distilled water at 30^{o}C at various extraction times

Fraction	GABA content (mg/100g db)			
	3	6	12	24
Germinated brown rice extracts	31.56±1.44^{b}	40.85±1.00^{a}	39.14±1.35^{a}	39.04±0.92^{a}
Germ extracts	230.40±1.61^{b}	271.23±0.45^{a}	272.44±3.94^{a}	272.87±1.39^{a}
Bran extracts	33.21±2.32^{d}	54.98±0.43^{c}	81.75±2.62^{b}	104.82±0.41^{a}
White rice extracts	5.88±0.17^{c}	5.65±0.50^{b}	10.78±0.61^{a}	10.86±0.13^{a}

$^{a-c}$ The different superscripts letters under the same raw were significantly different ($p<0.05$).

3. Development of functional drink from germinated brown rice

Consumer survey of functional drink

Demographic data

Three age groups were selected in the original design of the surveys and included 15-25, 26-45 and over 45 years old respondents. The sample was divided into these particular age groups as it was thought that they would have quite different functional drink and attitudes due to their life circumstances, experiences etc. The minimum age for respondents was set at 15 because at this age starting to gain a basic understanding of nutrition and understanding of how various basic nutrients affect their bodies. Moreover, they also can taste and answer the questionnaire.

The demographic profiles of three hundred respondents are shown in Table 19. There were 27.3% males and 72.7% females. Marital status of respondents were single (54.3%) and married (41.0%). The highest education indicated for most of them was bachelor degree (59.3%). The most of them had income less than 10,000 Bath/month (47.3%).

Table 19. The demographic profiles of the respondents in consumer survey

Respondent characteristic	Response (%)
Gender	
Female	72.7
Male	27.3
Age	
15-25 years old	33.3
26-45 years old	33.3
Over 45 years old	33.3
Marital status	
Single	54.3
Married	41.0
Other	4.7
Occupation	
Government official	35.0
Student	34.7
Employee	13.3
Other	17.0
Education	
Lower secondary school certificate	16.0
Vocational certificate/2 years of collage	8.0
Bachelor degree	59.3
Higher than Bachelor degree	16.7
Personal income (Baht per month)	
Less than 10,000 Baht	47.3
10,000-20,000 Baht	31.7
20,001-30,000 Baht	15.3
More than 30,000 Baht	5.7

Functional drink consumption behavior

Consumers were asked a number of questions relating to their functional drink consumption behavior. The consumer behaviors on functional drink of the respondents are shown in Table 20. The respondents drank functional drink at 3-4 times/week (36.3%), 1-2 times/week (28.3%) and daily (21.3%). Mostly drank in the morning (47.0%), afternoon break (34.3%), the evening (28.0%) and before bed time (27.7%). The objectives for drinking functional drink were improving their health (83.3%), refreshing (75.0%) and curing the disease (23.7%). Lappalainen *et al.* (1998) and Uutama-ang, (2006) reported that health is one of the frequently mentioned motivations behind food choices.

The favorite functional drinks of respondents are shown in Table 21. The most favorite functional drink was dairy product (85.7%), then a fruit or vegetable juice (76.9%) and the last was cereal product (70.3%).

Consumers were asked to state what factors influenced their purchase decisions and the results are shown in Table 22. From the table showed that the responds purchased the functional drink based on taste, nutritious and prices. Moreover, packaging, brand, advertisement and popularity seem to influence teenagers' decision than the young adults (26-45 years old) and adults (over 45 years old) while young adults also consider on academic supports. However, manufacturing company and country of production don't effect on the purchase' decision.

Table 20. The consumer behavior on functional drink of the respondents

Behavior	Response (%)
Drinking frequency	
Daily	21.3
3-4 times/week	36.3
1-2 times/week	28.3
< 1 time/week	14.0
Drinking time	
Morning	47.0
Moring break	12.3
Noon	15.0
Afternoon break	34.3
Evening	28.0
Before bed time	27.7
Objective of drinking	
Health benefit	83.3
Refreshing	75.0
Curing the disease	23.7
Beauty or diet	20.3
Anti-aging	8.7

Table 21. The favorite functional drink of respondents

Functional drink	Response (%)
Dairy product	85.7
Fruit or vegetable juice	76.0
Cereal product	70.3
Herb beverage	53.7
Dried herb	50.0
Instant tea	47.3
Cereal powder	43.3
Tea	42.3
Extract beverage	25.3
Fruit powder	23.7

Table 22. Factor affecting functional drink purchase decision

Factor	Response (%)
Taste	84.0
Nutritious	78.7
Price	62.7
Flavor	57.7
Convenient of consumption	49.3
Easily available	42.3
Packaging	25.0
Academic support	23.7
Brand	23.0
Easily to bring	18.0
Hearsay	14.3
Place of buying	14.0
Advertisement	12.3
Popularity	10.0
Manufacturing company and county of production	7.0

Consumer attitudes to functional drink from germinated brown rice

The attitudes of consumers to functional drink from germinated brown rice are shown in Table 23. The respondents thought that functional drink from germinated brown rice should be made from germinated brown rice mix (68.3%). The mixing ingredients were cereal (55.1%), fruit (36.1%) and dairy product (30.7%). They also thought that ready-to-drink (72.0%) was the type of functional drink.

Table 23. Attitudes to development of functional drink from germinated brown rice

Attitudes	Response (%)
Form of beverage	
Mix with other	68.3
Only GBR	31.7
Ingredient for mixing	
Cereal	55.1
Fruit	36.1
Dairy product	30.7
Herb	24.4
Type of beverage	
Ready-to-drink	72.0
Powder	12.7
Instant powder	12.7
Concentrate	2.7
Taste	
Sweet	42.3
Milk	24.7
Natural	18.3
Other	14.7

In conclusion, the product profile of functional drink from germinated brown rice being ready-to-drink mixed with cereal in glass bottle with sweet flavor was preferred.

Development of functional drink from germinated brown rice

Mock-up of functional drink

Mock-up of functional drink was selected from commercial functional drink which had difference formula. Formula#1 consisted of germinated brown rice, formula#2 consisted of extract brown rice and soy-protein and formula#3 consisted of soymilk. The sensory scores of commercial functional drink are shown in Table 24. The result showed that formula#3 had highest sensory score which selected for the development of functional drink from germinated brown rice.

Table 24. Sensory scores of commercial functional drink

Attributes	Formula		
	1	2	3
Appearance	6.73 ± 1.11^{c}	7.33 ± 0.48^{b}	7.83 ± 0.65^{a}
Color	6.57 ± 1.14^{b}	7.83 ± 0.53^{a}	8.03 ± 0.67^{a}
Viscosity	6.70 ± 1.06^{c}	7.40 ± 0.77^{b}	7.97 ± 0.67^{a}
Texture	6.80 ± 1.13^{c}	7.33 ± 0.80^{b}	7.93 ± 0.69^{a}
Flavor	5.77 ± 1.22^{c}	7.57 ± 0.94^{b}	8.17 ± 0.59^{a}
Taste	6.37 ± 1.54^{b}	7.60 ± 0.97^{a}	7.70 ± 0.70^{a}
Overall acceptability	6.40 ± 1.10^{c}	7.53 ± 0.73^{b}	8.20 ± 0.66^{a}

[a-c] The different superscripts letters under the same row were significantly different ($p<0.05$).

The formula of mock-up functional drink consisted of soymilk, sugar and milk powder. Therefore, the formula of functional drink will consist of germinated brown rice extracts, soybean, sugar and milk powder.

