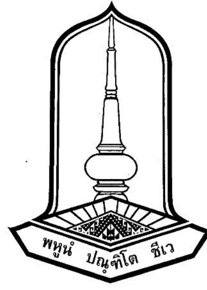

PROCESS DEVELOPMENT OF PARBOILED GLUTINOUS RICE (KHAO NIEW HANG) FOR IMPROVEMENT OF PHYSICAL, CHEMICAL AND NUTRITIONAL QUALITIES

PORNPISANU THAMMAPAT

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Technology

at Mahasarakham University

March 2015

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Mahasarakham University

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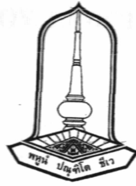

The examining committee has unanimously approved this dissertation, submitted by Mr. Pornpisanu Thammapat, as a partial fulfillment of the requirements for the Doctor of Philosophy in Food Technology at Mahasarakham University.

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ACKNOWLEDGEMENTS

A dissertation was granted by the Royal Golden Jubilee Ph.D. Program (RGJ) under the Thailand Research Fund (TRF) in 2012-2014, Mahasarakham University Scholarship for Graduates Research in 2014 and Faculty of Technology, Mahasarakham University Scholarship for Graduates Research in 2014.

The dissertation would not have been accomplished if without the help from several people. First of all, I would like to thank Asst. Prof. Dr. Rumpai Gaensakoo, Assoc. Prof. Dr. Sirithon Siriamornpun, Dr. Naret Meeso, Dr. Sudathip Inchuen and Assoc. Prof. Dr. Natthida Weerapreeyakul.

I was very fortunate to have many friends both within and outside the Faculty of Technology during my doctoral life. I thank them all for their being very supportive.

I wish to express my sincere gratitude and deep appreciation to advisor, Assoc. Prof. Dr. Sirithon Siriamornpun, for her guidance, patience, financial support and valuable suggestions in regard the main conduct of my research and the preparation of this dissertation. My deep appreciation is expressed to my co-advisor, Dr. Naret meeso for his supervision and valuable suggestions during my study. I also wish to thank Prof. Dr. Colin Wrigley and Assoc. Prof. Dr. Matthias Schreiner for the guidance and enhancement my manuscript submission.

I would like to thank the University of Natural Resources Life Sciences (BOKU, Austria) and Roi-Et Agricultural and Food Products, Co, Ltd. for granting permission to carry out this study and providing the facilities and materials to conduct the research. Finally, I wish to express a very special thanks to my parent for their love, sacrifice and patience support towards the success of my study.

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TITLE Process development of parboiled glutinous rice (Khao Niew Hang) for improvement of physical, chemical and nutritional qualities

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UNIVERSITY Mahasarakham University **YEAR** 2015

ABSTRACT

The aims of this study were mainly to (1) develop parboiled glutinous rice process for improvement of physical, chemical and nutritional qualities, (2) optimize condition of parboiling process for improvement of physical, chemical and nutritional qualities, (3) apply the parboiling process of glutinous rice on industrial scale as functional food and (4) develop product prototypes from parboiled rice bran oil as functional food.

The results showed that the total phenolic content, phenolic acids, γ-oryzanol, saturated fatty acid and mono-unsaturated fatty acid of the glutinous rice showed an increasing trend as NaCl content and soaking temperature increased, while α-tocopherol and polyunsaturated fatty acids decreased. Soaking at 3.0% NaCl provided the highest total phenolic content, phenolic acids and γ-oryzanol (0.2 mg GAE/g, 63.61 μg/g and 139.76 mg/100g, respectively) for the soaking treatments tested. Nevertheless, the amount of α-tocopherol and polyunsaturated fatty acid were found to be the highest (18.30/100g and 39.74%, respectively) in unsoaked rice. However, total phenolic content, phenolic acid and α-tocopherol content of glutinous rice decreased as steaming temperature and steaming time increased, while the amount of γ-oryzanol content increased.

In conclusion, this study has demonstrated that parboiling processes are the most important factors of parboiled rice. According to present data, we specially recommend the parboiling process for improvement of bioactive compounds and cooking quality. Besides, the greater nutritional values of parboiled rice are considered as a functional food.

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Key Words : glutinous rice; bioactive compounds; process development; parboiling process

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ชื่อเรื่อง การพัฒนากระบวนการผลิตข้าวเหนียวนึ่ง (ข้าวเหนียวฮาง) เพื่อปรับปรุงลักษณะทางกายภาพ เคมี และโภชนาการ

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บทคัดย่อ

งานวิจัยครั้งนี้มีวัตถุประสงค์ดังนี้ (1) พัฒนากระบวนการผลิตข้าวเหนียวนึ่ง เพื่อปรับปรุงลักษณะทางกายภาพ เคมี และโภชนาการ (2) ศึกษาสภาวะที่เหมาะสมในการผลิตข้าวเหนียวนึ่งเพื่อปรับปรุงลักษณะทางกายภาพ เคมี และโภชนาการ (3) เพื่อประยุกต์ใช้กระบวนการผลิตข้าวเหนียวนึ่งในระดับอุตสาหกรรมสำหรับอาหารเฉพาะทาง และ (4) พัฒนาผลิตภัณฑ์ต้นแบบจากน้ำมันรำข้าวนึ่งสำหรับอาหารเฉพาะทาง

ผลการวิจัยพบว่า ปริมาณสารฟีนอลทั้งหมด กรดฟีนอลิก แกมมา-ออริซานอล กรดไขมันอิ่มตัวและกรดไขมันไม่อิ่มตัวเชิงเดี่ยว มีปริมาณเพิ่มขึ้นเมื่อเพิ่มปริมาณเกลือและอุณหภูมิในกระบวนการแช่ ในขณะที่ปริมาณแอลฟา-โทโคฟีรอลและกรดไขมันไม่อิ่มตัวเชิงซ้อนลดลง การแช่ที่ปริมาณเกลือร้อยละ 3 ส่งผลให้ปริมาณสารฟีนอลทั้งหมด กรดฟีนอลิก และแกมมา-ออริซานอลสูงสุด (0.2 มิลลิกรัมกรดแกลลิกสมมูลย์ต่อกรัม, 63.61 ไมโครกรัมต่อกรัม และ 139.76 กรัมต่อ 100 กรัม ตามลำดับ) แต่ปริมาณแอลฟา-โทโคฟีรอลและกรดไขมันไม่อิ่มตัวเชิงซ้อนจะมีปริมาณสูงสุดในข้าวเหนียวที่ไม่ผ่านกระบวนการ (18.30 มิลลิกรัมต่อ 100 กรัม และร้อยละ 39.74 ตามลำดับ) อย่างไรก็ตามปริมาณสารฟีนอลทั้งหมด กรดฟีนอลิก แอลฟา-โทโคฟีรอล จะลดลงเมื่อนึ่งที่อุณหภูมิสูงเป็นระยะเวลานาน ในขณะที่ปริมาณแกมมา-ออริซานอลเพิ่มสูงขึ้น

โดยสรุป การศึกษาครั้งนี้แสดงให้เห็นว่ากระบวนการแปรรูปข้าวนึ่งเป็นปัจจัยที่สำคัญต่อข้าวนึ่ง จากข้อมูลดังกล่าวสามารถใช้กระบวนการผลิตข้าวนึ่งเพื่อเพิ่มปริมาณสารออกฤทธิ์ทางชีวภาพและคุณภาพการหุงต้ม นอกจากนั้นยังสามารถเพิ่มคุณค่าทางโภชนาการของข้าวนึ่งเพื่อเป็นอาหารเฉพาะทางได้

คำสำคัญ : ข้าวเหนียว สารออกฤทธิ์ทางชีวภาพ การพัฒนากระบวนการผลิต กระบวนการผลิตข้าวนึ่ง

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CHAPTER 1

INTRODUCTION

1.1 Background

Rice is an important economic crop and export product of Thailand. Due to its excellent reputation in appearance and cooking quality, Thai rice has gained increasing popularity throughout world food markets (Zhai et al., 2001). It is consumed as cooked milled kernels. Cooking of milled rice is an important operation usually done to provide desired softness in the kernels for direct consumption (Yadav and Jindal, 2007). However, it is sensitive to heat and fragile during handling, causing poor milling yield. Therefore, improvement of milling quality of damaged grain is needed.

Parboiling is a hydrothermal treatment applied to raw paddy that can enhance the physical, chemical and organoleptic quality of rice. This process involves the three basic processes of soaking, steaming and drying (Heinemann et al., 2005). The major reasons for parboiling rice include higher milling yields, higher nutritional value and resistance to spoilage by insects and mould (Chukwu and Oseh, 2009). Parboiling results in significant changes in the physico-chemical and cooking characteristics of rice grain. Parboiling fills the void spaces and cements the cracks inside the endosperm, making the grain harder and minimizing internal fissuring and thereby reducing breakage during milling (Corrêaa et al., 2007). The market value of parboiled rice as a product depends largely on its physical qualities after processing.

The three steps of parboiling – soaking, steaming and drying – are generally achieved by soaking paddy in cold water for typically 24–48 h until the kernels are saturated. The soaked paddy is then boiled at 100°C for typically 1 h to obtain 80% gelatinized starch. Finally the boiled paddy is sun-dried until the moisture content is reduced to 14%.

Parboiled rice is one of the most popular rice products in Asia and becomes more important not only by the fact of improved nutritional value but also by the improved cooking and processing properties which are desired from the industrial point of view (Sareepuang et al., 2008). Apart from the nutritional importance of parboiled

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rice there are plenty of advantages and effects which make parboiling attractive. In rice cultivation countries generally paddy (raw rice) is used for parboiling, in Asia, Europe, and America there is a tendency to parboil also brown rice. Moreover, parboiled rice product in Thailand tends to increase, especially in health food and green organic products (Bualuang et al., 2011). Widowati et al. (2010) reported that parboiling process increased rice amylose content from 15.44-26.32% to 19.35-27.25% and dietary fiber content from 4.68-7.57% to 8.19 10.27%, but reduced in vitro starch digestibility from 62.31-78.63% to 35.52-49.74%.

There are presently a lot of traditional and industrial methods where the basic steps like soaking, thermal treatment (steaming or cooking) and drying often only differ by the application of different techniques and process parameters (Miah et al., 2002). However, various studies on rice parboiling have reported different conditions for the parboiling process, depending on the purpose of the studies; most of them studied the effects of parboiling on texture, protein, color, mineral, bioactive compounds and on the antioxidant activities in rice (Dutta and Mahanta, 2012; Buggenhout et al., 2013). Nevertheless, there is little scientific literature about parboiling process on chemical and nutritional qualities of glutinous rice. Therefore, the objective of this work is to process development of parboiled glutinous rice (Khao Niew Hang) for improvement of physical, chemical and nutritional qualities. Furthermore, the aim of this study will be to develop parboiled rice process in industrial scale with respects to maintaining its nutritional qualities.

1.2 Objectives of the research

The objectives of the study are:

1. To develop parboiled glutinous rice process for improvement of physical, chemical and nutritional qualities.
2. To optimize condition of parboiling process for improvement of physical, chemical and nutritional qualities.
3. To apply the parboiling process of glutinous rice on industrial scale as functional food.

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4. To develop product prototypes from parboiled rice bran oil as functional food.

1.3 Expected outcomes

1. Obtain an optimization condition of parboiling process for improvement of physical, chemical and nutritional qualities.

2. Obtain the parboiling process on industrial scale as functional food.

3. Product prototype on industrial scale as functional food.

1.4 Hypothesis of the research

1. Parboiled glutinous rice from different processing processes has different physical, chemical and nutritional qualities.

2. Parboiling process suitable for the prediction of the study responses on industrial scale as functional food.

1.5 Scope of the research

1. Study an appropriate process on physical, chemical and nutritional qualities of parboiled rice using mathematic model.

2. Study an appropriate condition on industrial scale as functional food.

3. Evaluation of the physical, chemical and nutritional qualities of parboiled glutinous rice product.

4. Product prototypes from parboiled glutinous rice as functional food.

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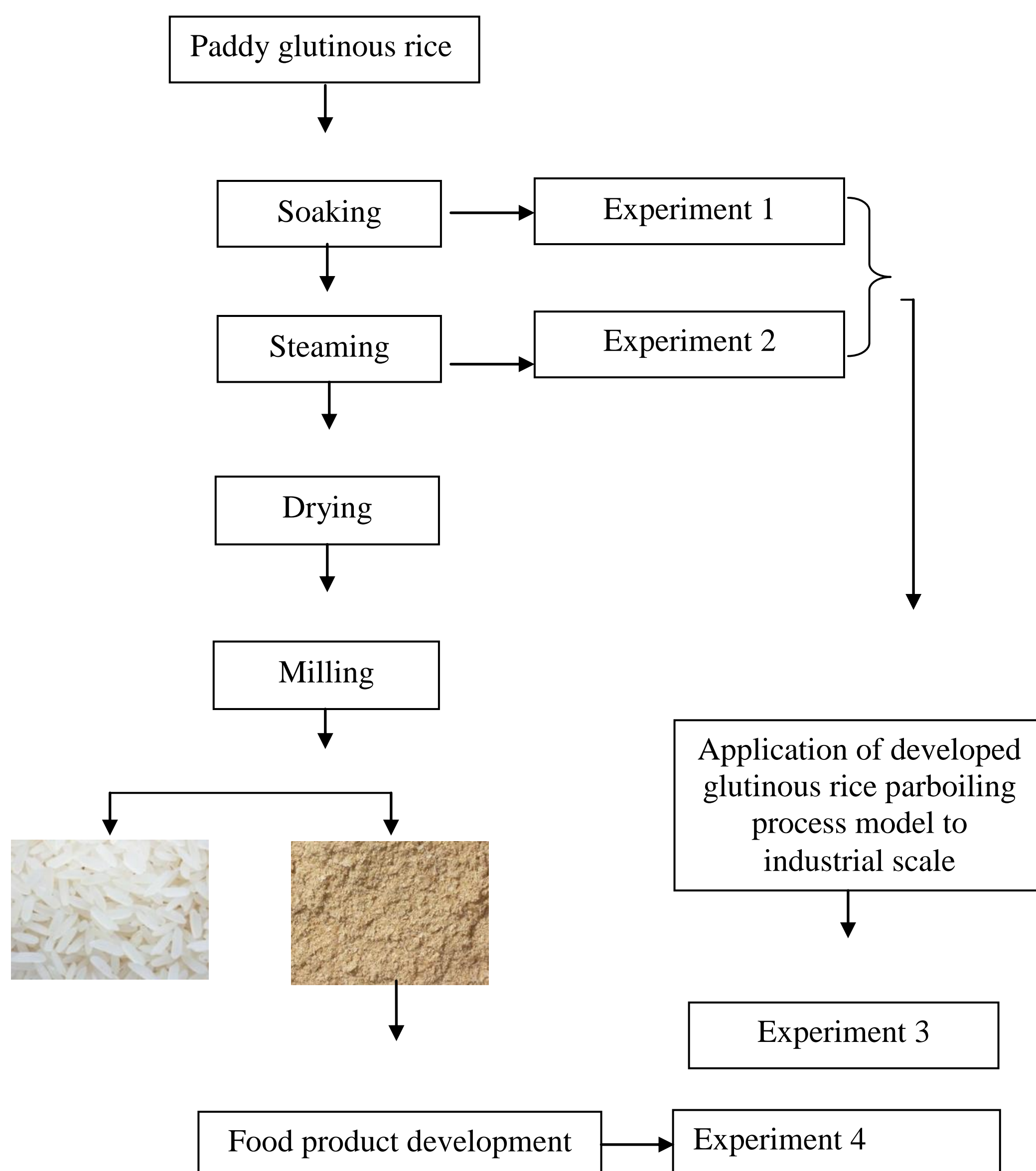
Figure 1.1 Flow chart of experiment procedure



1.6 Defined words

1. Parboiled rice refer to rice that has been partially boiled in the husk. This modifies the starch and permits the retention of much of the natural vitamins and minerals in kernels. The rice is usually slightly yellowish, although the color largely fades after cooking.