Optimum formula of functional drink from germinated brown rice extracts

Eighteen formulas of functional drink from germinated brown rice extracts were evaluated for their sensory characteristics and the results are shown in Table 25. Based on the mean sensory score of all attributes the formula #15 (79.2% of germinated brown rice extract, 14.3% of soybean, 5.2% of sugar and 1.3% of milk powder) had the highest sensory score while formula #14 (83.3% of germinated brown rice and 16.7% of soybean) had the lowest. It was also observed that all of formula without sugar (formula #3, 8, 11, 13 and 14) were rated dislike moderately for attributes of taste whereas the formula having the levels of sugar ranged 3.2-5.3% (formula #2, 6, 15 and 17) were rate like moderately. However, the high level of sugar at 6% (formula #1, 4, 5, 7, 10, 12 and 16), the attributes of taste were decreased. This indicated that the levels of sugar range 3.2-5.3% were required to mask the favorable taste.

Regression models and goodness-of-fit obtained from the sensory attributes of functional drink are listed in Table 26. The model used for appearance, viscosity, texture, flavor, taste and overall acceptability were quadratic equations, while the model used for color was a special cubic equation. They were significant, which was evident from the probability values ($p<0.05$); the lack of fit was not significant ($p>0.05$). The coefficients of determination (R^2) varied between 0.8062–0.9920, explaining 80–99% of variability in response. Hu (1999) suggested that for a good fit of model, R^2 should be at least 0.75. Therefore, all regression had a good fit. Zhou *et al.* (2007) optimized formulation of pure culture in Tibetan kefir using the mixture design and found that the regression model of four responses were quadratic equations with R^2 reached 0.97-0.99.

Table 25. Sensory scores of functional drink from germinated brown rice

Formulation	Appearance	Color	Texture	Viscosity	Flavor	Taste	Overall acceptability
1	6.83±1.37^{bcde}	7.23±1.07^{abc}	7.00±0.83^{abcd}	6.83±1.09^{bcd}	7.17±1.09^{ab}	6.17±1.09^{c}	7.17±0.70^{ab}
2	7.33±0.76^{ab}	7.57±0.77^{a}	7.33±0.66^{ab}	7.30±0.70^{ab}	7.13±0.82^{ab}	7.07±0.98^{a}	7.37±0.72^{a}
3	7.23±0.68^{abc}	7.20±0.66^{abc}	7.00±0.83^{abcd}	7.20±0.89^{abcd}	7.17±0.38^{ab}	3.27±0.91^{e}	6.70±0.92^{cde}
4	6.83±1.09^{bcde}	6.57±0.50^{ef}	7.00±1.31^{abcd}	6.83±1.09^{bcd}	6.20±1.27^{d}	6.17±0.87^{c}	6.90±0.92^{abc}
5	7.07±0.87^{abcde}	6.63±0.72^{ef}	6.90±0.80^{bcd}	7.03±0.67^{abcd}	6.13±0.78^{d}	6.20±1.19^{c}	6.70±0.53^{cde}
6	7.17±0.79^{abcd}	6.97±0.72^{cde}	7.17±0.75^{abc}	7.20±0.92^{abcd}	7.13±0.73^{ab}	7.13±0.82^{a}	7.20±0.89^{ab}
7	6.80±0.76^{cde}	6.57±0.73^{abcde}	7.17±0.87^{abc}	6.87±0.68^{abcd}	4.07±0.91^{ef}	6.17±0.59^{c}	5.80±0.66^{f}
8*	7.33±0.71^{ab}	7.10±0.92^{bcd}	7.03±0.81^{abc}	7.00±1.08^{abcd}	7.10±0.92^{ab}	3.80±0.81^{d}	6.97±0.96^{ab}
9	7.33±0.76^{ab}	7.43±0.63^{ab}	7.23±0.77^{abc}	7.23±0.90^{abc}	7.23±0.68^{ab}	6.50±0.78^{bc}	7.20±0.81^{ab}
10*	7.00±1.23^{bcde}	7.37±0.85^{abc}	6.83±0.53^{cd}	6.90±0.61^{abcd}	6.77±0.90^{bc}	6.17±0.70^{c}	6.90±0.80^{abc}
11	6.10±0.92^{f}	6.63±0.67^{ef}	6.57±0.50^{de}	6.77±0.77^{cd}	4.17±1.60^{e}	3.67±1.27^{de}	5.17±0.79^{g}
12	6.70±0.79^{de}	7.47±0.86^{ab}	7.03±0.61^{abc}	6.70±0.70^{d}	7.13±0.73^{ab}	6.13±0.73^{c}	6.97±0.67^{bc}
13	7.33±0.66^{ab}	7.20±0.89^{abc}	7.07±0.83^{abc}	7.03±0.81^{abcd}	6.87±0.86^{bc}	3.17±0.91^{e}	6.47±0.94^{cde}
14*	5.50±0.73^{g}	6.50±0.63^{f}	6.23±0.82^{e}	6.10±0.84^{e}	3.67±0.92^{f}	3.33±0.92^{de}	6.13±0.94^{ef}
15	7.57±0.94^{a}	7.47±0.68^{ab}	7.43±0.57^{a}	7.37±0.61^{a}	7.43±0.73^{a}	7.30±1.18^{a}	7.37±1.03^{a}
16	6.70±0.70^{def}	7.33±0.84^{abc}	7.13±0.68^{abc}	7.17±0.79^{abcd}	6.83±0.75^{bc}	6.20±0.71^{c}	6.30±0.65^{ef}
17	6.63±0.49^{e}	6.70±0.65^{def}	6.87±0.86^{abc}	7.20±0.96^{abcd}	6.57±0.68^{cd}	7.23±0.82	6.97±0.96^{ab}
18	7.33±0.48^{ab}	7.33±0.48^{abc}	7.27±0.83^{abc}	7.17±0.79^{abcd}	7.23±0.73^{ab}	6.87±0.86^{ab}	680±0.85^{bc}

***** 8, 10 and 14 were replicated design point of 3, 1 and 11, respectively.

$^{a-g}$ The different superscripts letters under the same column were significantly different ($p<0.05$).

Table 26. The regression models of sensory evaluation of functional drink from germinated brown rice

Attributes	Regression model	R^2	*p**	Lack of fit (*p*)
Appearance	6.95A + 6.86B + 7.98C - 16.67D - 0.81AB -1.60AC + 31.97A -0.25BC + 32.94BD + 22.67CD	0.8790	0.0070	0.5391
Color	7.29A + 7.50B + 7.48C – 24.15D - 0.31AB – 1.31AC + 39.58AD – 0.62BC + 39.05CD + 38.81CD + 10.32ABC + 0.37ABD – 21.29ACD – 39.47BCD	0.9859	0.0485	0.7909
Texture	6.94A + 7.24B + 6.96C – 5.91D + 1.21AB + 0.56AC + 17.76AD + 0.52BC + 16.47BD + 14.67CD	0.8293	0.0313	0.3944
Viscosity	6.89A + 6.66B + 6.86C - 5.37D - 1.22AB + 0.76AC + 16.33AD + 1.71BC + 17.47BD + 14.61CD	0.8062	0.0299	0.9816
Flavor	7.13A + 7.77B + 7.55C - 61.32D - 4.79AB - 2.63AC + 87.12AD - 2.05BC + 84.18BD + 76.41CD	0.9076	0.0176	0.0875
Taste	6.18A + 6.09B - 2.53C + 1.18D + 0.27AB + 17.09AC + 6.79AD + 17.70BC + 7.02BD + 25.24CD	0.9920	<0.0001	0.8749
Overall acceptability	7.07A + 7.40B + 6.01C – 28.47D – 3.90AB + 0.73AC + 46.07AD + 0.40BC + 45.14BD + 45.43CD	0.8419	0.0245	0.8333

A = germinated brown rice, B= soybean, C = sugar, D = milk powder

*p** : Probability level

The optimization technique provides product formulation for sensory evaluation (Gacula, 1993). The optimum formula was determined by superimposing the contour plots of all the responses (Sin *et al*. 2006). Deshpande *et al*. (2008) optimized formulation of peanut-soy beverage using response surface methodology which generated contour plots in optimum region having consumer acceptability rating $\geq$ 5.0. Karen *et al*. (2009) were chosen and superimposed based on sensory acceptability which had acceptability scores $\geq$ 6.8 (moderately like on the 10 cm bidirectional labeled affective magnitude scale) to obtain a predicted optimum formulation of mayonnaise-type spreads. In this study we want this product to contain the maximum GABA content, so the optimum area should contain the highest germinated brown rice and had maximum sensory attributes. By overlaying all the responses, the optimized regions of functional drink consist of 78.6-83.3% of germinated brown rice extracts, 10.0-14.4% of soybean, 5.0-6.3% of sugar and 0.8-1.7% of milk powder. The optimized formula with highest of desirability composed of 83.3% of germinated brown rice extracts, 10.0% of soybean, 5.0% of sugar and 1.7% of milk powder which selected for developed of functional drink.

To confirm the prediction, the sensory score of the optimized formula of functional drink was evaluated. The predicted and experimented values of sensory score of functional drink are shown in Table 27. The results showed that the predicted error was less than 10% of experimented value, indicating that the model had a good fit with the target ratio formula.

Table 27. Sensory scores with predicted and experimented value of functional drink from germinated brown rice

Attributes	Predicted value	Experimented value
Appearance	7.44	7.30±0.65
Color	7.29	7.43±0.50
Viscosity	7.39	7.17±0.70
Texture	7.22	7.50±0.51
Flavor	8.01	7.67±0.48
Taste	7.30	7.17±0.65
Overall acceptability	7.65	7.33±0.48

Therefore, it can be concluded that functional drink composed of 83.3% of germinated brown rice extracts, 10.0% of soybean, 5.0% of sugar and 1.7% of milk powder.

4. Properties of functional drink from germinated brown rice

The optimum formula of functional drink from germinated brown rice was pored into glass bottle (240 mL per bottle) with twist off cap. Heat penetration and sterility test was conducted by Agro-Industry Development Center for Export (ADCET). The functional drink was processed at 118°C to standardized F_0 value of 7.3. Heat penetration and sterility test are shown in Table 28, which indicated that the appropriate processing temperature of functional drink was 118°C for 24 min.

Table 28. Heat penetration and sterility test of functional drink from germinated brown rice

Parameter	Result
Bottle size (mL)	250
Maximum filling weight (mL)	240
Net weight (mL)	240
Vacuum (inch/Hg)	14
Head space (mm.)	31.3
Process temperature/time	118°C/24 min
Initial temperature (°C)	34.8
Come-up time (min)	10
Heating parameter	
fh	5.8
f_2	-
J	1.20
Xbh	-
Lethality (F_0) Criteria	7.3 (Formula)
Sterility test	No microbial growth

Properties of functional drink from germinated brown rice

Feature of functional drink from germinated brown rice is shown in Figure 22, which had white color with L*, a* and b* values of 77.37, 2.61 and 19.42, respectively. The pH value was 6.46, total soluble solid was 13°brix, viscosity 2.52 cP and total acidity was 0.0094 % as citric acid.

Figure 22. Feature of functional drink from germinated brown rice

The nutrition compositions and nutrition label of functional drink are shown in Table 29 and Figure 23, respectively. One serving size (240 mL) was composed of energy 170 Kcal, protein 10.0 g, fat 4.5 g, carbohydrate 22.0 g, dietary fiber 1.0 g, sugar 17 g, sodium 35 mg, vitamin B1 0.14 mg, vitamin B2 0.07 mg, calcium 65 mg and iron 1.0 g. DeMan *et al*. (1987) reported that soymilk contained 3.0 % protein, 1.5 % fat and 1.5 % carbohydrate.

Table 29. Nutrition compositions of functional drink from germinated brown rice

Compositions	Amount per 100 mL	Amount per serving (240 mL)
Total energy (Kcal)	70	170
Energy from fat (Kcal)	16	40
Total fat (g)	1.8	4.5
Saturated fat (g)	0.3	0.5
Cholesterol (mg)	Not detected	Not detected
Protein (Nx6.5) (g)	4.3	10
Total carbohydrate (g)	9.1	22
Dietary fiber (g)	0.5	1
Sugars (g)	7.2	17
Sodium (mg)	15	35
Vitamin B1 (mg)	0.06	0.14
Vitamin B2 (mg)	0.03	0.07
Calcium (mg)	27	65
Iron (mg)	0.4	1.0
Ash (g)	0.5	1.2
Moisture (g)	89.3	-
GABA (mg)	17.21	41.30
γ-oryzanol (mg)	Not detected	Not detected
Ferulic acid (mg)	Not detected	Not detected
Phytate (mg)	Not detected	Not detected

Nutrition Information			
Serving size: 1 bottle (240 mL)			
Servings Per Container: 1			
Amount per serving			
Total energy 170 Kcal (Energy from fat 40 Kcal)			
			Product Percent of Thai RDI*
Total fat	4.5 gm.		7 %
Saturated fat	0.5 gm.		2 %
Cholesterol	0 mg.		0 %
Protein	10 gm.		
Total carbohydrate	22 gm.		7 %
Dietary fiber	1 gm.		4 %
Sugar	17 gm.		
Sodium	35 mg.		1 %
	Product Percent of Thai RDI*		
Vitamin A	0 %	**Vitamin B1**	10 %
Vitamin B2	4 %	**Calcium**	8 %
Iron	6 %		
* Percent of Thai Recommended Dairy Intakes for population over 6 years of age are based on 2,000 Kcal diet.			
Total fat	Less than	65 gm.	
Saturated fat	Less than	20 gm.	
Cholesterol	Less than	300 mg.	
Total carbohydrate		300 gm.	
Dietary fiber		25 gm.	
Sodium	Less than	2,400 mg.	
Energy (Kcal) per gm. : fat = 9; protein = 4; carbohydrate = 4			

Figure 23. Nutrition label of functional drink form germinated brown rice

The GABA content of functional drink is 41.40 mg/serving which from germinated brown rice and soybean. Khampang *et al*. (2009) reported that GABA content in soybean was 3.34 mg/100g and increased to 13.80 mg/100g after soaking in water for 6 hours. In this study, soybean soaked in water for 16-18 hours. These process was perhaps induced the GABA accumulation in soybean. Okada *et al*. (2000) reported that GABA content intake of 26.4 mg per day improved the symptoms of menopause or mental disorder. Nakamura *et al.* (2009) reported that chocolate which enriched with 28 mg of GABA was considered to have a psychological stress-reducing effect. Based on this report, an effective amount of GABA can be obtained by drinking less than one severing of functional drink.