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2. Bioactive compounds refer to extra-nutritional constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health.

3. Antioxidant components refer to micro-constituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals. Food such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phenolic compounds.

4. Response surface methodology (RSM) refers to a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). An experiment is a series of tests, called runs, in which changes are made in the input variables in order to identify the reasons for changes in the output response.

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CHAPTER 2

LITERATURE REVIEW

2.1 Rice

Rice (*Oryza sativa*) is considered a sacred in Asia and is still the most important food for more than 50% of the world population. Archeological evidence has proved that rice was planted and used at least 4,000 years BC. There are approximately 20 species of the genus Oryza and only one (*Oryza sativa*) supplies virtually all the rice consumed nowadays. *Oryza rufipogon* (Perennial) and *Oryza nivana* (annual) are considered the ancestral species. In developing and developed Asian countries, the yearly per capita intake of rice is approximately 81 and 24 kg, respectively (Serna-Saldivar, 2010). Production data indicate that about 90% of the world's rice production is harvested in Asia. Rice is planted in tropical and subtropical regions of the world, close to or neighboring the equatorial line where is high relative humidity and rainfall. The agronomics of rice is different from other cereals because this plant is generally planted on flooded soils and by transplanting seedlings previously grown in nurseries. However, most of the rice in the world is directly planted and irrigated. Planting rice demands high labor for sowing, irrigation, other agronomic practices, and harvesting. Mechanization is difficult, especially in terraced paddy fields. Threshing is usually manually done by beating the harvested against the ground of logs or by having animals or humans tread upon the heads. Winnowing to remove the chaff is traditionally done by tossing the rice from rattan trays. The wind blows the lighter chaff while the denser rice settles nearby. However, the high labor and energy pays off because rice is the cereal crop with the second highest average yield-estimated to have been 4.2 tons/Ha in 2007 (FAO, 2009). Three major groups of rice are widely recognized: Japonica, Indica, and Javanica. The first two are the most relevant. Japonicas are usually high yielding and produce short caryopses, which upon cooking yield sticky rice. These rices are popular in Asia. On the other hand, Indica usually yield less compared to their Japonica counterparts, producing long caryopses that upon cooking, yield drier, firmer, and non-glutinous rice (Marshall and Wadsworth, 1994; Champagne, 2004).

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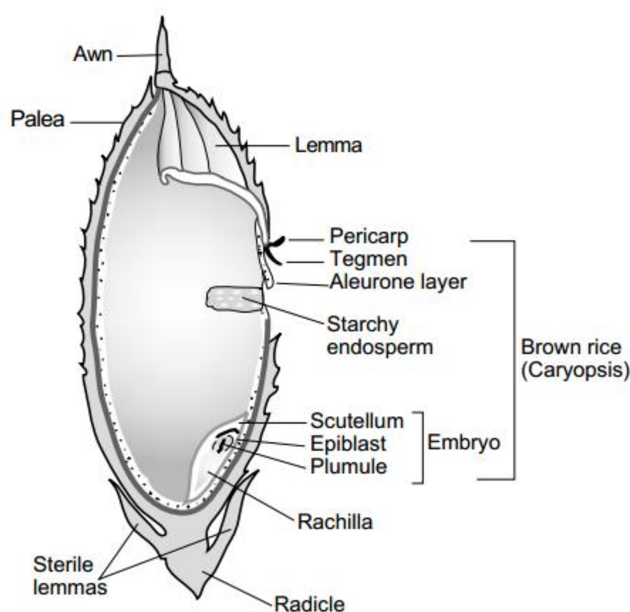
Rice (*Oryza sativa*) is widely consumed in the world, and the most common type (> 85%) has a white pericarp. Other types have a colored pericarp, and the most common are green, black, and red. The black and red varieties are planted mainly in South Asia and other countries (Simmons and Williams, 1997). A rice grain consists of the hull (including the awn, lemma and palea) and the rice caryopsis, also known as brown rice (Figure 2.1) (Juliano and Bechtel, 1985). The four layers of the caryopsis including pericarp, seed coat, nucellus, and aleurone, along with much of the embryo comprise the bran portion of the rice grain. Rice is a good source of calories provided by its high content of starch and high nutritional quality protein; it is hypoallergenic and easily digested (Mazza, 1998). Rice is a good source of the vitamins B, thiamine, riboflavin and niacin, but contains little to no vitamin C, D or beta-carotene, the precursor of vitamin A. The amino acid profile of rice is high in glutamic and aspartic acids, but low in lysine (FAO, 1993). The main anti-nutritional factors, most of which are concentrated in the bran, are phytate, trypsin inhibitor, oryzacystatin and haemaglutinin-lectin. The different layers of rice seed (outer hull, caryopsis, aleurone, subaleurone and endosperm) and the embryo contain differ amounts of nutrients. Dietary fiber, minerals and B vitamins are highest in the bran and lowest in the aleurone layers; the rice endosperm is rich in carbohydrate and contains a fair amount of digestible protein, with an amino acid profile which compares favorably to other grains (FAO, 1993).

Figure 2.1 Structure of rice grain



2.2 Rice Starch

2.2.1 Granule structure of rice Starch

The endosperm, the starchy inner portion of rice kernel, is composed of tightly packed compound starch granules with protein interspersed as spherical bodies and crystalline structures. The starch content is particularly high, up to 77.6% in milled rice (Juliano and Bechtel, 1985). Starch is the major dietary source of carbohydrates and is the most abundant storage polysaccharide in plants. It is present in high amounts in roots, tubers, cereal grains and legumes and also occurs in fruit and vegetable tissues. Starch is a polymer of glucose linked together by α-D-(1-4) and/or α -D-(1-6) glycosidic bonds. The starch granule mass comprises 70% amorphous regions, which consists of amylose and branching points of amylopectin molecules, and 30% crystalline, which is mainly composed of the outer chains of amylopectin (Reddy and Bhotmange, 2013). Starch granules are relatively dense, insoluble, and swell only slightly in cold water. Rice starch has the smallest particle size and the whitest color of all the commercial starches. The average rice starch granule is between 2-8 microns in mature grain (Figure 2.2A). These granules are polyhedral and constitute approximately 90% of milled rice (dry weight). Starch granules consist of alternating semi-crystalline and amorphous (Figure 2.3). However, rice contains only compound granules in amorphous rings. The amorphous region accounts for 70% of the starch granule (Oostergetel and Van Bruggen, 1993), and consists of free amylose, lipid-complexed amylose, and some branch points of amylopectin (Hizukuri, 1996). The conformation of chains in the amorphous domains appears to be mainly a single helix or random coil (Gidley and Bociek, 1988). Dang and Copeland (2003) suggested that the growth rings in rice are approximately 400 nm apart. Based on scanning electron microscopy observations, one block-let in the semi-crystalline growth ring contains several amorphous and crystalline lamellae (Figure 2.2B). Donald et al. (1997) reported that the cross striations within the growth rings of rice starch (as observed by AFM) correspond to the block-let of amorphous and crystalline lamellae. These block-let have an average size of 100 nm in diameter and are proposed to contain 280 amylopectin side chain clusters. Amorphous lamellae contain branch points of the amylopectin side chains and possibly some amylose, whereas semi-crystalline lamellae are constituted of amylopectin double

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helices (Figure 2.2C). The other minor components in rice starch are lipids and proteins, and calcium, potassium, magnesium, and sodium in the ionic form (Vandeputte and Delcour, 2004). Starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS) (Zhu et al., 2011). Variations in amylose and amylopectin ratio and molecular structure are reported to be greatly affected by genetic, environmental, and agronomic conditions (Lawal et al., 2011). These 2 principal components greatly influence the functional properties of rice starches.

Figure 2.2 Granule structure of rice starch and lamellar structure of a starch granule

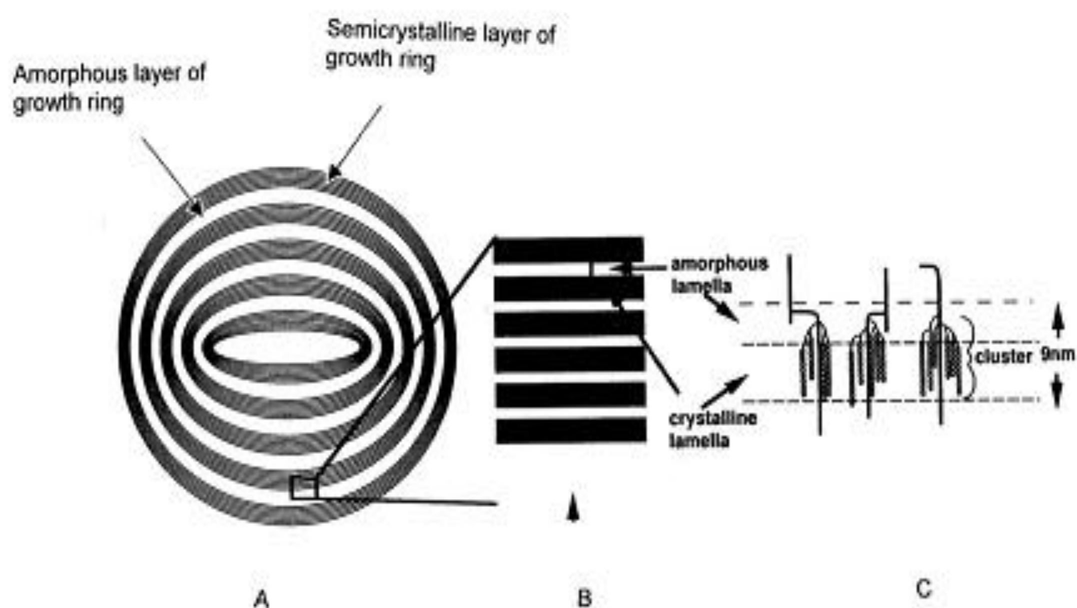
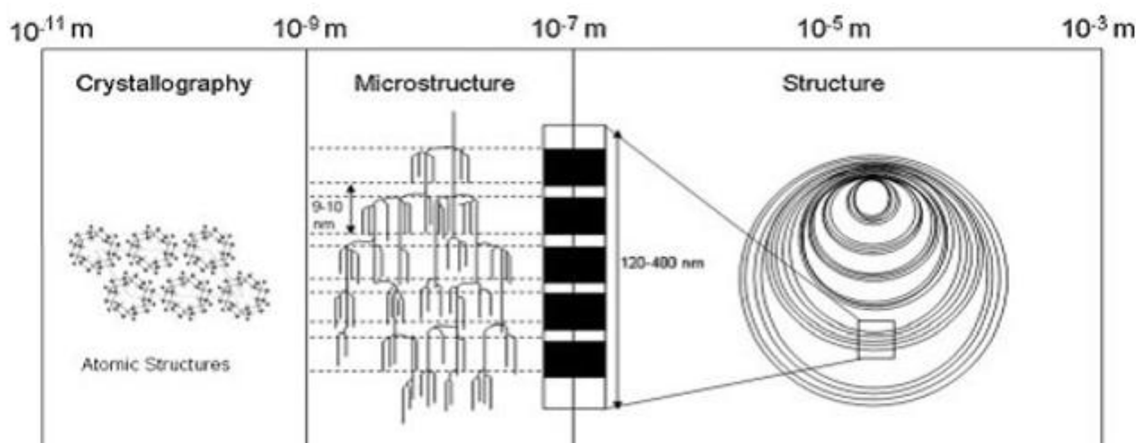


Figure 2.3 The range of elastic neutron scattering techniques, corresponding size range, and complementary methods shown in relation to the hierarchical structure of starch



2.2.2 Chemical composition of rice starch

Rice starch is made up of two distinct polymers, amylose and amylopectin; 98.50% of the starch granule is α-glucans. Both amylose and amylopectin are glucose polymers linked by the α-(1,4) linkage (Takeda et al., 1993). Amylopectin also contains 4-5% of α-(1,6) linkages, leading to a branched molecule (Cura and Krisman, 1990). The average chain length for cereal amylopectin is 20-26 glucose units. The rice starch type can vary in certain genotype from regular waxy rice (0-2%), very low amylose rice (5-12%), low amylose rice (12-20%), medium amylose rice (20-25%) and high amylose rice (25-33%) (Juliano, 1992). Rice starch, as other starch, is composed of two polymeric forms of glucose : amylose and amylopectin (Figure 2.4). These two molecules are organized into a radically anisotropic, semi-crystalline structure in the starch granule (Lineback, 1984). Amylose isolated from tuber and root starches, such as potato and tapioca have larger molecular sizes than those isolated from cereal starches, such as maize, rice and wheat (Takeda et al., 1986). The conformation of amylose has been the subject of controversy and has been shown to vary from helical to an interrupted helix, to a random coil. In alkaline solutions (KOH) and in dimethyl sulfoxide (DMSO) amylose probably has an expanded coil conformation, while in water and neutral aqueous potassium chloride solutions it is a random coil with short, loose helical segments (Banks and Greenwood, 1971).

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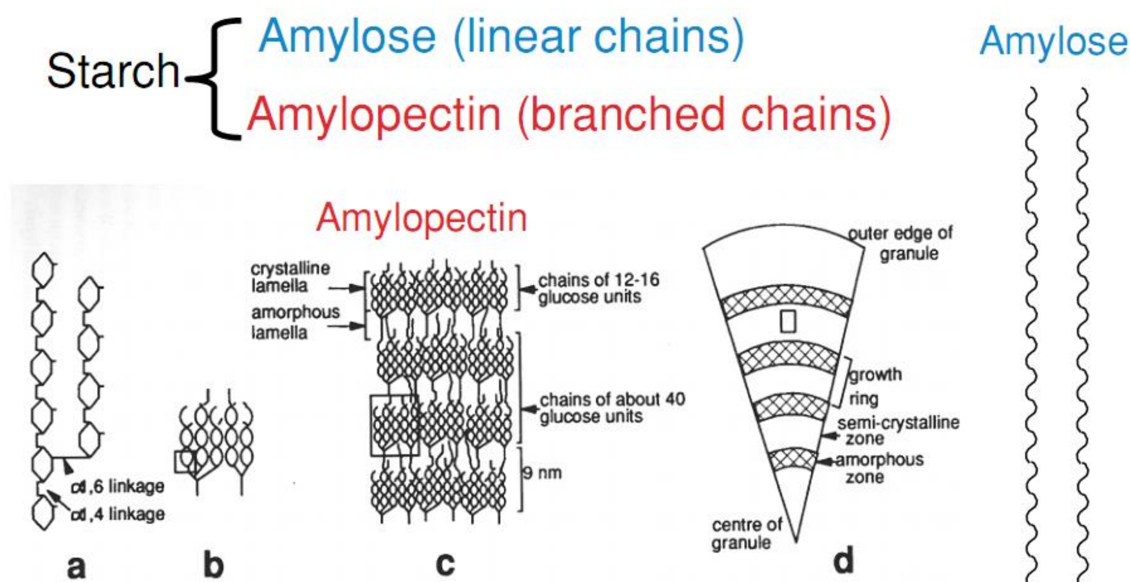
Figure 2.4 Representative partial structures of amylose and amylopectin



The boxes within the diagrams in panels a, b, c and d represent the area occupied by the structure in the preceding panel. a) Structure of two branches of an amylopectin molecules, showing individual glucose units. b) A single cluster within an amylopectin molecule, showing association of adjacent branches to form double helices. c) Arrangement of clusters to form alternating crystalline and amorphous lamellae. The crystalline lamellae are produced by the packing of double helices in ordered arrays. Chains of 12–16 glucose units span one cluster, chains of about 40 glucose units span two clusters. d) Slice through a granules, showing alternating zones of semicrystalline material, consisting of crystalline and amorphous lamellae, and amorphous material.