However, γ-oryzanol, ferulic acid and phytate were not found in functional drink. This was perhaps due to insoluble in the water extraction of bio-compounds such as γ-oryzanol (Patel and Naik, 2004). In addition, some compound may be lower than limit of detection such as free ferulic acid which had only 0.1% of total ferulic acid. Moreover, heat processing may be affected to amount of bio-active compounds particularly phytate which reduced after food processing. Chansuwan (2005) reported phytate content in rice was cooked in rice steamer decreased 11.70-87.44%. Hussain *et al.* (2006) also reported that the phytic acid of chickpea was reduced to a range value of 16-60% and 16-64% by roasting and autoclaving, respectively depending upon the cultivar.

Development of functional drink from germinated brown rice was studied by some researcher. Sutinium (2007) developed the instant nutritious beverage from germinated jasmine brown rice for aged consumer. The optimum formulation of the instant drink consisted of 20% germinated jasmine brown rice flour, 20% of caster sugar, 44.6% of skim milk powder, 5.1% of vanilla powder and 10% of inulin. Functional ingredients added in instant nutritious drink were vitamin B6, vitamin B12, folic acid and L-carnitine. One serving size (35g) of instant nutritious beverage composed of energy 120 Kcal, protein 5.64 g, dietary fiber 3.72 g, carbohydrate 24 g, fat 0.18 g, vitamin B6 1 mg, vitamin B12 0.82 μg, folic acid 59.3 μg and Ca 203 mg.

Lerswanichwatana (2003) also developed of functional drink from germinated rice. The optimum formulation of dry mix drink consists of germinated

rice powder 33.65%, instant milk powdered 14.42%, non-dairy creamer 9.62%, gum arabic 9.62%, sugar 28.85% and cocoa powder 3.84%. The dry mix drink had brown color with the color in L a b system of 69.18 6.52 and 12.11 respectively. Proximate composition of the dry mix drink were 0.478% of nitrogen, 13.44% of protein, 4.82% of fat, 1.25% of dietary fiber, 2.06% of ash, 74.26% of carbohydrate, 0.65 mg/100g of thiamin and 0.69 mg/100g of riboflavin content.

Anawachkul and Jiamyangyuen (2009) also developed of yogurt with enhanced level of GABA. The 30% germinated red rice flour was selected to add in yogurt formula. The enriched yogurt contained 4.09 mg/100g of GABA, which was significantly higher than that of commercial yogurt which was not detected.

Consumer acceptability of functional drink from germinated brown rice

Two hundred consumers participated in consumer acceptability test of functional drink from germinated brown rice. The surveys conducted at the University, government offices and National Agricultural Exhibition. The demographic profiles of the respondents are shown in Table 30. There were 15.5% males and 84.5% females. Marital status of respondents were single (54.0%) and married (40.0%). The ages were divided into three groups included 33.5% of 15-25 years old, 47.5% of 26-45 years old and 19.0% of older than 45 years old. Most of them (50%) earned a bachelor degree and secondary school certificate (20.5%). The most of them had income less than 10,000 Baht per month (45.5%).

Germinated brown rice consumption behaviors of consumer were evaluated and the results shown in Table 31. The first question was asked about the consumer ever heard about germinated brown rice or not. The results showed that 85% of respondents ever heard or known about germinated brown rice. Newspaper, television or radio was the main source of information. Most of respondent (99.5%) interested in germinated brown rice because of its nutritious (92.0%). Half of respondent even ate germinated brown rice in form of cooked rice (26.5%) and beverage (32.5%).

Table 30. The demographic profiles of the respondents in consumer test

Respondent characteristic	Response (%)
Gender	
Female	84.5
Male	15.5
Age	
15-25 years old	33.5
26-45 years old	47.5
Over 45 years old	19.0
Marital status	
Single	54.0
Married	40.0
Other	6.0
Occupation	
Student	28.5
Government official	26.0
Business owner	18.5
Employee	11.5
Other	15.5
Education	
Lower secondary school certificate	20.5
Vocational certificate/2 years of collage	12.5
Bachelor degree	50.0
Higher than Bachelor degree	17.0
Personal income (Baht per month)	
Less than 10,000 Baht	45.5
10,000-20,000 Baht	38.5
More than 20,000 Baht	16.0

Table 31. The consumer behavior on germinated brown rice of the respondents

Behavior	Response (%)
Have you ever heard germinated brown rice	
Yes	85.0
No	15.0
Source of information	
Newspaper/TV/radio	70.0
Friend or personal contact	38.0
Internet/E-mail	34.5
Food exhibition	28.5
Interest in germinated brown rice	
Yes	99.5
No	0.5
Reason for interested in germinated brown rice	
Nutritious	92.0
Hearsay	29.0
Popularity/in trend	11.5
Advertisement	8.5
Have you even ate germinated brown rice	
Yes	59.0
No	41.0
Form of germinated brown rice	
Beverage	32.5
Cooked rice	26.5

Acceptance test of functional drink from germinated brown rice were evaluated by the consumer who drinks and is not allergic to soybean. The consumer acceptance test of developed functional drink from germinated brown rice was conducted by 200 respondents and sensory score are shown in Table 32. Overall acceptability of the product was rated at like very much range (7.72). The other attributes were rated like very much range such as color rating (7.66), flavor rating (7.51), taste rating (7.63) and texture rating (7.55). Resurreccion (1998) pointed that hedonic rate should be more than 6 for acceptance of food products. Therefore, this functional drink from germinated brown rice was accepted by consumer. Confirmation was obtained by the acceptance question, 99.5% of consumer accepted this product by itself that means it has a high potential in the market.

Table 32. Sensory scores of functional drink from germinated brown rice by consumers

Attributes	Sensory score
Appearance	7.74±1.00
Color	7.66±1.00
Flavor	7.51±1.18
Taste	7.63±1.13
Texture	7.55±1.07
Overall acceptability	7.72±0.90

Consumer acceptance ratings are based on a 9-point hedonic scale

5. Shelf life of the functional drink from germinated brown rice

This experiment was conducted to investigate the quality of functional drink during storage at 25, 35 and 45°C for 12 weeks. The main chemical components (total soluble solid, pH, acidity, color, viscosity and GABA) and sensory evaluations were measured every week. Changes of pH, total soluble solid, viscosity, acidity and GABA were ranged from 6.45 to 6.46, 13.0 to 13.2 ^{o}Brix, 2.51 to 2.53 cP, 0.0093 to 0.0095% and 17.67 to 17.47 mg/100 mL, respectively, but not significantly different ($p>0.05$). However, total color difference (ΔE) was significantly different ($p<0.05$) during storage at various temperatures as shown in Figure 24.

Sensory evaluation of the functional drink at various temperatures were also investigated and the result showed a decreased in attributes of sensory especially appearance, color and overall acceptability ($p<0.05$) which varied changed from 8.00 to 5.33, 7.87 to 5.07 and 7.93 to 5.73, respectively (as shown in Figure 25). However, change of flavor, viscosity, texture and taste were ranged from 7.80 to 6.20, 7.87 to 6.27, 7.60 to 6.20 and 7.87 to 6.33, respectively, but not significantly different ($p>0.05$) during storage at various temperatures.