2.2.3 Rice amylose

Amylose, a linear polymer, is composed almost entirely of α-1,4-linked D-glucopyranosyl units; many amylose molecules have a few α-1,6-linked D-glucopyranose branches, about 0.3% to 0.5% (Whistler and BeMiller, 1997) and at times less than 0.1% (Ball et al., 1996). Rice amylose is a mixture of branched and linear molecules with a degree of polymerization (DPn) of 1,100-1,700 and 700-900, respectively (Hizukuri et al., 1989). The branched fraction in rice constituted 25-50 % by number and 30-40% by mass of amylose. Rice amyloses have a β-amylolysis of 73-81%, indicating them to be slightly branched molecules with three to four chains on

average (Hizukuri et al. 1988). This β-amylolysis limit is considerably higher than that of amylopectin (55-60%). The location of amylose in a starch granule is still in dispute. Various possible locations have been listed: amorphous lamellae, amorphous growth ring, or interspersed or co-crystallized with amylopectin molecules (Hoover et al., 2010).

These structural properties of amylose from rice are similar those of wheat and maize, as indicated in Table 2.1. The amylose content of the starch granule varies with the botanical source of the starch and is affected by climatic and soil conditions during grain development (Morrison and Azudin, 1987). High temperatures decrease the amylose content of rice, whereas cool temperatures have the opposite effect. Amylose content of rice is specified as waxy (0-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (25-33%) (Juliano, 1992). Amylose is a roughly linear molecule containing about 99% α- (1,4) and about 1% α- (1,6) bonds with a molecular weight of 500-20,000 (Figure 2.4).

Table 2.1 Structural properties of amylase

Source	β- Amylolysis limit (%)	Avg. DP_n	Avg. Number Chain	Avg. Chain Length	Branched Molecules (%)
Wheat	88	1,300	4.8	270	27
Maize	82	930	2.7	340	44
Rice: Indica	73	1,000	4.0	250	49
Rice: Japonica	81	1,100	3.4	320	31

Source: Hizukuri et al. (1989)

Amylose can form an extended shape (hydrodynamic radius 7-22 nm) but generally tends to wind up into a rather stiff left-handed single helix or form even stiffer parallel left-handed double helical junction zones (Swinkels, 1985). Single helical amylose has hydrogen-bonding 2 and 6 atoms on outside surface of the helix with only the ring oxygen pointing inwards. Hydrogen bonding between aligned chains causes retrogradation and releases some of the bound water (syneresis). The aligned chains

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may then form double stranded crystallites that are resistant to amylases. These possess extensive inter- and intra-strand hydrogen bonding, resulting in a fairly hydrophobic structure of low solubility. Single helix amylose behaves similarly to the cyclodextrins by processing a relatively hydrophobic inner surface that holds a spiral of water molecules, which are relatively easily lost to be replaced by hydrophobic lipid or aroma molecules. It is also responsible for the characteristic binding of amylose to chains of charged iodine molecules (e.g. the polyiodides; chains of I3- and I5- forming structures such as I93- and I153-; note that neutral I2 molecules may give polyiodides in aqueous solution and there is no interaction with I2 molecules except under strictly anhydrous conditions) where each turn of the helix holds about two iodine atoms and a blue color is produced due to donor-acceptor interaction between water and the electron deficient polyiodides (Davies et al., 1980).

2.3.4 Rice amylopectin

Rice amylopectins are highly branched polymer having on the average 96% α- (1,4) bonds and 4-5% α-(1,6) bonds (Figure 2.4). Enzymatic techniques have been used to obtain structural information and develop models of amylopectin. This branching is determined by branching enzymes that leave each chain with up to 30 glucose residues. Each amylopectin molecule contains a million or so residues, about 5% of which form the branch points. There are usually slightly more outer unbranched chains (called A-chains) than 'inner' branched chains (called B-chains). There is only one chain (called the C-chain) containing the single reducing group. The molecule may have 10,000 – 100,000 individual chains and the ratio of unbranched to branched chains is about 1.0. Approximately 22-25 chains form each cluster, comprising the crystalline regions of starch granules (Figure 2.5). In waxy rice, 80-90% of the amylopectin chains probably constitute a single cluster, while the remaining 10–20 % from intercluster connections, which are mainly between adjacent clusters (Lineback, 1993).

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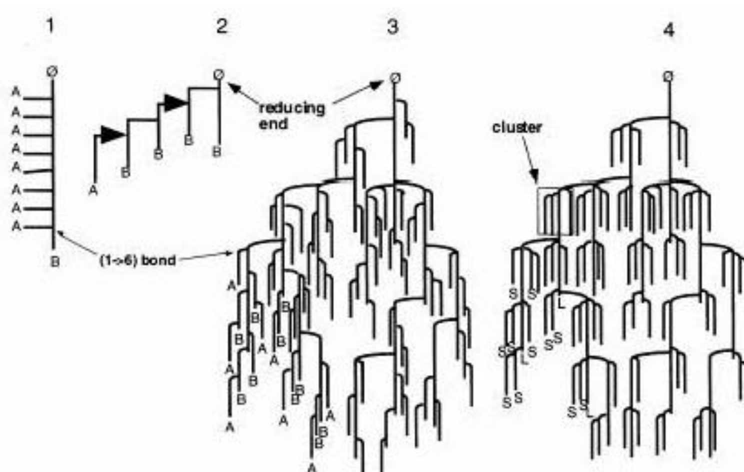
Figure 2.5 Cluster model of amylopectin (= Reducing chain-end). Solid lines indicate (1,4)- α-D- glucan chain; arrows indicate α-(1,6) linkage



Amylopectin molecules are highly branched, of high molecular weight and constitute the skeleton of the starch granule (Kossmann and Lloyd, 2000). Peat et al. (1956) defined the basic structure of amylopectin in terms of linear A, B, and C chains. The chains (outer chains) are attached through their potential reducing end to B chains. The latter are linked in the same way and carry one or more A chains. The C chain contains the single reducing group of the amylopectin molecule and carries other chains. Based on the A-, B-, C-chain terminology of Peat et al. (1956) and Hizukuri (1986) the cluster model has been refined. Amylopectin has a polymodal distribution with A (Chain length, CL 12 to 16) and B chains, namely B_1 (CL 20 to 24), B_2 (CL 42 to 48), B_3 (CL 69 to 75), and B_4 (CL 104 to 140) chains. A and B_1 chains form a single cluster, whereas B_2, B_3 and, B_4 chains extend into 2, 3, and more than 4 clusters. Hanashiro and et al. (2002) suggested that C chains are very similar among botanical sources and range in size from 10 to 130 glucose units, with the majority being around 40 glucose units (Hanashiro et al., 2002). The following composition was proposed for waxy rice amylopectin: A (CL 13), B_1 (CL 22), B_2 (CL 42), B_3 (CL 69), and B_4 (CL 101) (Hizukuri, 1986). Enevoldsen and Juliano (1988) have been reported waxy and nonwaxy (low amylose) rice amylopectin to have similar molar ratios of A to B chains (1.1 to 1.5).

Each amylopectin molecule contains up to two million glucose residues in a compact structure with hydrodynamic radius 21-75 nm(Lineback, 1993). The molecules

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are oriented radically in the starch granule and as the radius increases so does the number of branches required filling up the space, with the consequent formation of concentric regions of alternating amorphous and crystalline structure (Figure 2.6). The structural properties of amylopectin, with amylose, vary depending on source (Table 2.2). Rice amylopectin have β-amylolysis limits of 56-58%, chain lengths of 18-21, and widely different DP and linear chain distribution values (Takeda et al., 1987; Hizukuri et al., 1989). Low average DP values are observed for indica rice (4,700), as compared to those for japonica rice (12,800) and waxy rice (18,500) amylopectin.

Figure 2.6 Idealized diagram of the consequent formation of concentric regions of alternating amorphous and crystalline structure of amylopectin. (A) Shows the essential features of amylopectin. (B) Shows the organization of the amorphous and crystalline regions (or domains) of the structure generating the concentric layers that contribute to the "growth rings" that are visible by light microscopy. (C) Shows the orientation of the amylopectin molecules in a cross section of an idealized entire granule. (D) Shows the likely double helix structure taken up by neighboring chains and giving rise to the extensive degree of crystallinity in granule

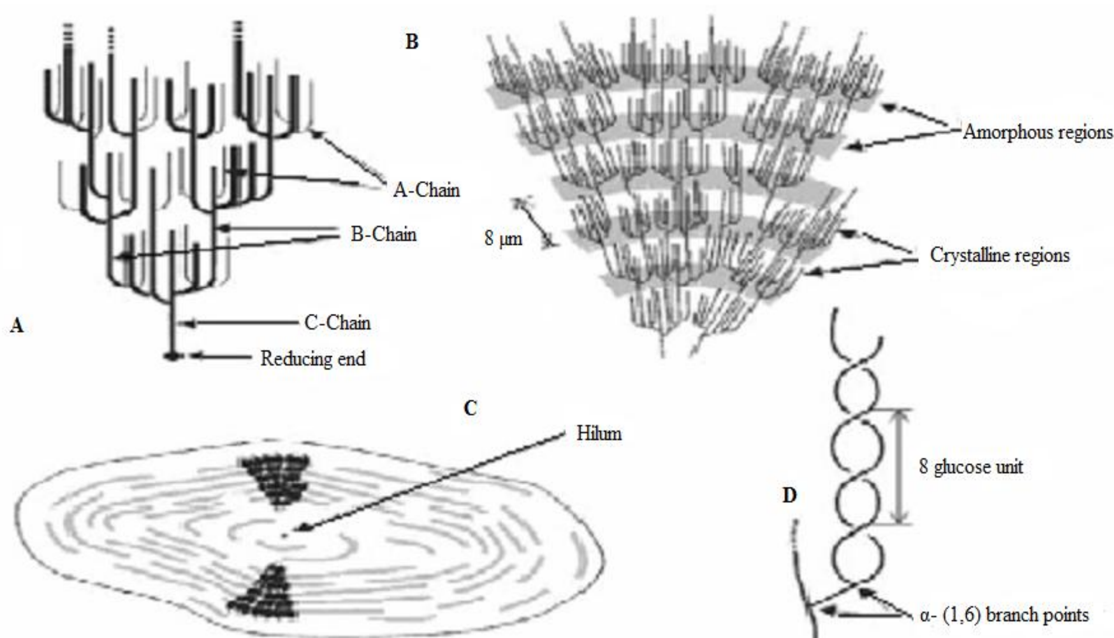


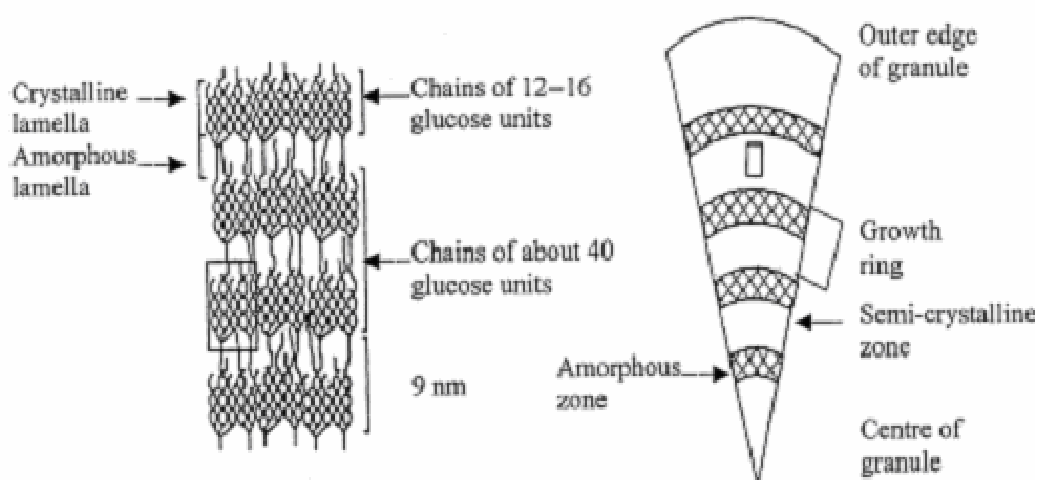
Table 2.2 Structural properties of amylopectin

Source	Avg. Degree of Polymerization	Avg. Number Chains	Avg. Chain Length	Avg. External Chain Length	Avg. Internal Chain Length
Maize	8,200	370	22	15	6
Rice: Indica	4,700	220	21	14	6
Japonica	12,800	670	19	13	5
Waxy rice	18,500	1,000	18	12	5
Potato	9,800	410	24	15	8

Source: Hizukuri et al. (1988)

Amylose and amylopectin are assembled in a cluster structure, in which the granules are composed of starch molecules laid down in concentric rings (Figure 2.7). The molecules that comprise a layer are deposited in a radial fashion with some sections in highly ordered crystalline regions. These radically ordered crystallites are linked by less structured amorphous regions. Hydrogen bonding is likely to be a significant force in both regions. Linear portions of amylopectin constitute the crystalline regions, whereas the branch points and amylose are the main components of the amorphous portion (Blanshard, 1987).

Figure 2.7 Amorphous and semi-crystalline regions in the starch granule



2.2.5 Minor components of rice starch

The most abundant components of starch are amylose and amylopectin, which constitute almost of 100% starch dry matter. Apart from these main components, smaller amounts of other components such as proteins, free fatty acids, other lipids and phosphate groups may also be present in amounts depending on the botanical source and starch isolation procedure (Morrison and Karkalas, 1990). Non-waxy rice starches (12.2-28.6% amylose) contain 0.9-1.3% lipids comprising 29-45% fatty acids and 48% lysophospholipids (Jane et al., 1992). Waxy rice starches (1.0-2.3% amylose) contain ineligible amounts of lipids. Starch proteins are mostly either storage protein or biosynthetic or degradative enzymes. Rice storage proteins exist mainly as protein bodies (PB), PB I (prolamin) or PB II (glutelin) (Juliano, 1984). Biosynthetic enzymes are most probably entrapped within the starch granules following starch synthesis (Glaszmann, 1987). Besides lipids and proteins, phosphorus is an important non carbohydrate component of rice starch. In waxy rice starches, it is mainly present as phosphate-monoesters (0.003% on dry basis), whereas non-waxy rice starches predominantly contain phospholipids phosphorus 0.048% on dry basis (Jane et al., 1992). Other mineral components of starch are calcium, potassium, magnesium and sodium in their ionic form (Juliano and Viliareal, 1993).

2.2.6 Properties of starch

2.2.6.1 Granular swelling

Rice, more than any other cereal, exhibits very wide ranges of cooking quality and rheological properties that are largely determined by the swelling, gelatinization, pasting, and retrogradation characteristics of its starch (Juliano 1985). Being a diploid cereal, rice has numerous stable starch variants commonly classified as high-, intermediate-, and low-amylose and waxy (zero-amylose). Each of these types normally includes varieties with low, intermediate, and high gelatinization temperatures (GT). Most starch granules are insoluble in water. When dry starch granules are placed in water, a small amount of water is absorbed (exothermic process). If the temperature is increased, the amount of absorbed water increases, until a certain temperature (the onset of gelatinization) the water uptake is reversible, but then the changes are irreversible. The sequence of events during swelling of starch is presented in (Figure 2.8). Swelling power and solubility provide evidence of the magnitude of interaction

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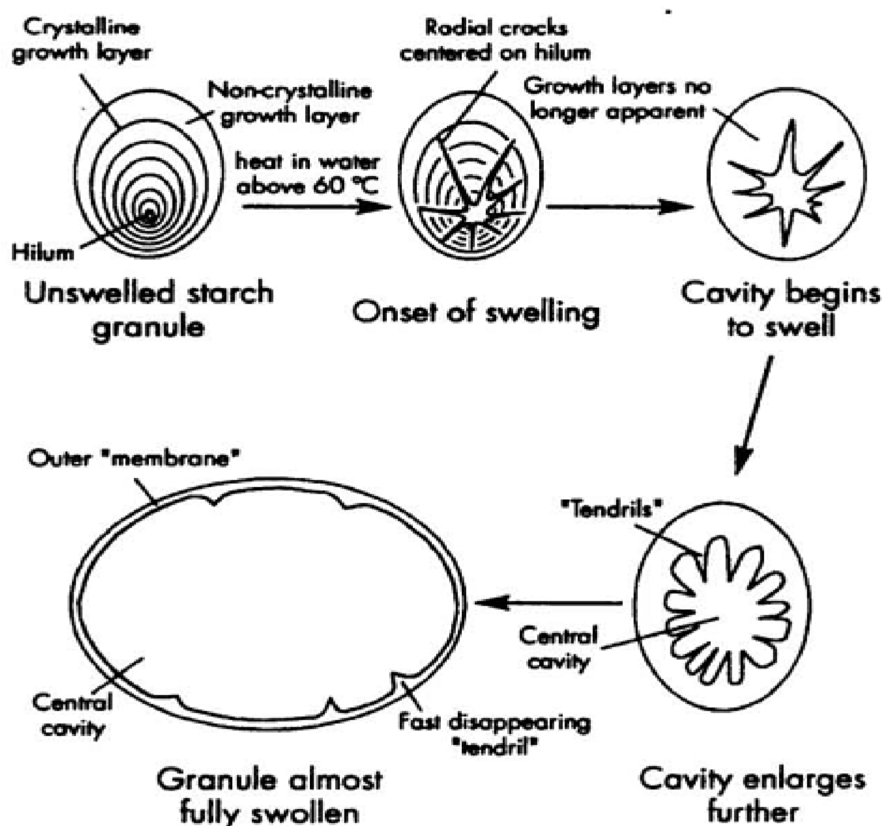
between starch chains within the amorphous and crystalline domains. Jenkins et al. (1994) showed that the initial absorption of water and the location of swelling occur primarily within the amorphous growth ring rather than the amorphous lamellae. The extent of this interaction is influenced by the amylose/amylopectin ratio and by the characteristics of amylose and amylopectin in terms of molecular weight distribution and conformation. Granular swelling has been shown to be influenced by amylose content (Tester and Morrison. 1990), granular size (Vasanthan and Bhathy, 1996), temperature (Colonna and Mercier, 1985), starch damage (Karkalas et al., 1992), bound lipid content (Gudmundsson and Eliasson, 1987) and crystallinily (Robin et al., 1975).

Figure 2.8 The process of swelling of starch granule in hot water



2.2.6.2 Rice starch gelatinization

Gelatinization describes the irreversible collapse (disruption) of molecular order within a starch granule when heated in excess water. Starch undergoes an order-disorder phase transition called gelatinization over a temperature range

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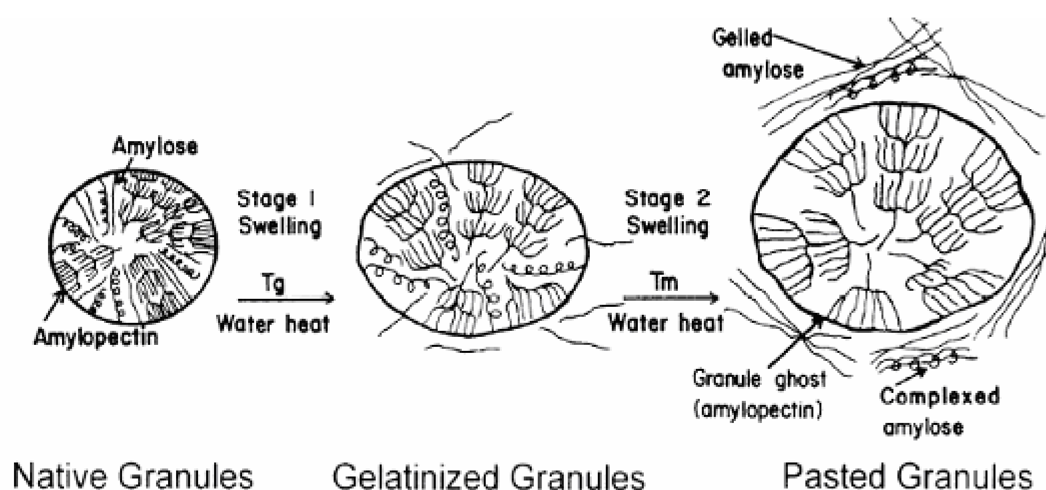
characteristic of the starch source. The above phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of optical birefringence, uptake of heat, loss of crystalline order, uncoiling and dissociation of double helices (in the crystalline regions) and amylose leaching (Evans and Haismann, 1982; Jenkins et al., 1994). Further heating of starch granules in excess water results is granule swelling and additional leaching of soluble component (primary amylose). Swelling of granules attains a maximum value at elevated temperatures and is subsequently followed by granular disruption and exudation of the granule contents into the suspension matrix (Whistler et al., 1984) as shown in Figure 2.9. Final gelatinization temperature (GT) of starch granules refers to the water temperature at which at least 90 percent of the starch granules have gelatinized or lost birefringence (Maltese cross) or swollen irreversibly in hot water. GT is classified for rice starch granules as low (55 to 69.5°C), intermediate (70 to 74°C) and high (74.5 to 80°C). GT is indexed in the breeding programmed by the alkali spreading value based on the degree of dispersion of six grains of milled rice in 10 ml of 1.7 percent potassium hydroxide after 23 hours soaking at 30°C (Swinkels, 1985).

Figure 2.9 Idealized diagram of the swelling and gelatinization of a starch granule



Waigh et al. (2000) have been proposed a model for gelatinization based on the side-chain liquid crystalline model for starch. In this model, the lamellae in starch are considered in terms of three components: 1) backbone, 2) side-chain and 3) double helices (Figure 2.10). It is the degree of mobility of those three components, coupled with the helix-coil transition, which gives starch its distinctive properties. Their postulate is as follows: 1) at low water contents (< 5% w/w) the amylopectin helices are in a glassy nematic state (Figure 2.10A). 2) Intermediate water contents (> 5%, < 40% w/w) have two steps in their breakdown and there are correspondingly two DSC endotherms. The first is thought to be due to the rearrangement of dislocation between constituent amylopectin helices leading to a semectic-nematic transition (Figure 2.10B). The second is the helix to coil transition as the amylopeclin helices unwind in an irreversible transition. 3) In excess water (40% w/w) lamellae break up and the helix to coil transition occurs at the same point, since free unassociated helices are unstable (Figure 2.10C).

A high GT value is uncommon, particularly in high amylose rice. A low ambient temperature during ripening may increase amylose content and independently reduce GT (Nikuni, 1969). The GT affects the degree of cooking of rice because of the cooking gradient from the surface to the core of the grain. Because GT correlates directly with cooking time, a low GT favors fuel conservation, provided eating quality is not adversely affected. GT also affects the molecular properties of amylopectin (Donald et al., 1997).

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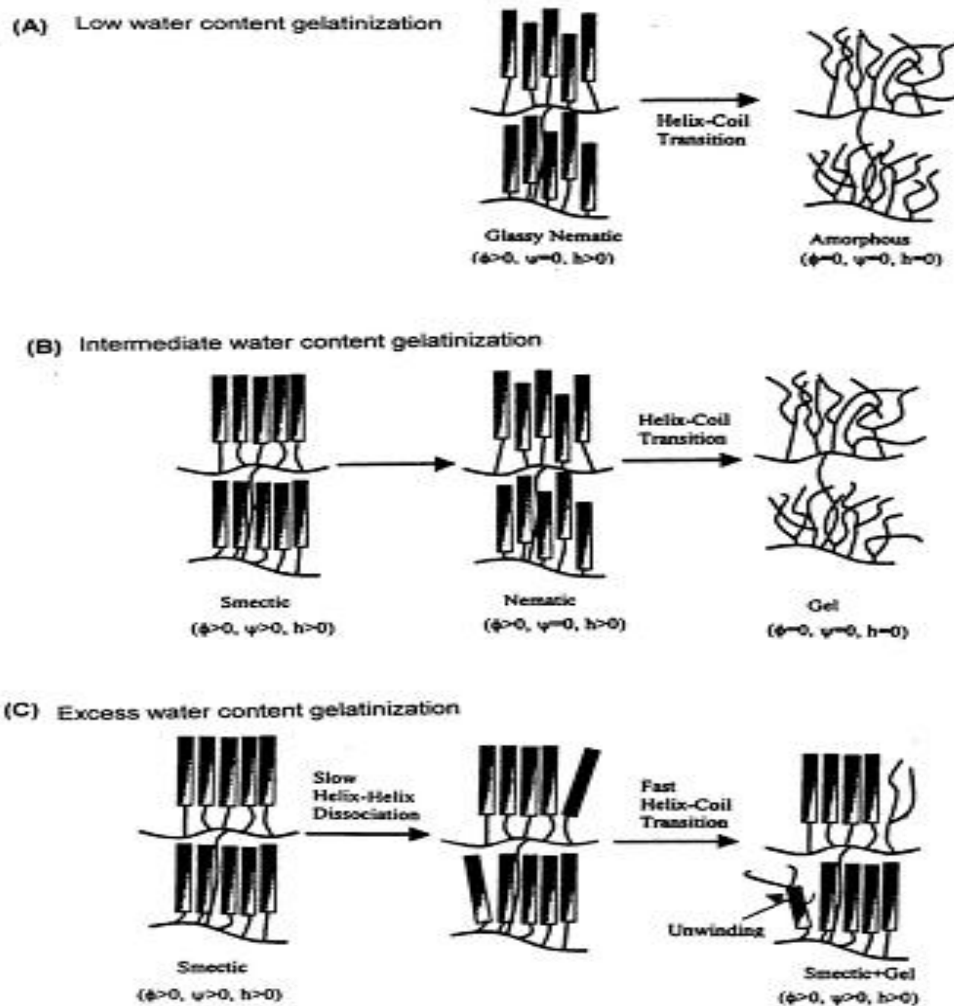
Figure 2.10 Models for gelatinization process based on water content available during gelatinization

(A) The single stage process in the gelatinization of starch at low water contents

(B) The two-stage process involved in the gelatinization of starch in limiting water (intermediate water content)

(C) The two stage process involved in the gelatinization of starch in excess water: relative values of the orientation = ϕ lamellar = φ and helical order parameter = h



2.2.6.3 Rice starch changes during gelatinization

Gelatinization in the narrowest sense is the thermal disordering of crystalline structures in native starch granules, but in the broader sense it includes related events such as swelling of the granules and leaching of soluble polysaccharides (Tester and Morrison, 1990). Gelatinization temperature (GT) and enthalpy (AH) are conveniently measured by differential scanning calorimetry (DSC), and this aspect has received much attention in recent years because it is experimentally convenient and precise. Rice starch begins to gelatinize between 85°C and 95°C, the exact temperature dependent is the specific varieties (Bhattacharya, 1979). For example, different starches exhibit different granular densities, which affect the granules that can absorb water. Since loss of birefringence occurs at the time of initial rapid gelatinization (swelling of the granule), loss of birefringence is a good indicator of the initial gelatinization temperature of a given starch. The largest granules, which are usually less compact, begin to swell first. Once optimum gelatinization of the grains has occurred, unnecessary agitation may fragment the swollen starch grains and cause thinning of the paste. Further, it occurs in those parts of the grain where the water content is sufficiently high (water to starch ratio $\geq$ 0.75) (Hoseney, 1990). Starch swell are transformed progressively to an essentially amorphous form with loss of organized structure. Ultimately granule structure is completely lost and a thin paste ($< \sim 4\%$) or gel ($> \sim 4\%$) is formed. Evidence of this loss of order can be seen by irreversible granule swelling, loss of birefringence, and loss of crystallinity.

2.2.6.4 Loss of birefringence

Starch granules show birefringence or a typical “maltese cross” when viewed in polarized light (Fitt and Snyder, 1984; Hoseney, 1990). The property of birefringence is brought about because the starch molecules are radially oriented within the granule. The level of orientation then determines the degree of birefringence. Birefringence, however, must not be confused with crystallinity; molecules can be very ordered without necessarily having three-dimensional crystalline order. When the starch is heated in water, birefringence in polarized light is lost when viewed under a microscope. The loss of birefringence is related to the water content, using 0.1 – 0.2 % suspensions and noting the temperature intervals for different level of loss (Kulp and Ponte, 2000). One of the most common methods for determining the gelatinization

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temperature range is to follow the loss of birefringence in excess water. The loss of birefringence occurs over a broader temperature interval when content is decreased (Lund, 1984).

2.2.6.5 Loss of crystallinity

The loss of crystalline order during heating is observed in x-ray diffraction patterns (Eliasson and Gudmundsson, 1996). The diffraction pattern disappears, and eventually a pattern indicative of a completely amorphous material is obtained (Zobel et al., 1988). The temperature range during which the crystallinity is lost and the rate which it is lost depend on the water content and or the type of starch (Liu et al., 1997). The temperature range increases with decreasing water content, and at water content below 50% the temperature for complete loss of crystallinity approaches 100°C. The loss of crystallinity seems to occur in two steps: at first the loss occurs at a very low rate, but then at a temperature typical of the starch the rate increases dramatically (Svensson and Eliasson, 1995).

2.2.6.6 Endothermic transitions

The starch gelatinization is an endothermic process with enthalpy values in the range 10-20 J/g. In a study of ten different starches (including A-, B- and C-starches), a relation between transition enthalpy and the amylopectin unit-chain distribution was found; that is, the enthalpy increased when the amylopectin unit-chain length increased. Another trend was a negative correlation between transition temperature and amylose content (Eliasson, 2006). During the last 10-15 years DSC has become the most important tool for studying starch gelatinization (Donaovan, 1979; Eliasson, 1980). DSC measures the gelatinization transition temperature and the enthalpy of gelatinization (ΔH). These DSC parameters are influenced by the molecular architechture of the crystalline region (Noda et al., 1996). The effect of water content on the glass transition (Tg) of the native and gelatinized rice starches was studied by DSC (Perdon et al., 2000) demonstrated that Tg increased as water content decreases from 27 to 3% (mass basis). Moreover, Tg of native starch was significantly higher than that of gelatinized starch in a low moisture content range (8-18%), and the difference became greater as the moisture content decrease (Noda et al., 2003).

With regard to amylopectin chain length distribution, Noda et al. (2003) revealed that vary short (DP<12) amylopectin chains related negatively, while

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somewhat longer ($12 < DP < 24$) amylopectin chains related positively to gelatinization onset (To), peak (Tp) and conclusion (Tc) temperatures of rice starch as measured by DSC. Whether amylopectin chains have a positive or negative influence on gelatinization temperature depends on the way they are packed into the lamellar structure of the starch granules (Chung et al., 2002). Short amylopectin chains may destabilize the lamellar structure in several ways. Starches with higher relative amounts of very short amylopectin chains will thus have lower molecular and crystalline order and a non-optimized packing within the crystalline lamellae. Consequently, they will most likely have lower gelatinization To, Tp, Tc. For gelatinization to occur the regions of amorphous starch must first melt or undergo glass transition (Slade and Levin, 1988). The heat energy required to completely gelatinize starch in rice is critical to the rice processor, who must optimize heat input, cooking time, and temperature.

2.2.6.7 Morphological changes

The swelling of starch granules and the solubilization of macromolecules are the overall effects of the gelatinization process. The process can be characterized by the swelling index and the solubility index. The high swelling power can be related to the sharp consistency increase observed on viscograms in the same temperature range. Simultaneously, a large amount of soluble material is recovered in the supernatant indicating a high solubilization of starch granules. Using light microscopy and scanning electron microscopy several authors observed the morphological changes occurring at the different temperatures (Lu et al., 1996). When the water molecules possessed sufficient kinetic energy to overcome the attractive forces between the hydrogen-bonded starch molecules within the granule, hydration of the grain occurred, with swelling of the amorphous regions between the crystallites. The size of the starch granules increased slightly as the temperature was raised from 35 to 55°C (Yeh and Li, 1996).