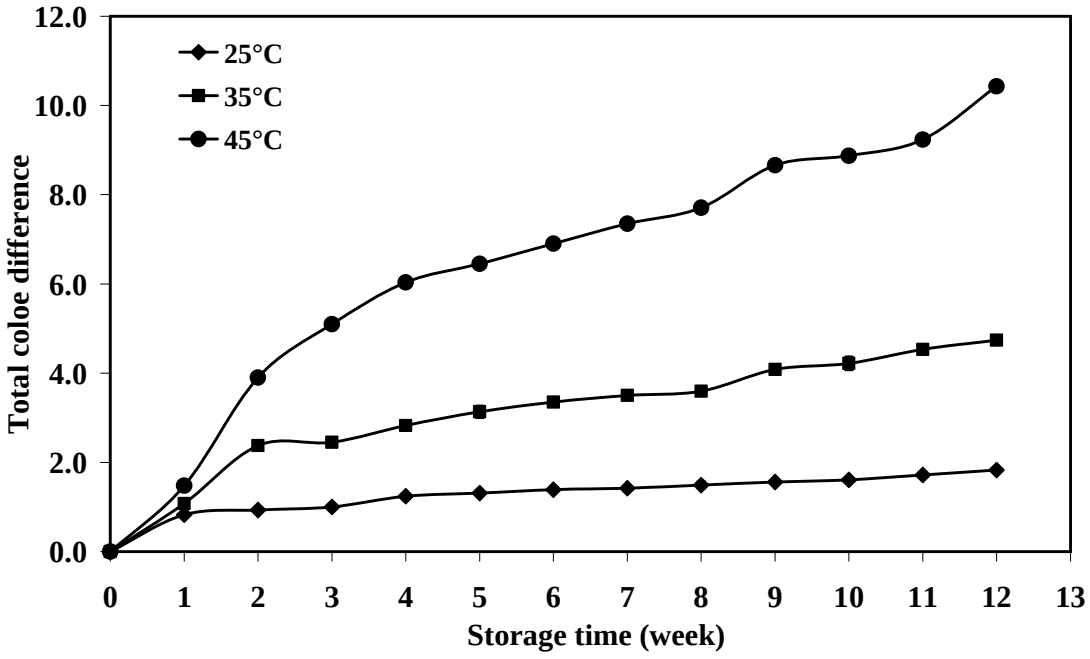


Figure 24. Changes in total color difference (ΔE) of functional drink from germinated brown rice during storage at various temperatures

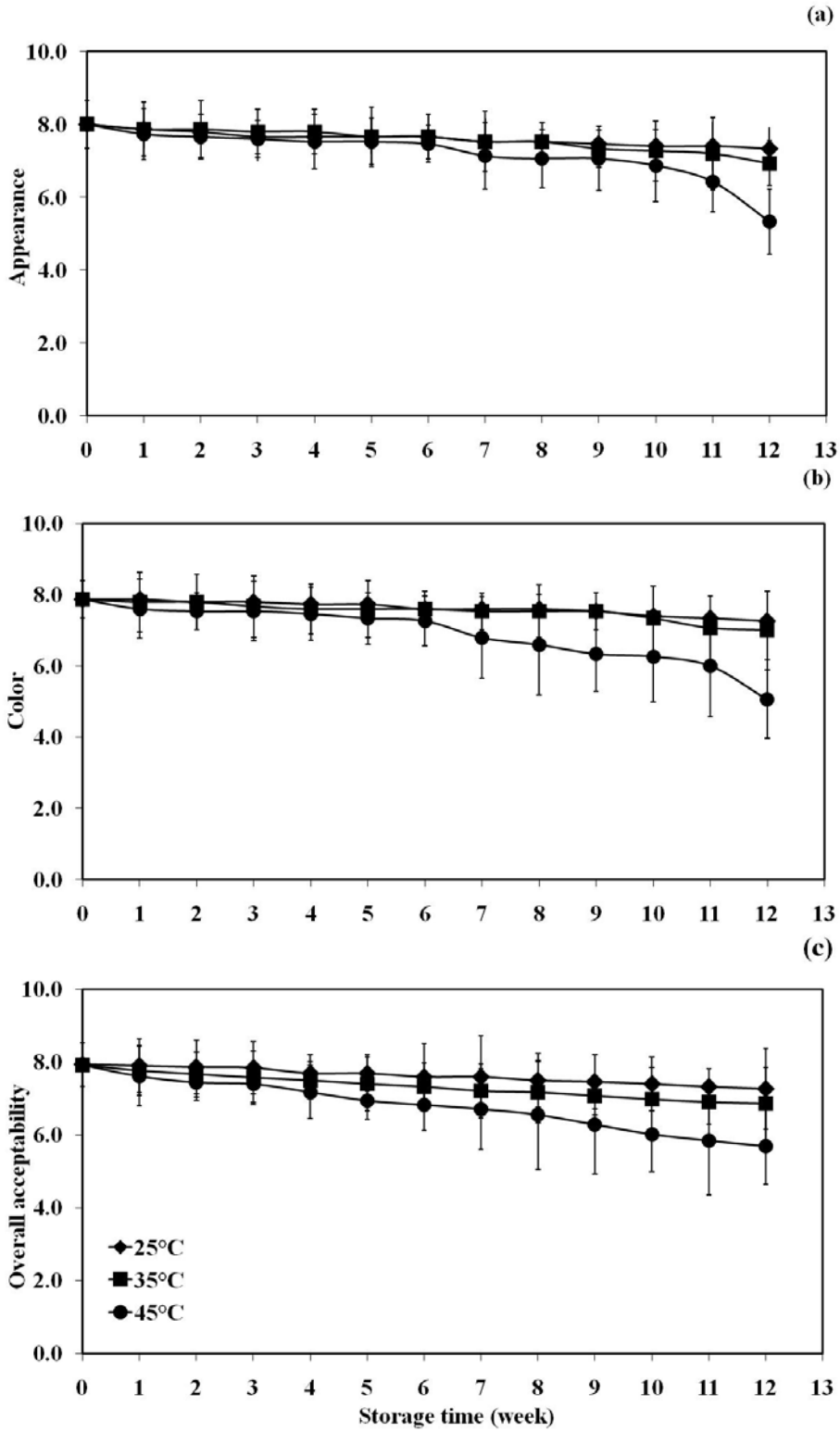


Figure 25. Changes in appearance (a), color (b) and overall acceptability (c) of functional drink from germinated brown rice during storage at various temperatures

The deterioration in specific color and overall acceptability has been use extensively in the determination of shelf life of foods. Change of the color affected to appearance, color and overall acceptability score from sensory evaluation with decreased as ΔE increasing. Based on the results of this experiment, ΔE and overall acceptability were chosen as the parameters for determining shelf life of functional drink.

For the mathematical modeling of ΔE and overall acceptability change of functional drink, zero-order (eq. 1) and first-order (eq.2) kinetic model were used (as shown inFigure 21).

$Q = Q_0 - kt$ Zero-order *Eq.1*

$Q = Q_0 \exp(-kt)$ First-order *Eq.2*

Where Q is indicator parameters at time t, Q_0 is Q at zero time, k is the constant and t is the time.

It was observed that ΔE was fitted to the first-order kinetic model (Figure. 26b); on the other hand, the values of overall acceptability followed a zero-order kinetic model (Figure 26c).The estimated kinetic parameters of these models and the statistical values of coefficients of determination R^2 are represented in Table 33. The kinetic rate constant of ΔE increased from 0.061 to 0.083 $week^{-1}$ and overall acceptability increased from 0.056 to 0.181 $week^{-1}$ as temperature increasing.