2.2.6.8 Rice starch retrogradation

Starch granules when heated in excess water above their gelatinization temperature undergo irreversible swelling resulting in amylose leaching into solution. In the presence of high starch concentration this suspension will form an elastic gel on cooling. The molecular interactions (mainly hydrogen bonding between starch chains) that occur after cooling have been called retrogradation (Kulp and Ponte, 2000). Starch

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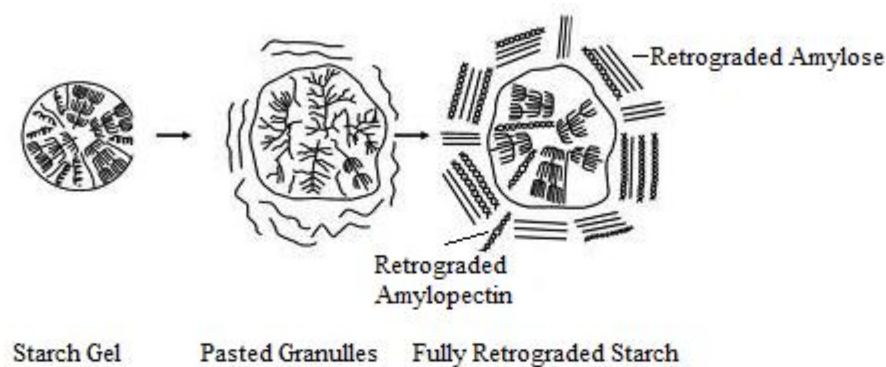
retrogradation is a process that occurs when the molecules comprising gelatinized starch begin to re-associate in an ordered structure (Figure 2.11). In the initial phases of retrogradation, two or more molecules may form a simple juncture point, which then may develop into more extensively ordered regions. Ultimately, under favorable conditions, crystalline order may appear and amylose precipitation from "solution" may occur in dilute solution. Retrogradation of gelatinized starch is a reorganization process that can involve either amylose or amylopectin, with amylose undergoing retrogradation at a more rapid rate than amylopectin (Jacobson et al., 1997).

Figure 2.11 Retrogradation during storage of cooked cereal starch paste leads to recrystallization of the amylose and amylopectin chains



1) Factors affecting rice starch retrogradation

Retrogradation refers to the occurrence where starch reverts or retrogrades to a more crystalline structure upon cooling. Both amylose and amylopectin may participate in a texture change that makes them somewhat more "gritty" with time (Vaclavik and Christian, 2014). Retrogradation can lead to an obvious increase in the firmness of frozen cooked rice (Yu et al., 2010) and stored baked goods (Sozer et al., 2011), making them unattractive to consumers. However, in some products, retrogradation can provide a desirable quality such as in the manufacture of bread crumb (Ottenhof and Farhat, 2004), rice stick noodles (Satmalee and Charoenrein, 2009) and resistant starch type III (Eerlingen et al., 1994). For this reason, numerous studies have been performed to examine the factors affecting the retrogradation of

starch. The rate of rice starch retrogradation depends on a number of variables, including the structures and ratio of amylose and amylopectin, storage temperature, starch concentration, botanical sources of the starch and the presence and concentration of other ingredients, such as salts, detergent and surfactants. The retrogradation kinetics of starch has received wide attention though the underlying mechanism of retrogradation has not been concluded. Lai et al. (2000) reported the retrogradation kinetics of pure amylopectin from 13 rice cultivars. Generally, the amylopectin systems showed two stages of retrogradation behavior during early (< 7 days) and late (>7 days) storage. Correlation analysis suggested that the kinetics of early stage retrogradation were more correlated than the late stage retrogradation with the number-average molecular weight and chain lengths of the amylopectin molecules. The proportion of short, long and extra-long chain fractions appeared to have greater effects on the enthalpy changes and late stage kinetics than the other structural factors.

1.1) Structures and ratio of amylose and amylopectin

Retrogradation is more likely to occur in high amylose starch. A large variability in susceptibility to amylases shown by raw starch granules also influences retrogradation. Potato starch and high amylose maize starch are known to be very resistant in vitro and incompletely absorbed in vivo, whereas most cereal starches are slowly but virtually completely digested and absorbed in vivo (Sajilata et al., 2006).

1.1.1) Retrogradation mechanism of amylose

Amylose is a linear polysaccharide composed of 1,4-linked α-D-glucopyranosyl residues by definition, but the actual specimens that are isolated and purified from starch, include small amount of branched molecules (Takeda et al., 1993). The amylose molecules are notoriously unstable, and retrogradation results in increase of turbidity and eventual precipitation. Consequently, the retrogradation occurs because the amylose molecules shrink, which is caused by a decrease in kinetic energy and Brownian motion of the polymer and water molecules (Tako et al., 2014). This shrinkage results in new formation of intra- and inter-molecular hydrogen bonding between both the hemiacetal oxygen atom and the adjacent OH-6 of the D-glucopyranosyl residues, and the O-6 and OH-2 of D-glycopyranosyl residues on different molecules (Figure 2.13). Much more intense intra- and inter-molecular

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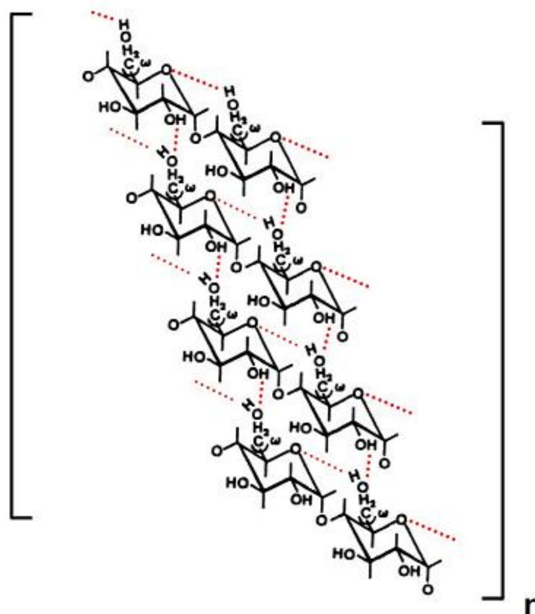
hydrogen bonding leads to precipitation of the amylose molecules in aqueous media (Tako and Hizukuri, 1995).

Figure 2.12 Retrogradation mechanism of amylose. The dotted lines represent hydrogen bonding



The 1,4-linked α-D-glucopyranosyl amylose is the linear fraction of starch. Commercial samples of amylose usually occur in retrograded, water insoluble form, which can be solubillized by pressure cooking at 150-160°C. On cooling, amylose solutions of 2% or higher concentrations, prepared in this way, rapidly gel, while at concentrations lower than 2% precipitation occurs (Schoch, 1969). Amylose is also soluble in dilute alkali, and rapid neutralization of concentrations greater than 2% results in gel formation. Both the gelation of amylose from concentrated solutions and precipitation from dilute solution can be termed retrogradation. The gels and precipitates formed result from the inherent tendency of amylose molecules to undergo conformational ordering and to tendency of amylose molecules to undergo conformation ordering and to subsequently align or aggregate (Gidley and Bulpin, 1989). The rate of retrogradation increases with increasing amylose concentration and with decreasing temperature and is greatest at pH 5-7. In addition, the retrogradation rate has a sharp maximum at amylose degree of polymerization (DP) of

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80, shorter and longer molecules being much more soluble (Ring et al., 1987; Jacobson et al., 1997).

Goodfellow and Wilson (1990) studied the retrogradation of amylose using Fourier Transform Infrared (FT-IR). They found that helix formation must occur before the creation of crystallites; therefore double helix must form at the short or early on in the gelation process. Thus in summary, a same time as phase separation, to create a gel network, with subsequent aggregation of these helices producing crystallinity would be consistent with the experimental data (Figure 2.12).

1.1.2) Retrogradation mechanism of amylopectin

The high solubility and thermal stability of rice amylopectin molecules may be attributed to large number of branching short side-chains (A) on long chains (B2-B3). Consequently, the short side-chains (A) of rice amylopectin molecules prevent intermolecular hydrogen bonding and are likely to dissolve easily in water compared with those of wheat amylopectin molecules. The branching structure of the wheat and rice amylopectin molecules corresponds to the cluster model in which highly branching side-chains (A and B1) and less branching regions are involved along long chains (B2-3). Insoluble fragments of wheat amylopectin molecules also attributed to an involvement of a little large number of super-long chains, 2% - 4% (Shibanuma et al., 1994) than those of rice amylopectin molecules, 1.5% (Tako and Hizukuri, 1999; Tako and Hizukuri, 2000). The super-long chains without side chains, like amylose, on amylopectin molecules are liable to associate with neighboring long chains of polymer molecules with hydrogen bonding resulted in formation of the insoluble fragments in aqueous solution. The hydrogen bonding occurs due to a decrease in kinetic energy and Brownian motion of the polymer and water molecules during storage (Figure 2.13).

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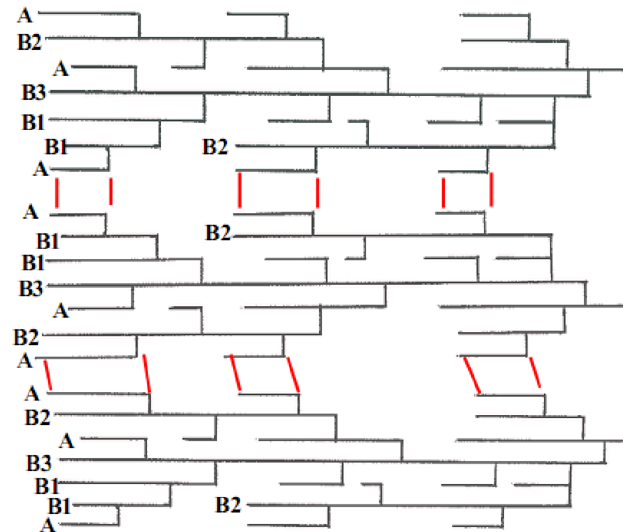
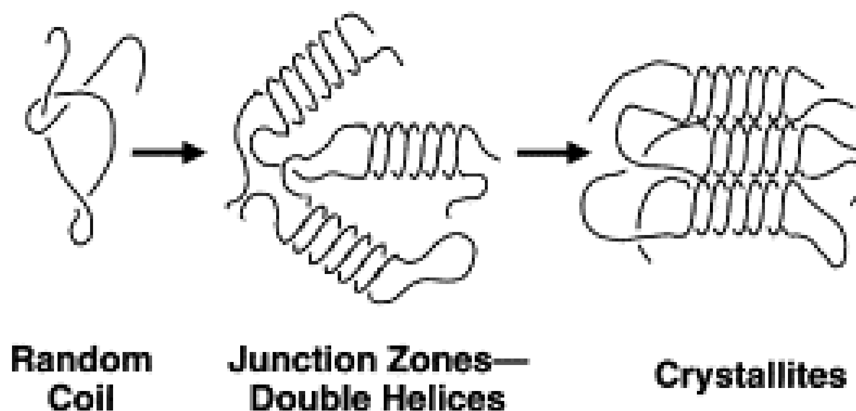
Figure 2.13 Association sites shown in red lines between amylopectin molecules. After storage for about 10 days, much more intense intermolecular association between amylopectin molecules takes place and water molecules also take part in the association due to decrease in kinetic energy and Brownian motion



The IR results for amylopectin suggest an initial fast change followed by a much slow change. Previous IR work by Wilson et al. (1987) in which the spectra for amylopectin from 6 to 340 hour were obtained suggest that FT-IR and G monitor the same process-namely the crystallization of the amylopectin side chains followed by a slow aggregation of these helices to produce crystallinity (Figure 2.14)

Figure 2.14 Schematic diagrams showing the process occurring the gelation and retrogradation of amylose and amylopectin



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Lu et al. (1997) reported the retrogradation of amylopectin from Taiwan rice varieties. They reported that the short chains of amylopectin with DP 10-15 glucose units would result in more retrograded crystalline building blocks. On this basic as well, it would require more energy to disorder the greater number of double helical linkages of retrograded amylopectin. Thus, the plateau enthalpy would be higher (Sander et al., 1990). Gidley and Bulpin (1987) suggested that a chain length of at least 10 units is required for crystallinity development and, by inference, for the formation of double helices. On the other hand, short chains with DP 6-9 glucose units are known to inhibit retrogradation (Shi and Seib, 1992). The retrogradation of waxy rice starch appears to be directly proportional to the mole fraction of unit chains of amylopectin with DP 14-24 and inversely proportional to mole fraction DP 6-9. Once crystalline regions are formed, their reversal is dependent on molecular type: amylose, 100°C to 120°C; amylopectin, as low as 50°C for samples having a low degree of order. When formed in gels, crystalline regions act as physical cross links in a gel network. In reviewing crystallinity, the X-ray polymorphic forms have been described in terms of structure, their function in granules, changes that occur with annealing, loss due to melting (gelatinization), and recrystallization upon retrogradation. Through macro-crystalline domains, crystallinity also contributes to granule ultra-structure. These structures are often revealed by etching granule examination. Most studies require the use of transmission or scanning electron microscopy but large growth rings can be seen with an optical microscope. Observed structures show periodic density fluctuations that vary in size and shape, depending on the extent to which amorphous regions or imperfect crystallites have been disrupted (Maningat and Juliano, 1980).

1.1.3) Retrogradation mechanism of starch

The water molecule (H_2O) can participate in four hydrogen bonds, in which two bonding involve the two hydrogens and the lone pair of electrons of the oxygen and the hydrogen of two neighboring water molecules. This tetrahedrally directed bonding of water molecules, however, is partially involved in gelatinized starch solutions due to the higher kinetic energy of the short side chains (A and B1) of amylopectin molecules (Tako et al., 2014).

The inter-molecular interaction between rice starch and water, and between water molecules are shown in Figure 2.15. The starch molecules

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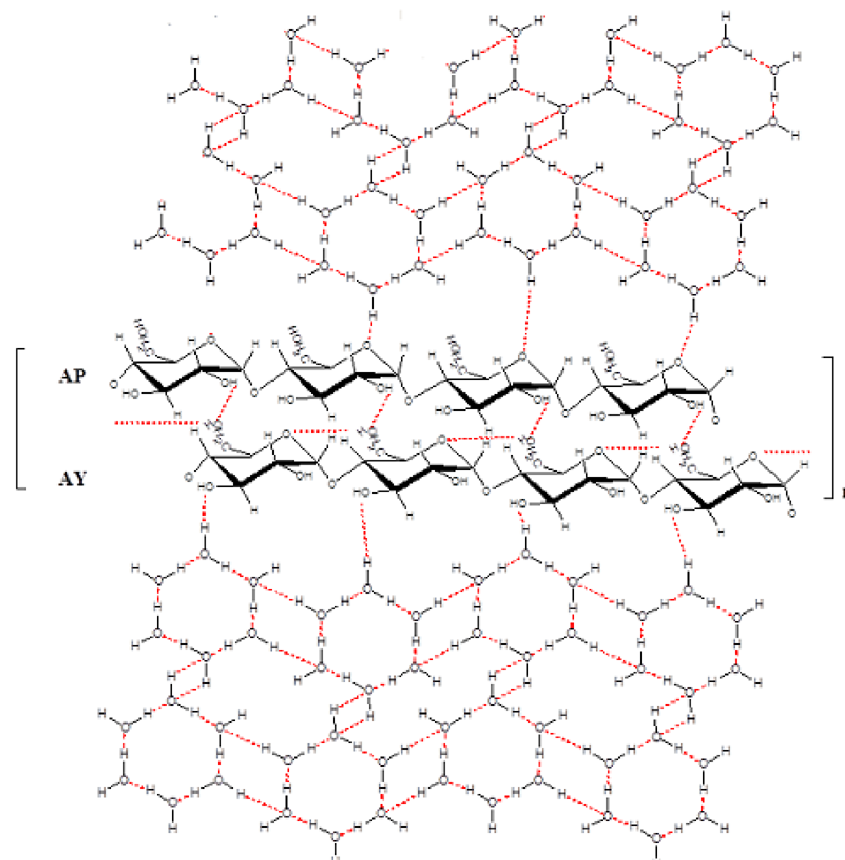
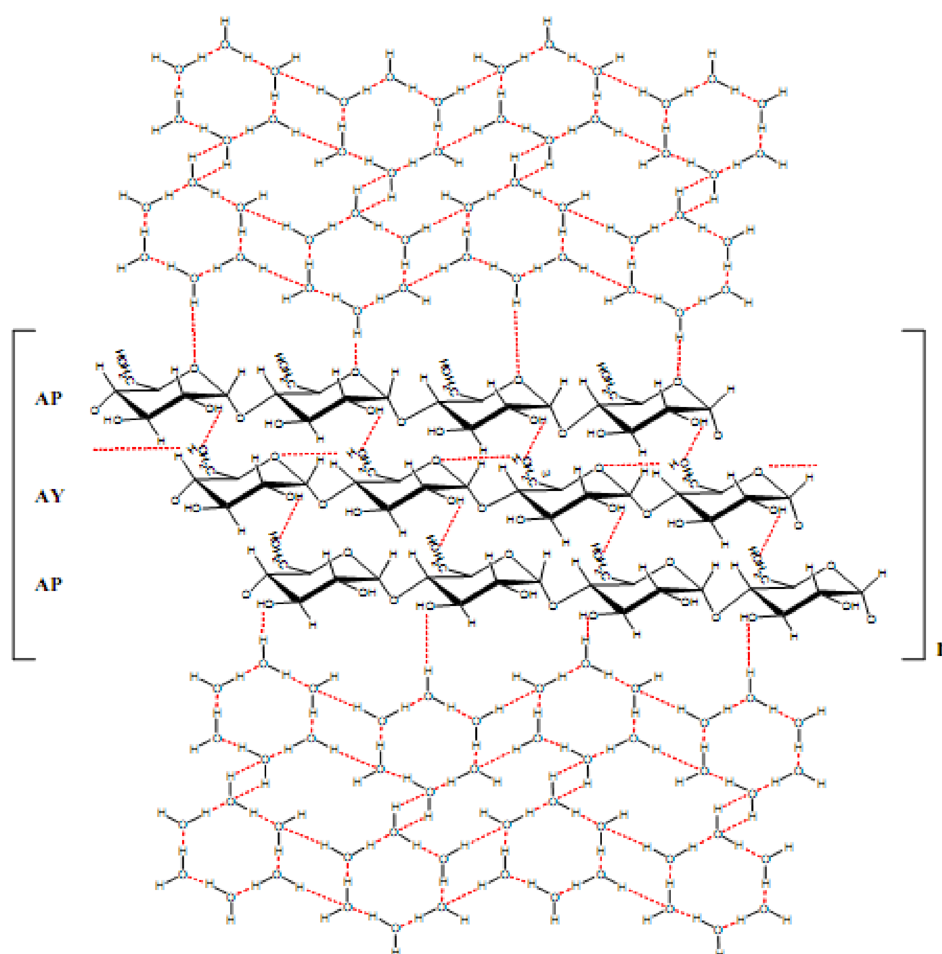
(4%) that adopt a hexagonal conformation involving intra- and inter-molecular hydrogen bonding changed approximately large number of the liquid water molecules into a network of tetrahedrally hydrogen-bonded water molecules in part, even at room temperature, which results in gelatinization. The hemiacetal oxygen (>O) and hydroxyl groups of the starch molecules may be responsible for arranging a network of tetrahedrally hydrogen-bonded water molecules. This network is formed by a cage effect leading to the lower energy state of the lone pair of electrons on the water molecules and extends into hydrogen bonding between water molecules even at room temperature, although the lowest energy has been established at a temperature of 0˚C pure water molecules (Tako et al., 2014).

Figure 2.15 Gelatinization mechanism of rice starch. The dotted red lines represent hydrogen bonding. AY, Amylose; AP, short chain (A or B1) of amylopectin molecules. Water molecules are associated in part with the A or B1 chain of amylopectin molecules caused by a little high kinetic energy of the short side chains



. For the retrogradation process, much more intense intermolecular hydrogen bonding takes place between amylose and amylopectin and between amylopectin molecules, as shown in Figure 2.16. After saturation of intermolecular hydrogen bonding between amylose and amylopectin molecules, an inter-molecular association may also take place between amylopectin molecules due to hydrogen bonding. This bonding is caused by a decrease of kinetic energy and Brownian motion of amylopectin and water molecules during storage. At this stage, side-by-side association between the O-3 and the OH-3 of D-glucopyranosyl residues on different amylopectin molecules may also take place (Figure 2.17).

Figure 2.16 Retrogradation mechanism of rice starch. The dotted lines represent hydrogen bonding. AY, Amylose; AP, short chain (A or B1) of amylopectin molecules.



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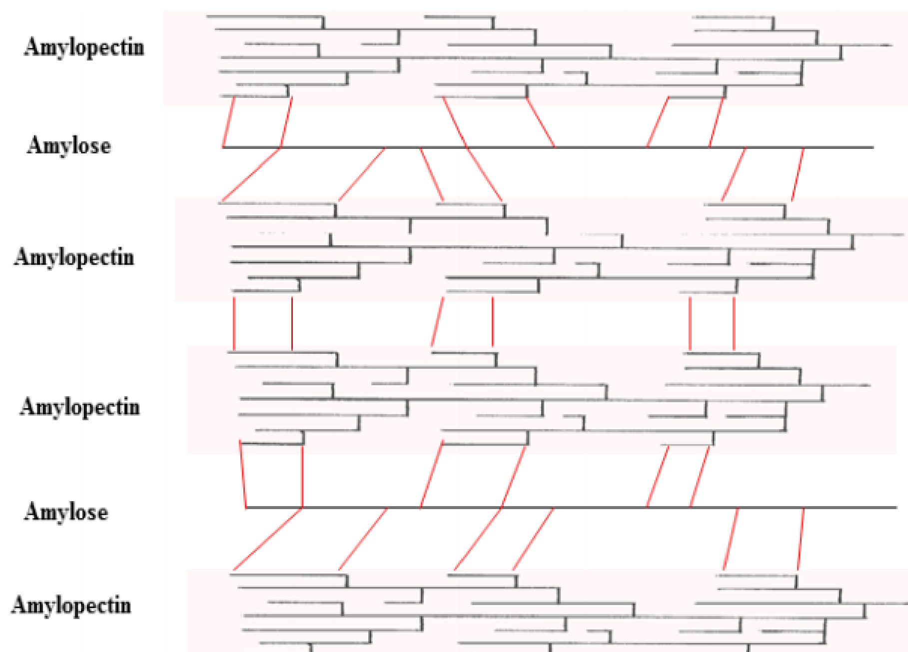
Cereal amylose may form double-helical associations with glucose units of 40-70, while amylopectin forms shorter double helices (Miles et al., 1985; Goodfellow and Wilson, 1990; Liu et al., 1997). The latter can be attributed to restrictions imposed by the branching structure of the amylopectin molecules and the chain lengths of the branches, Thus, amylose, is responsible for short-term changes while amylopectin is responsible for the longer term rheological and structural changes of starch gel. Double helices may associate and organize into crystallites, most of which are related to association of the amylopectin chain which comprise the bulk of the starch component of rice.

Figure 2.17 Association sites shown with red lines between amylose and amylopectin molecules of rice starch. Two or more short side-chains (A or B1) of amylopectin molecules take part in the interaction with an amylose molecule.



1.2) Starch concentration

Starch concentration influences the extent of retrogradation. The maximum extent of retrogradation is obtained in a starch concentration about 60% (w/w). Retrogradation process is very sensitive to temperature (Zeleznak and Hoseney,

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1986). The aging of wheat starch gels at 4, 21, and 30°C was studied with DSC (Lengton and LeGrays, 1981). Crystallinity increased with time and crystallization occurred at the highest rate and to the greatest extent in storage at 4°C. Retrogradation is very sensitive to the water content in starch gels. Lengton and LeGrays (1981) observed that crystallization during aging occurred only in gels with starch content between 10 and 80%, and maximum crystallization, measured with DSC, occurred in gels with 50-55% starch. Other DSC studies have confirmed that maximum crystallinity occurs in gels with 50-60 % starch (w/w) (Zeleznak and Hoseney, 1986).

The recrystallization process depends on the glass temperature (Tg) of amorphous gel because the mobility of the chains determines their amorphous gel. As a plasticizer, water controls the Tg of the amorphous gel. At very low water content, the Tg is above room temperature and the amorphous gel is in a highly viscos glassy state that effectively binders molecular mobility. Recrystallization increases with increasing water content (depress of Tg below room temperature) up to 45-50%, because of progressively more effective plasticization (increase molecular mobility); with further increase of water content up to 90%, it decreases, apparently due to excess dilution (Slade and Levine, 1988).

One can visualize the gel as starch chains with layers of water molecules attached by hydrogen bonding. As the starch paste is cooled, the starch chains become less energetic and the hydrogen bones become stronger, giving a firmer gel. As a gel ages or if it is frozen and thawed, the starch chains have a tendency to interact strongly with each other and thereby force water out of the system. The squeezing of water out of the gel is called 'syneresis'. Longer storage gives rise to more interaction between the starch chain and eventually to formation of crystals. This process, called 'retrogradation' is the crystallization of starch chains in the gel. Because the crystalline areas differ from the non-crystalline areas in their refractive index, the gel becomes more rigid or rubbery, perhaps partially as a result of crystallization and partially just from the interaction of the starch chains (Hoseney, 1990).

1.3) Storage temperature

Retrogradation is greatly affected by storage temperature. Compared to storage at room temperature, storage of starch gels containing 45-50% water at low temperature but still above the glass temperature (Tg~5.0°C) increases the

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retrogradation, especially during the initial day of storage, compared to starch gels stored at ambient temperature (Gudmundsson, 1994). Storage temperature below Tg virtually inhibits recrystallization. Higher temperatures (above 32-40°C) effectively reduce retrogradation. Colwell et al. (1969) studied the effect of storage temperature (-1 to 43°C) on concentrated starch gels by DSC. They found that the mechanism of starch crystallization (instantaneous nucleation followed by rod-like growth of crystals) is the same over the whole range of temperature. Moreover, at higher storage temperatures, a more symmetrically perfect crystalline structure is found. Biliaderis and Zawastowski (1990) studied the effect of temperature on rigidity of 5% (w/w) amylose and 40% waxy maize starch gels. They observations further support the notion that amylase gelation mainly involves rapid formation of double helical junction zones upon cooling of amylose solutions, whereas a network-based structure for amylopectin is established mainly as a result of separation of partially crystalline structure.

1.4) Botanical sources of the starch

The degree of starch retrogradation and the properties of the starch crystallites formed are influenced not only by the storage time and temperature, but also by starch concentration (Jang and Pyun, 1997) and the botanical origin of the starch (Varavinit et al., 2003). The botanical source is one of great importance for the retrogradation of starch gels (Gudmundsson and Eliasson, 1993). They do not concern differences in amylase content, because it has even been observed in starches with very similar amylase content. This indicates that structural differences found in the amylopectin molecule may be the cause of differences in the recrystallization rate. Amylopectin from cereal has also been shown to retrograde to a lesser extent than pea, potato and canna amylopectin which has been attributed to shorter average chain length in the cereal amylopectin (Kailcheusky et al., 1990). The structural differences in cereal amylopectin as related to retrogradation can be related either to differences in the amorphous regions or to differences in the ratio of short to long chains and the ratio of A-chains to B-chains. A greater amount of short chains more than fifteen glucose units and increase ratio of A- chains to B-chains probably promote retrogradation. It has also been reported that very short chain (6-9 glucose units) can inhibit or retard retrogradation of starch gels (Levine and Slade, 1986)

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2.3 Parboiling Process

Parboiling process involves the hydrothermal treatment of paddy before milling. The advantage of the parboiling process stems from the gelatinization of rice starch and hardening of rice kernel that it brings about. As a result, breakage losses during milling of rice can be minimized. Parboiling is a process developed for improving rice quality. It consists of soaking, steaming and drying of the rough rice. (The major reasons for parboiling rice include higher milling yields, higher nutritional value and resistance to spoilage by insects and mold (Bhattacharya, 1985; Elbert et al., 2000; Chukwu and Oseh, 2009). Parboiling results in significant changes in the physico-chemical and cooking characteristics of rice grain. Parboiling fills the void spaces and cements the cracks inside the endosperm, making the grain harder and minimizing internal fissuring and thereby reducing breakage during milling (Corrêaa et al., 2007). The market value of parboiled rice as a product depends largely on its physical qualities after processing. The three steps of parboiling-soaking, steaming and drying – are generally achieved by soaking paddy in cold water for typically 24-48 hours until the kernels are saturated. The soaked paddy is then boiled at 100°C for typically 1 hour to obtain 80% gelatinized starch. Finally the boiled paddy is sun-dried until the moisture content is reduced to 14%. Recently, more sophisticated procedures such as dry-heat parboiling and pressure parboiling have been applied (Bello et al., 2004).

Parboiling with gelatinizing of rice starch and elimination and filing rice seed chaps, results in improved resistance of seeds against exerted tensions during paddy threshing operations. Also, nick percentage is reduced significantly, operation percentage increases and because of leakage and penetration of bran into the rice seed, bran percentage is reduced significantly and crust percentage is reduced slightly to, which justifies operation percentage improvement (Kshirod et al., 1966). Parboiling process consists of 4 steps are soaking, steaming drying and milling.

1. Soaking : The purpose of this stage is to absorb water up to 40% and facilitate cooking and heat transferring during next steps (Ramachandra et al., 2000).

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2. Steaming : The purpose of steaming stage is to complete parboiling operations and gelatinizing rice starch, which is done by two methods of steaming under pressure and steaming under atmosphere pressure (Pillaiyar, 1985).

3. Drying: For this purpose, dryers was used with ambient temperature, during which, paddies were wide spread in a shelter and after three days paddies are dried out and final humidity reaches near 14% (Roy et al., 2006).

4. Milling : The purpose of a rice milling system is to remove the husk and the bran layers, and produce an edible, white rice kernel that is sufficiently milled and free of impurities. Depending on the requirements of the customer, the rice should have a minimum of broken kernels.

2.4 Phenolic compounds

Phenolic compounds are defined as substances possessing a benzene ring bearing one or more hydroxyl substituents, including their functional derivatives (Waterman and Mole, 1994). Phenols have many favorable effects on human health. They reduce the risk of heart diseases by inhibiting the oxidation of low-density lipoprotein (LDL) (Bonilla et al., 1999). A large range of low and high molecular weight phenols exhibiting antioxidant properties have been studied and proposed to be used as antioxidants against lipid oxidation. This is particularly true for those phenolics with multiple hydroxyl groups that are generally the most efficient for preventing lipid oxidation. Phenolic compounds are also known to possess antibacterial, antiviral, anti-mutagenic and anti-carcinogenic properties (Moure et al., 2001). Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. Phenolic compounds in plants may generally and conveniently be divided into three major classes based on their sizes. These are phenolic acids, flavonoids and tannins (Scalbert and Williamson, 2002).