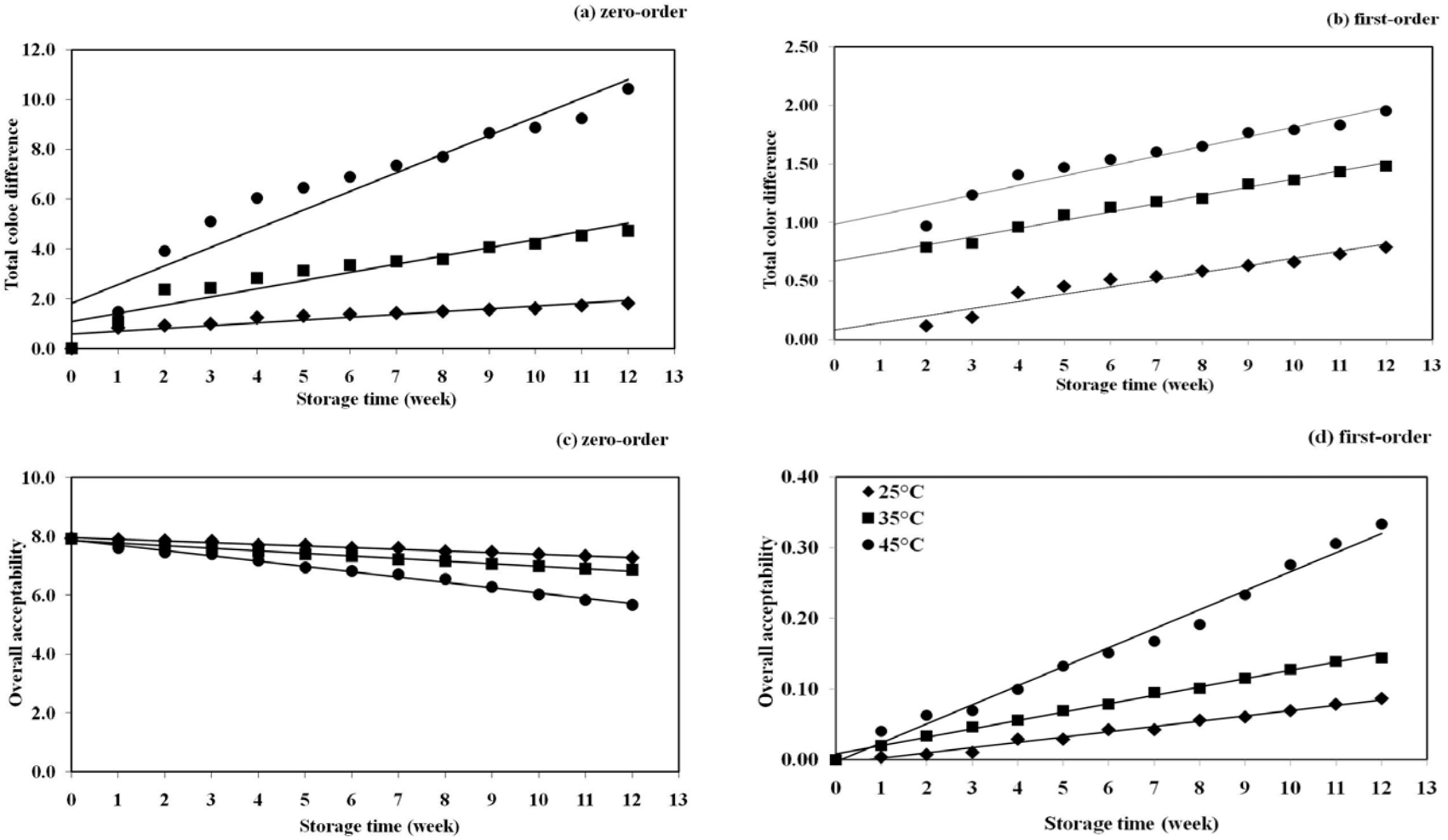


Figure 26. Kinetic of changes of ΔE (a and b) and overall acceptability (c and d) at various temperatures for zero-order and first-order

Table 33. The estimated kinetic parameter and the statistical values of zero-order and first-order for ΔE and overall acceptability from various temperature

Parameter	Temperature	Zero-order		First-order	
	(°C)	k ($week^{-1}$)	R^2	k ($week^{-1}$)	R^2
ΔE	25	0.112	0.821	0.061	0.928
	35	0.328	0.889	0.070	0.980
	45	0.748	0.913	0.083	0.929
Overall	25	0.056	0.985	0.007	0.985
acceptability	35	0.087	0.991	0.011	0.994
	45	0.181	0.991	0.026	0.986

Color of the product and sensory score to levels unacceptable by legislation or industrial practice often defines product shelf life. The deterioration in specific sensory attributes and/or overall quality has been use extensively in the determination of shelf life of foods. However, the use of different sensory tests, that utilize different scales of measurements and the product-specific reduction in sensory quality perceived to be tolerated by consumers had resulted in the use of different cut-off points to mark end of shelf-life of foods. On a nine-point hedonic scale, scores of 4.5 in sunflower kernels (Fritsch *et al*., 1997), score of 5 in fruit juice drinks (Alves *et al*. 2001; Polydera *et al*., 2005) were used to mark sensory failure. In this study, a score of 5 for overall acceptability was used to indicate product failure. For ΔE = 10 was used to indicate product failure because a perceptible change of overall acceptability and color score (score <5) was noted around this point.

The shelf life of functional drink at different storage temperature was therefore calculated through eq.2 for ΔE and eq.1 for overall acceptability which substituted *Q* with 10 of ΔE and 5 of sensory (Table 34).

Table 34. Shelf life of functional drink from germinated brown rice at different storage temperatures

Temperature (°C)	Shelf life (week)		
	Based on ΔE	Based on overall acceptability	Average
25	40.80	52.32	46.56
35	31.79	33.68	32.74
45	23.02	16.19	19.61

In order to evaluate the shelf life of the functional drink at these three temperatures, an average value of the different shelf life for each attribute was obtained. Shelf-life of functional drink at 25, 35 and 45°C were 46.56, 32.74 and 19.61 weeks, respectively. These three values can be adjusted to a shelf life plot (Figure 27) by calculating the regression of the log shelf life versus temperature to obtain the following equation (Labuza and Schmidl, 1985):

$$\log \theta = 2.149 - 0.018\,T \qquad (R^2 = 0.988)$$

when T is the storage temperature (in °C) and θ is the shelf life (in weeks) of functional drink.

This regression enables us to estimate the shelf-life of the product at storage conditions different from those used in this study. For instance, the shelf life of this product at normal storage conditions (30°C) would be 40.64 weeks (or 9.5 months).