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2.4.1 Phenolic acids

Phenolic compounds are the most active antioxidant derivatives in plants which mostly found in the outer layers of plants, such as the peel, shell and hull, contain large amounts of polyphenolic compounds to protect the inner components (Bors et al., 2001). There are two classes of phenolic acids:hydroxybenzoic acids and hydrocynamic acids (Dykes and Rooney, 2007). A number of the phenolic acids are linked to various cell-wall components, such as arabinoxylans and proteins (Hartley et al., 1990). They are known to be good natural antioxidants and exert various physiological effects in humans, such as preventing oxidative damage of lipid and low-density lipoproteins (Morton et al., 2000), inhibiting platelet aggregation (Daniel et al., 1999) and reducing the risk of coronary heart disease and cancer (Newmark, 1996; Martinez-Valverde et al., 2000). Ferulic acid, *p*-coumaric acid, caffeic acid, vanillic acid and syringic acid are all examples of phenolic acids (Pratt and Hudson, 1990). Structures of some phenolic acids are shown in Figure 2.18.

It is widely accepted that phenolic compounds significantly contribute to the overall antioxidant properties of grain. Phenolic acids have been strong inhibitors of carcinogenesis at the initiation and promotion stages induced by different compounds (Kaul and Khanduja, 1998).

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Figure 2.18 Structures of some phenolic acids

Benzoic acid derivatives

Name of acid	Position of the functional groups		
	R_1	R_2	R_3
Gallic	OH	OH	OH
p- Hydroxybenzoic	H	OH	H
Protocatechuic	OH	OH	H
Vanillic	OCH_3	OH	H
Syringic	OCH_3	OH	OCH_3

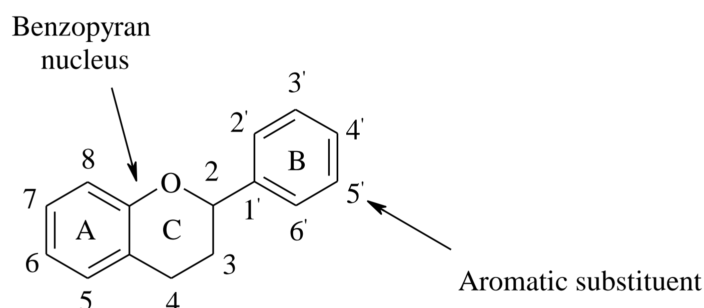
Cinnamic acid derivatives

Name of acid	Position of the functional groups		
	R_1	R_2	R_3
p-Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	OCH_3	OH	H
Sinapic	OCH_3	OH	OCH_3

2.4.2 Flavonoids

Flavonoids are a subset of polyphenol antioxidants, which bear the C_6–C_3–C_6 structure (Madhavi et al., 1996). The C_6-C_3 is from cinnamic acid and the other C_6 fragment is from 3 molecules of malonyl-coenzyme A (Hahn et al., 1984). The general flavonoid structure may be described as consisting of a benzopyran nucleus with an aromatic substituent at carbon number 2 of the C ring (Waterman and Mole, 1994) (Figure 2.19).

Figure 2.19 Basic structures of flavonoids



Flavonoids are commonly found in edible fruit, leaves and other parts of plant foods as either glycosides (esterified to a sugar molecule) or aglycones (not esterified to a sugar molecule). The subgroups are classified based on the substitutional pattern of the C ring and the position of the B ring. The major subgroups include flavonols, flavanones, flavanols (or flavans) and flavones (Figure 2.20). Flavonols, such as quercetin and kaempferol, have a carbonyl at C-4, double bond between C-2 and C-3, and hydroxyl at C-3; flavanones (e.g. taxifolin) have a carbonyl at C-4, no double bond between C-2 and C-3 and no hydroxyl at C-3; flavanols (e.g. catechin) have no carbonyl at C-4, no double bond between C-2 and C-3 and a hydroxyl at C-3 and flavones, such as apigenin and luteolin, have a carbonyl at C-4, a double bond between C-2 and C-3 and no hydroxyl at C-3 (Sugihara et al., 1999). Flavonones give rise to other family members such as anthocyanins by undergo a series of transformation that affects the heterocyclic ring (Cao et al., 1996), which are responsible for the color of fruits, legumes and vegetables (Mazza and Miniatti, 1993).

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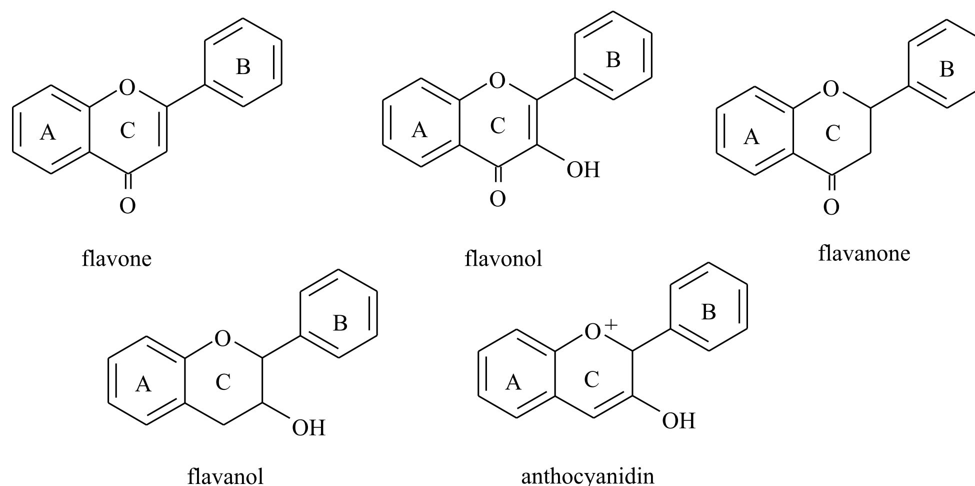
Figure 2.20 Structure of major flavonoid sub-groups



2.4.3 Tannins

Tannins are substances of vegetable origin capable of transforming fresh hide into leather (Hahn et al., 1984). Tannins are rich in phenolic hydroxyl groups. They are divided into two classes, namely hydrolysable tannins and condensed tannins (Waterman and Mole, 1994). Hydrolysable tannins are phenolic carboxylic acids esterified to sugars such as glucose. They are called hydrolysable tannic since they break down into sugars and a phenolic acid (gallic or ellagic acid) upon hydrolysis with acid, alkali or hydrolytic enzymes (tannase) (Hahn et al., 1984). Condensed tannins are polymers of flavan-3-ol units and are also known as proanthocyanins (or proanthocyanidins) because they yield anthocyanins upon heating in acidic media (Santos-Buelga and Scalbert, 2000). The structures of condensed tannins and hydrolysable tannins are shown in Figure 2.21 (Krause et al., 2005).

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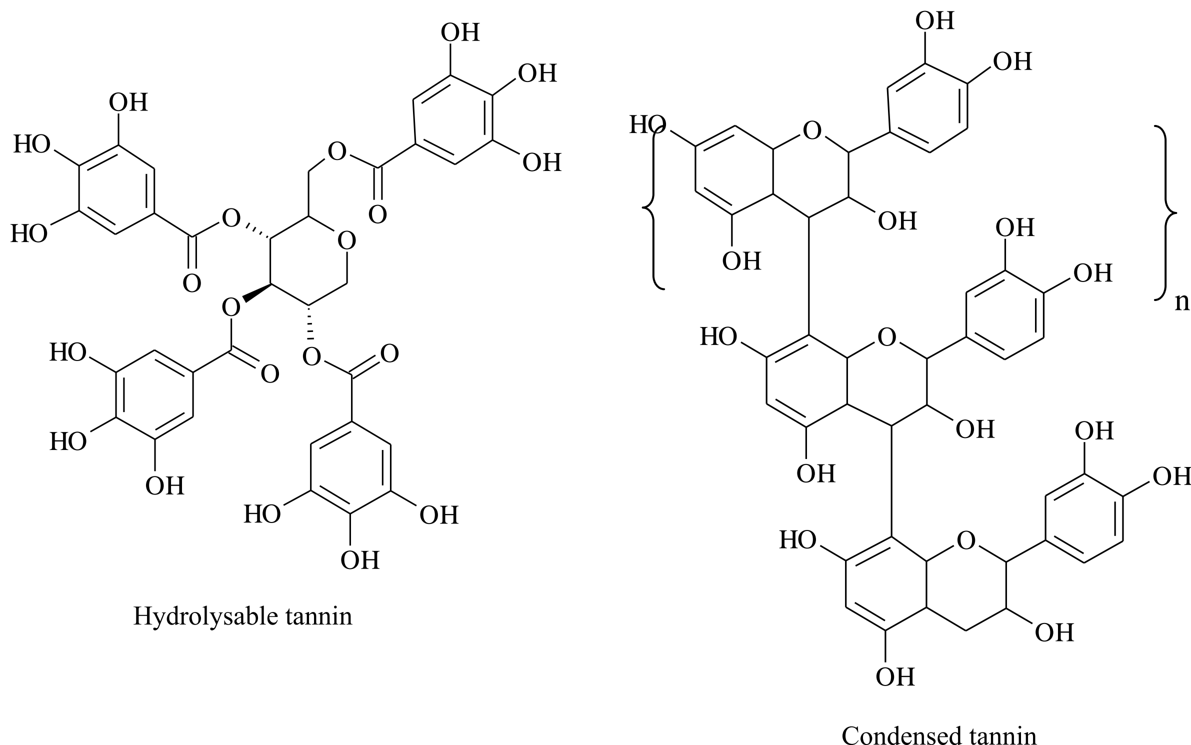
Figure 2.21 Structures of hydrolysable tannins and condensed tannins



2.4.4 Biosynthesis of phenolic compounds in plants

All plants produce an amazing diversity of secondary metabolites. One of the most important groups of these metabolites are phenolic compounds. Phenolic compounds are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups (Michalak, 2006). Phenolics display a wide variety of structures, ranging from simple moieties containing a single hydroxylated aromatic ring to highly complex polymeric substances (Strube et al., 1993; Harborne, 1994). Biosynthesis of phenolic compounds in conifers has very high activity. This is especially true for lignin formation. The original substance for synthesis of phenypropanoids is phenylalanine, one of the end products of the shikimate pathway (SKP). The SKP is defined as seven metabolic steps beginning with the condensation of phosphoenolpyruvate (PEP) and erythrose 4-phosphate (Ery4P) and ending with the synthesis of chorismate. It is the common route leading to the production of three aromatic amino acids: phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp). Higher plants use these amino acids not only as protein building blocks, but also as precursors for a large number of secondary metabolites, among them plant pigments, flavonoids, auxins, phytoalexins, lignin and

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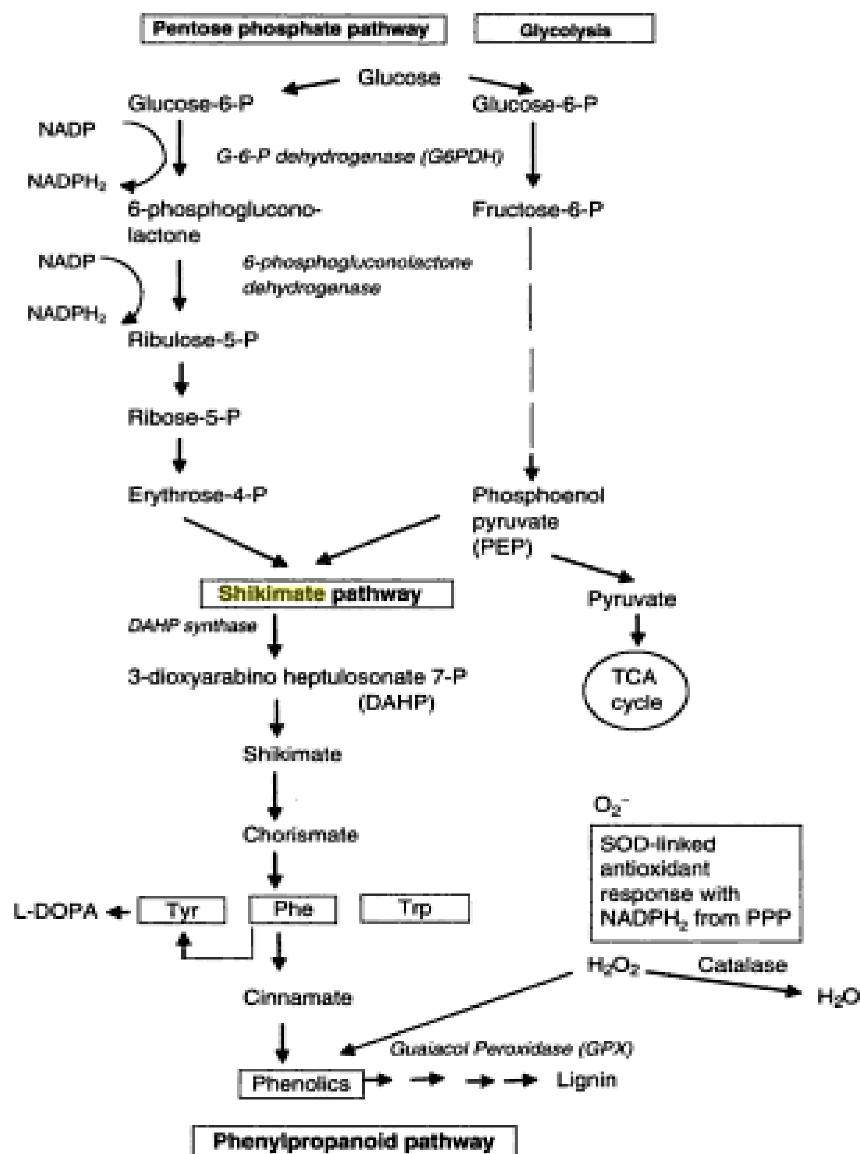
tannins (Herrmann, 1995). The biosynthetic pathways of some flavonols and phenolic acids are shown in Figure 2.22. The biosynthesis and accumulation of secondary compounds can be an endogenously controlled process during developmental differentiation (Macheix et al., 1990) or it can be regulated by exogenous factors such as light, temperature and wounding (Bennet and Wallsgrove, 1994; Dixon and Paiva, 1995). Phenylalanine, produced in plants via the SKP, is a common precursor for most phenolic compounds in higher plants (Macheix et al., 1990; Strack et al., 1997).

Figure 2.22 Biosynthetic pathways of phenolic compounds



2.5 Phenolic compounds as antioxidants

Due to the increased prevalence of chronic degenerative diseases, people are more aware of their food consumption. This is to prevent the occurrence of the diseases that will affect their quality of life and economic status. Many nutritional factors are widely considered to be critical for human health. Among them, free radicals have been of concern as one of the factors contributing to chronic degenerative disease (Bray, 2000). Phenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kähkönen et al., 1999). Recent studies on epidemiological have suggested that increased consumption of whole cereal grains, fruits and vegetables is associated with reduced risks of chronic diseases (Hu, 2002). Cereal grains and their products are one of the most commonly consumed food items and a staple in Thailand diet. Cereal grains are important sources of energy, protein, dietary fiber, minerals, vitamins and phytochemicals such as phenolic acid, phytic acids, lignans and phytoestrogens (Slavin et al., 1999). Phenolic acids, particularly ferulic acid, *p*-coumaric acid and vanillic acids, are predominant in bran layer of grains and are mainly present as a covalently bound form with insoluble polymers. Phenolic acids are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because of their stable radical intermediates, which prevent the oxidation of various food ingredients, particularly fatty acids and oils (Cuvelier et al., 1992; Maillard et al., 1996). Recent studies have reported the antioxidant activities of black rice (Hu et al., 2003), oat (Handelman et al., 1999), sorghum (Awika et al., 2003), millet (Dykes and Rooney, 2006), buckwheat (Holasova et al., 2002) and cereal bran products (Yu et al., 2002). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans et al., 1995). All polyphenols are capable of scavenging singlet oxygen and alkyl radical through electron donating properties, thus generating a relative stable phenoxyl radical (Santos-Buelga and Scalbert, 2000).