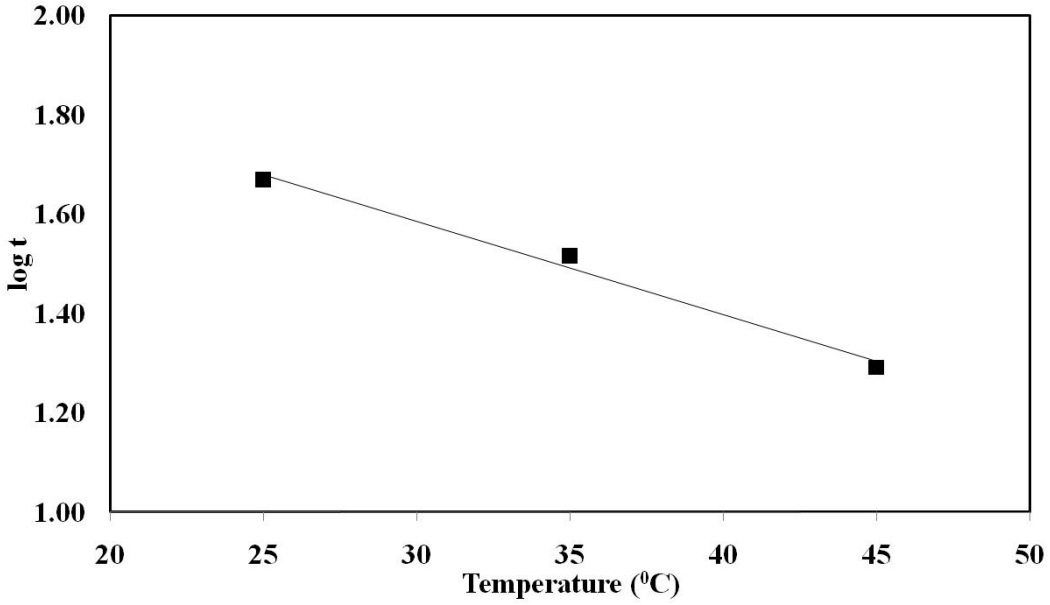


Figure 27. A shelf life plot of functional drink from germinated brown rice at 25, 35 and 45°C

In conclusion, the shelf life study, based on selected and accelerated properties of functional drink, color and sensory evaluation relationship useful to measure quality decay during some storage of functional drink.

CHAPTER 4

CONCLUSION

Conclusion

Three Thai rice varieties var. Niaw Dam Peuak Dam, Sangyod Phatthalung and Chiang Phatthalung were classified as low, intermediate and high amylose content, respectively. Sangyod Phatthalung had the highest of protein and dietary fiber. The optimum germination conditions of the Southern Thailand rice which giving the highest of GABA content were soaked in citrate buffer pH 3.0 at room temperature (30±2^{o}C) for 5 hours and germinated in closed vessel 36 hours for Sangyod Phatthalung and Chiang Phatthalung and 48 hours for Niaw Dam Peuak Dam. Compared to regular brown rice, the GABA content in germinated brown rice increased 9.43-16.74 times. In addition, the germinated brown rice contained more protein, total dietary fiber and ferulic acid while γ-oryzanol content was in the same level for both brown rice and germinated brown rice. On the contrary, germinated brown rice contained phytate content lower than of brown rice. As the result indicating that, the germination process was an important technique for enhancing nutrients and bio-active compounds in brown rice

The germinated brown rice of Sangyod Phatthalung was contained 10.64% of germ, 7.45% of bran and 81.91% of white rice. The optimum extraction method for GABA from germinated brown rice and its fractions (germ, bran, and white rice) were the highest in distilled water at 30^{o}C. Different lengths of extraction time affect to the GABA content. The highest GABA content of germinated brown rice germ, bran, and white rice were obtained when extracted for 6, 6, 24 and 12 hours, respectively. GABA extract from germinated brown rice may be used as a functional ingredient in functional food products such as beverages or confectioneries.

The functional drink in formula made from germinated brown rice extracts was developed using a consumer affective sensory evaluation study. The optimum formula obtained was 83.3% of germinated brown rice extracts, 10.0% of

soybean, 5.0% of sugar and 1.7% of milk powder which 99.5% of consumer accepted. Shelf life of functional drink storage at 30^{o}C was 40.64 weeks (or 9.5 months).

Future Work

1. Using germination technique for enhancing nutrients and bio-active compounds in other cereal.
2. Development of food product from germinated brown rice.
3. Nutraceutical effect of germinated brown rice and functional drink.

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APPENDIX

Appendix A

Consumer Survey

This survey is a part of the research of Miss Jiraporn Banchuen, Graduate student in the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkha University. Please feel free to answer the questions. Your name and personal identification is not required.

Instruction

Please read each of the following questions. Then place a tick in the box that best reflects your opinions about the particular question. Please answer any sections where you are asked to give reasons for your selection or actions. With some questions you may be asked to tick more than one box. The instruction to tick more than one box will be provided after the question.

PART I: Functional drink behavior

> ***Functional drink*** *are liquids for drinking that are formulated to provide nutrients for special proposes such as dietary purposes, sport drink for athletes, meal replacement drinks for dieters, meal replacement drinks for general adults and special drinks for diabetic*

1. Do you normally drink functional drink?

- ❑ Yes **(Please continue with the next question)**
- ❑ No **(Please state reasons and go to PART II)**

  Why do you not drink functional drink?

  .....

  .....

2. How often did you drink functional drink?

- ❑ Daily
- ❑ 3-4 times/week
- ❑ 1-2 times/week
- ❑ Less than once a week

3. What time of the day do you normally drink functional drink?

(**You may tick as many boxes as you like)**

- ❑ Morning
- ❑ Morning break
- ❑ Noon
- ❑ Afternoon break
- ❑ Night
- ❑ Before bed time
- ❑ Other (Please specify).....

4. What is your purpose for drinking functional drink?

(**You may tick as many boxes as you like)**

- ❑ Health benefit
- ❑ Refreshing
- ❑ Curing the disease
- ❑ Beauty or diet
- ❑ Anti-aging
- ❑ Other (Please specify).....

5. What is your favorite functional drink?

(**You may tick as many boxes as you like)**

- ❑ Dairy product
- ❑ Instant tea
- ❑ Fruit or vegetable juice
- ❑ Dried herb
- ❑ Cereal product
- ❑ Cereal powder
- ❑ Tea
- ❑ Herb beverage
- ❑ Extract beverage
- ❑ Other (Please specify).....

6. What would make you buy functional drink?

(**You may tick as many boxes as you like)**

- ❑ Taste
- ❑ Flavor
- ❑ Nutritious
- ❑ Brand
- ❑ Packaging
- ❑ Price
- ❑ Convenient of consumption
- ❑ Easily to bring
- ❑ Easily available
- ❑ Place of buying
- ❑ Advertisement
- ❑ Hearsay
- ❑ Popularity
- ❑ Manufacturing company
- ❑ County of production
- ❑ Academic support
- ❑ Other (Please specify).....

PART II: Consumers attitudes to functional drink from germinated brown rice

Germinated brown rice *is brown rice germinated after rice soaked in water for a certain period. Germinated brown rice great of nutrients such as γ-aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol and prolylendopeptidase inhibitor.*

1. Do you agree if functional drink which made from germinated brown rice?

- ❑ Yes **(Please continue with the next question)**
- ❑ No **(Please state reasons and go to PART III)**

Why do you not agree?

.....

.....

.....

2. What type of functional drink do you want?

- ❑ Ready-to-drink
- ❑ Dry powder
- ❑ Instant powder
- ❑ Concentrate
- ❑ Other (Please specify).....

3. What type of functional drink do you want?

- ❑ Only germinated brown rice
- ❑ Mix with other

If mix, what kinds of ingredient do you like to mix?

- ❑ Dairy product
- ❑ Cereal
- ❑ Herb
- ❑ Other (Please specify).....

4. What taste of functional drink do you want?

- ❑ Natural
- ❑ Sweet
- ❑ Milk
- ❑ Fruit
- ❑ Chocolate
- ❑ Other (Please specify).....

5. What taste of functional drink do you want?

- ❑ Glass bottle
- ❑ Plastic bottle
- ❑ Can
- ❑ Plastic bag
- ❑ Other (Please specify).....

6. The optimum price of functional drink per unit

- ❑ Less than 20 Bath
- ❑ 20-30 Baht
- ❑ 31-40 Baht
- ❑ 41-50 Baht
- ❑ Other (Please specify).....

7. If functional drink containing germinated brown rice is commercialized, would you like to buy them?

- ❑ Yes
- ❑ No

PART III: Demographic data

1. Gender

❑ Male ❑ Female

2. Age

❑ 15-25 years old ❑ 26-45 years old ❑ Over 45 years old

3. Marital statue

❑ Single ❑ Married ❑ Other

4. Occupation

❑ Student ❑ Government official
❑ Business ❑ Agricultural/Farmer
❑ Housewife ❑ Employee
❑ Unemployed ❑ Other (Please specify).....

5. Education

❑ Lower secondary school certificate
❑ High school ❑ Vocational certificate/2 years of collage
❑ Bachelor degree ❑ Higher than Bachelor degree

6. Your monthly income

❑ Less than 10,000 Baht ❑ 10,000 – 20,000 Baht
❑ 20,001 – 30,000 Baht ❑ More than 30,001 Baht

Appendix B

Consumer Acceptance Test

This questionnaire for consumer acceptance test is a part of the research of Miss Jiraporn Banchuen, Graduate student in the Department of Food Technology, Faculty of Agro-Industry, Prince of Songkha University. Please feel free to answer the questions. Your name and personal identification is not required.

Instruction

Please read each of the following questions. Then place a tick in the box that best reflects your opinions about the particular question. Please answer any sections where you are asked to give reasons for your selection or actions. With some questions you may be asked to tick more than one box. The instruction to tick more than one box will be provided after the question.

PART I: Germinated brown rice behavior

> ***Germinated brown rice*** *is brown rice germinated after rice soaked in water for a certain period.*

1. Have you ever heard about “germinated brown rice”?

❑ Yes ❑ No

If yes, what is the source of your information about “germinated brown rice”? (**You may tick as many boxes as you like)**

❑ Newspaper/TV/radio ❑ Internet/E-mail

❑ Food exhibition ❑ Friend or personal contact

❑ Other (Please specify).....

2. Do you interest in the “germinated brown rice”?

❑ Yes **(Please continue in question No. 3)**

❑ No **(Please continue in question No. 4)**

3. Why you are interested in “germinated brown rice”?

(You may tick as many boxes as you like)

- ❑ Nutritious
- ❑ Hearsay
- ❑ Popularity/in trend
- ❑ Advertisement
- ❑ Other (Please specify).....

4. Why you don’t interest in “germinated brown rice”?

(You may tick as many boxes as you like)

- ❑ Don't like the new product
- ❑ Difficult to available
- ❑ Don’t ensure in nutrition
- ❑ Don’t like the brown rice
- ❑ Don’t known the product
- ❑ Other (Please specify).....

5. Have you even had “germinated brown rice”?

- ❑ Yes
- ❑ No

If yes, what form of germinated brown rice had you have?

(**You may tick as many boxes as you like)**

- ❑ Cooked rice
- ❑ Beverage
- ❑ Instant or powder
- ❑ Soup
- ❑ Other (Please specify).....

PART II: Acceptance test of functional drink from germinated brown rice

I am going to ask you to taste functional drink from germinated brown rice with soy milk which produced. To avoid your fatigue, this part will be split to two subunits. Before I get you to taste functional drink, I need to know whether there are any foods that you don't eat or if you are allergic to any foods so that I don't give you some product to taste which you don't like or are allergic to.

1. Do you eat foods that made from soy bean?

❑ Yes ❑ No

2. Do you allergic foods that made from soy bean?

❑ Yes ❑ No

(If you don't eat or allergic foods that made from soybean, please STOP and continue in PART IV)

3. I am going to you taste functional drink from germinated brown rice with soy milk and then I want you to tell me how much you like its appearance, its color, its flavor, its taste, its texture and then how much you like the product. I am going to use a liking score (dislike extremely-like extremely) for describing the product.

Appearance: anything of product you can see such as color, viscosity, etc.

Color: color of product when you see.

Flavor: flavor of product when you sniff.

Taste: feeling you can feel when you taste product such as sweetness or fatty.

Texture: feeling you can feel when you taste product such as smoothly, sticky or viscosity.

Overall: overall liking.

Please test the functional drink sample and weight your liking score by put ✓ in the box which is directly to your opinion.

Liking score	Appearance	Color	Flavor	Taste	Texture	Overall liking
Like extremely						
Like very much						
Like moderately						
Like slightly						
Neither like nor dislike						
Dislike slightly						
Dislike moderately						
Dislike very much						
Dislike extremely						

4. Do you accept this product?

❑ Yes ❑ No (Please state reasons)

If no, why do you not accept this functional drink?

.....

.....

.....

5. Do you want to buy this product?

❑ Yes ❑ No

Part III: Information of functional drink from germinated brown rice

Functional drink from germinated brown rice with soy milk

Functional drink from germinated brown rice with soy milk is produce from brown rice varieties Sangyod Phatthalung which germinated and mixes with soybean for added value. The product was sterilized and can keep at room temperature more than 1 year.

These functional drink have 40 mg of GABA (γ-aminobutyric acid). GABA is a neurotransmitter in the brain and the spinal cord of mammals. It can lower hypertension, promote the sleepiness, anti-stress effect in humans, and has the benefit for human.

After reading this information, please answer the questions.

1. Do you accept this product?

❑ Yes ❑ No

2. Do you want to buy this product?

❑ Yes ❑ No

Other comments, if any

……
……
……
……

PART IV: Demographic data

1. Gender

❑ Male ❑ Female

2. Age

❑ 15-25 years old ❑ 26-45 years old ❑ Over 45 years old

3. Marital statue

❑ Single ❑ Married ❑ Other

4. Occupation

❑ Student ❑ Government official

❑ Business ❑ Agricultural/Farmer

❑ Housewife ❑ Employee

❑ Unemployed ❑ Other (Please specify).....

5. Education

❑ Lower secondary school certificate

❑ High school ❑ Vocational certificate/2 years of collage

❑ Bachelor degree ❑ Higher than Bachelor degree

6. Your monthly income

❑ Less than 10,000 Baht ❑ 10,000 – 20,000 Baht

❑ 20,001 – 30,000 Baht ❑ More than 30,001 Baht

VITAE

Name Miss Jiraporn Banchuen

Student ID 4883012

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Food Science and Nutrition)	Prince of Songkla University	2000

Scholarship Awards during Enrolment

1. A scholarship by the Agricultural Research Development Agency (Public Organization)
2. The grant-in-aid for dissertation from Prince of Songkla University

List of Publications and Proceedings

Publications

Banchuen, J., Thammarutwasik, P., Ooraikul, B., Wuttijumnong, P. and Sirivongpaisal, P. 2009. Effect of germinating processes on bioactive component of Sangyod Muang Phatthalung rice. Thai Journal Agricultural Science. 42: xx-xx (*Article in press*)

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Poster Presentation

Banchuen, J., Thammarutwasik, P., Ooraikul, B., Wuttijumnong, P. and Sirivongpaisal, P. 2009. Bio-active compounds of germinated Brown rice cv. Sangyod Phattalung. The 8th National Horticultural Congress. May, 6-9 2009. The Empress Hotel, Chiangmai, Thailand.

Petty Patent

Banchuen, J., Thammarutwasik, P., Wuttijumnong, P. and Sirivongpaisal, P. 2010. Petty patent: Production of ready to drink which consist of the extracts from germinated brown rice cv. Sangyod Phattalung and cereals. (No. requires 1003000031, 14 January 2010).