Generally the efficacy of phenolic compounds as antioxidants depends on the structure, a number of factors such as the number of hydroxyl groups bonded to the

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aromatic ring, the site of bonding, matual position of hydroxyls in the aromatic ring (Sroka and Cisowski, 2003) and their ability to act as hydrogen or electron donating agents and free radical scavengers. A relationship exists between the efficacy of phenolic compounds as antioxidants and their chemical structure. The configuration and total number of hydroxyl groups substantially influence the mechanism of antioxidant activity (Heim et al., 2002). The phenolic ring with hydroxyl groups are the main structural features required for antioxidant activity. In order for phenolic compounds to act as antioxidants, their hydroxyl groups have to be in free form. This is because the attachment of an external group reduces the antioxidant power of the phenolic compounds as they lack hydrogen atom for donation (Farag et al., 2003).

Phenolic acids are known to be scavengers of oxygen species. The position of the hydroxyl groups in the aromatic ring is important in the efficiency of phenolic acids as antioxidants (Sroka and Cisowski, 2003). For instance, the presence of hydroxyl group in the para position of phenolic acid is important for high antioxidant activity (Pannala et al., 1998; Pannala et al., 2001). Phenolic acids have been shown to be strong inhibitors of carcinogenesis at the initiation and promotion stages induced by different compounds (Kaul and Khanduja, 1998).

The antioxidant activity of phenolic acids depends on the degree of hydroxylation. The derivatives of cinnamic acids are generally more effective than the derivatives of benzoic acid (Marinova and Yanishlieva, 2003). The presence of the CH=CH-COOH group in cinnamic acid derivatives ensures greater efficiency than the COOH group in benzoic acids (Madhavi et al., 1996). The double bond has been reported to participate in stabilizing the phenolxyl radical by resonance (Cuvelier et al, 1992) according to Marinova and Yanishlieva (2003). Hydroxycinnamic acid ester serve as antioxidants *in vitro*, and it has been suggested that they may serve as natural antioxidants for lipids *in vivo* (Daniels and Martin, 1967; Rice-Evans et al., 1997; Daniel et al., 1999). Although more attention has been paid to flavonoids as potential natural antioxidants. Phenolic acid esters, such as chlorogenic acid, also perform well as antioxidant, particularly in protecting lipids from peroxidation (Rice-Evans et al., 1997).

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CHAPTER 3

METHODOLOGY

This research was experimental research to develop parboiled glutinous rice process for improvement of physical, chemical and nutritional qualities, including soaking, steaming, cooling and drying processes. Moreover, this study includes model equations that predict the optimization conditions based on the process factors. Finally, application of parboiled rice bran as functional food prototype was examined. Therefore, experimental design was presented as followes:

3.1 Experimental plan
3.2 Instruments and equipments
3.3 Materials
3.4 Chemicals
3.5 Methods
3.6 Statistical analysis

3.1 Experimental plan

This research is divided into four experiments including (1) determination an appropriate soaking process on chemical and nutritional qualities of parboiled glutinous rice, (2) determination an appropriate steaming process on chemical and nutritional qualities of parboiled rice, (3) determination of physical, chemical and nutritional qualities of parboiled glutinous rice and (4) develop product prototypes from parboiled rice bran oil as functional food.

1. Experiment 1 was to develop soaking process for improvement of chemical and nutritional qualities.

2. Experiment 2 was to develop steaming process for improvement of chemical and nutritional qualities.

3. Experiment 3 was to determine physical, chemical and nutritional qualities of parboiled glutinous rice.

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4. Experiment 4 was to develop product prototypes from parboiled rice bran oil as functional food.

Completely randomized design (CRD) was used for experiment plan in this study. Analysis of variance was used to test any difference in resulting from these methods. Duncan method was used to determine significant differences at $p < 0.05$.

Mathematic model of hexagonal rotatable design was used for experiment plan in this study. Triplicate reactions were carried out at all designed points except at the central point (0,0) where four replications were performed to allow the estimation of the 'pure error'. All experiments were carried out in a randomized order to minimize the effect of unexplained variability in the observed responses due to extraneous factors.

3.2 Instruments and equipments

1. Gas chromatography system with flame ionization detector (GC-2014, Shimadzu)
2. High performance liquid chromatography system with diode array detector (LC 20A, Shimadzu)
3. Gas chromatography-Mass spectrometry
4. Ultraviolet-Visible spectrophotometer (Lambda 12, Perkin Elmer, USA)
5. Rotary evaporator (Buchi)
6. Centrifuge (Rotina 48 R)
7. Column Inetsil ODS-3, C18 (4.6 mm x 250 mm, 5 µm)
8. Column DB-Wax (0.25 mm x 60 m)
9. Sep-pack (C18)
10. Hot air oven (Memmert)
11. Incubator shaker
12. Beaker
13. Erlenmenyer flask
14. Volumetric flask
15. Pipette and Pasteur pipette
16. Vial

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3.3 Materials

Samples

Paddy rice of *Oryza sativa* L., cultivar RD-6 (a popular glutinous rice cultivar for consumption in the north and northeast region of Thailand) were used for analyses in this research.

3.4 Chemicals

1. 2,2-Diphenyl-1-picrylhydrazyl , DPPH (Fluka)
2. Folin-Ciocalteu's reagent (Fluka)
3. Ferrous sulphate (Carlo)
4. Sodium sulphate (Merck)
5. Absolute ethanol (BDH)
6. Standard α-tocopherol (Fluka)
7. Standard phenolic acids(gallic, ferulic, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids (Sigma)
8. γ-Oryzanol (food grade, 99.9% purity) (Wakayama, Japan).
9. Acetic acid (Fisher Scientific)
10. Phosphoric acid (BDH)
11. Methanol (Merck)
12. Acetonitrile (Merck)
13. Sodium hydroxide (Fluka)
14. Sodium azide (Merck)
15. Calcium chloride dehydrate (Merck)
16. Pancreatic α-amylase (Megazyme)
17. Amyloglucosidase (Megazyme)
18. Glucose Determination Reagent (GOPOD) (Megazyme)
19. Resistant Starch (Megazyme)
20. Sodium chloride (Merck)
21. Pyridine (Merck)
22. BSTFA+TCMS (99:1) (Supelco)

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23. Chloroform (Lab-Scan)
24. Diethyl ether (Lab-Scan)
25. Standard fatty acids (Fluka)
26. Ethanol 95% (Imported denatured ethyl alcohol 95%)
27. Hexane (commercial grade, Etalmar)
28. Hexane (HPLC grade, Lab-Scan)
29. Urea (Ajax Finechem)
30. BHT (Sigma-aldrich)
31. Hydrochloric acid (Merck)
32. Sulfuric acid (Merck)

3.5 Methods

The experimental methods in this research study were divided into four experiments as follows:

1. Experiment one: develop soaking process for improvement of chemical and nutritional qualities.

In this study, completely randomized design (CRD) was employed to study the effect of soaking process on glutinous rice. Analysis of variance was used to test any difference in resulting from these methods. Duncan method was used to determine significant differences at $p < 0.05$.

For mathematic model, the hexagonal rotatable design was employed to study the responses, such as the γ-oryzanol (Y variable). The soaking temperature (X_1) and salt content (X_2) were independent variables employed to optimize Y variables.

1.1 Soaking conditions

The soaking process was performed according to the methods of Han and Lim (2009) with some modifications. To study the effect of NaCl content on chemical and nutritional qualities, 500 g of each paddy rice sample was soaked in solutions of 0.0-3.0% (w/v) NaCl with a ratio 1:3 w/v for 6 h at ambient temperature. To study the effect of soaking temperature on bioactive compounds and fatty acids of glutinous rice, paddy rice samples (each 500 g) were soaked in distilled water with a ratio 1:3 w/v at 30-60°C for 6 h. After the paddy rice was soaked, it was immediately

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dried in a 70% relative humidity (RH) cabinet at 50°C for 12-14 h until the moisture content of the soaked rice was less than 15±1% (wet basis). These grains were milled to separate the husks from brown rice. Then the brown rice were ground and passed through a 180 μm sieve screen. Moisture content was determined by the conventional method of drying the samples at 105°C overnight until reaching constant weight. This and all other analyses were performed in triplicate. Analytical results were expressed on a dry matter basis. The samples were stored at -20°C prior to analysis.

1.2 Extraction and determination of phenolic compounds

1.2.1 Determination of total phenolic content

The phenolic compounds present in rice fractions were analyzed by the method of Butsat and Siriamornpun (2010). Briefly, samples were extracted with 80% aqueous methanol (1:10, w/v) at 25°C in a shaking incubator at 150 rpm for 16 h. The mixtures were centrifuged at 2500 rpm for 20 min and the supernatants were collected. The residues were re-extracted under the same conditions, and supernatants from both extractions were combined. The total phenolic content (TPC) of rice extracts was determined using the Folin-Ciocalteu method with some modifications (Kubola et al., 2011; Butsat and Siriamornpun, 2010). Briefly, 200 μl of diluted extract solution was mixed with 800 μl of freshly diluted Folin-Ciocalteu reagent. The mixture was shaken for 1 min, then 2 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the mixture was shaken once again for 30 sec. The final mixture was increased to 5 ml with deionized water and then allowed to stand for 2 h at ambient temperature. The absorbance at 760 nm was measured. Using gallic acid as a standard, TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

1.2.2 Phenolic acids composition by HPLC-DAD

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A Diode-array detection; chromatographic separations were performed on a LUNA C-18 column (4.6 × 250 mm i.d., 5 μm). The elution system was performed using 1% acetic acid in water (A) and acetonitrile (B) as mobile phase. Gradient elution was performed as follows: 0–40 min, from 0 – 70% B with a flow rate 1 ml/min; 40–45 min, from 70 – 80% B with a flow rate 1 ml/min; 45–55 min, from 80–85% B with a flow rate 1.2 ml/min; 55–57 min, from 85– 90% B with a flow rate 1.2 ml/min; 57–75 min 90% B with a flow rate 1.2 ml/min. Operating conditions were as

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follows: column temperature, 40°C, injection volume, 20 µl, UV-Diode Array detection at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds and were detected using an external standard method.

1.3 Extraction and determination of γ-oryzanol and α-tocopherol contents

γ-Oryzanol and α-tocopherol were extracted according to the method of Butsat and Siriamornpun (2010) with some modifications. Each sample (1 g) was extracted with acetone at a ratio of 1:10 w/v, vortexed at maximum speed for 1 min then centrifuged at 2500 rpm for 20 min, after which the solvent was removed. The residue was further extracted twice, and the supernatants were combined before evaporating them to dryness under nitrogen gas. The determinations were made in triplicate.

The contents of γ-oryzanol and α-tocopherol were determined using HPLC. The crude extracts were dissolved in the mobile phase and filtered through a 0.45 µm pore size syringe-driven filter. The RP-HPLC system (Shimadzu) consisted of an auto sampler and column oven equipped with Inertsil ODS (4.6 mm × 250 mm, 5 µm) with mobile phase of acetonitrile/methanol(20:80, v/v), flow rate 1.5 ml/min and photodiode-array detector at 292 nm for the analysis of α-tocopherol and at 325 nm for the analysis of γ-oryzanol. To construct the calibration curves, standard solutions of γ-oryzanol and α-tocopherol, were used.

1.4 Analysis of fatty acid composition

Lipids were extracted according to the method of Bligh and Dyer (1959). Approximately 5 g of well-ground samples was extracted with 50 ml of chloroform-methanol (2:1 v/v) containing10 mg/l of BHT. Then, the samples were stored in a fume hood overnight. Each sample was filtered and transferred into a separate funnel and to this was added 15 ml of physiological saline solution. The samples were shaken well to allow the phases to separate; the lower phase was then evaporated. The fatty acids were esterified into methyl esters using the acid catalyzed methylation method. In detail, 3 ml HCl–methanol reagent and 1 ml toluene reagent were added to the extracted lipid samples of 100 mg. They were then heated at 70°C for

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2 h. Fatty acid methyl esters were extracted in 2 ml hexane and stored at -25°C before chemical analysis. The fatty acid methyl esters were analyzed on a SHIMADZU (GC-2014) gas chromatograph with a flame ionization detector (FID). The esters were separated on a 60 m × 0.25 mm i.d. wall-coated open tubular fused silica capillary column coated with DB-WAX. Column injector and detector temperature were 250 and 270°C, respectively. The carrier gas used was nitrogen operated at 1.27 ml/min. The temperature program was set to 150–180°C at 20°C/min, then from 180°C to 220°C at 2.5°C/min, held at 220°C for 3 min, then from 220°C to 230°C at 10°C/min, held at 230°C for 3 min, and from 230°C to 235°C at 5.0°C/min and held at 235°C for 10 min (Thammapat et al., 2010). Individual methyl esters were identified against the retention time of standard methyl esters (Sigma; St. Louis, USA).

2. Experiment two: develop steaming process for improvement of chemical and nutritional qualities.

In this study, completely randomized design (CRD) was employed to study the effect of steaming process on glutinous rice. Analysis of variance was used to test any difference in resulting from these methods. Duncan method was used to determine significant differences at $p < 0.05$.

For mathematic model, the hexagonal rotatable design was employed to study the responses, such as the γ-oryzanol (Y variable). The steaming temperature (X_1) and steaming time (X_2) were independent variables employed to optimize Y variables.

2.1 Steaming conditions

To study the effect of steaming conditions on chemical and nutritional qualities of glutinous rice, 500 g of paddy rice soaked sample at optimization condition was steamed with steaming temperature of 100-120°C and steaming time of 10-20 min. After the paddy rice was steamed, it was immediately dried in a 70% relative humidity (RH) cabinet at 50°C for 12-14 h until the moisture content of the soaked rice was less than 15±1% (wet basis). These grains were milled to separate the husks from brown rice. Then the brown rice were ground and passed through a 180 µm sieve screen. Moisture content was determined by the conventional method of drying the samples at 105°C overnight until reaching constant weight. This and all other analyses were performed in triplicate. Analytical results were expressed on a dry matter basis. The samples were stored at -20°C prior to analysis.

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2.2 Extraction and determination of phenolic compounds

2.2.1 Determination of total phenolic content

The phenolic compounds present in rice fractions were analyzed by the method of Butsat and Siriamornpun (2010). Briefly, samples were extracted with 80% aqueous methanol (1:10, w/v) at 25°C in a shaking incubator at 150 rpm for 16 h. The mixtures were centrifuged at 2500 rpm for 20 min and the supernatants were collected. The residues were re-extracted under the same conditions, and supernatants from both extractions were combined. The total phenolic content (TPC) of rice extracts was determined using the Folin-Ciocalteu method with some modifications (Kubola et al., 2011; Butsat and Siriamornpun, 2010). Briefly, 200 µl of diluted extract solution was mixed with 800 µl of freshly diluted Folin-Ciocalteu reagent. The mixture was shaken for 1 min, then 2 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the mixture was shaken once again for 30 sec. The final mixture was increased to 5 ml with deionized water and then allowed to stand for 2 h at ambient temperature. The absorbance at 760 nm was measured. Using gallic acid as a standard, TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

2.2.2 Phenolic acids composition by HPLC-DAD

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A Diode-array detection; chromatographic separations were performed on a LUNA C-18 column (4.6 × 250 mm i.d., 5 µm). The elution system was performed using 1% acetic acid in water (A) and acetonitrile (B) as mobile phase. Gradient elution was performed as follows: 0–40 min, from 0 – 70% B with a flow rate 1 ml/min; 40–45 min, from 70 – 80% B with a flow rate 1 ml/min; 45–55 min, from 80–85% B with a flow rate 1.2 ml/min; 55–57 min, from 85– 90% B with a flow rate 1.2 ml/min; 57–75 min 90% B with a flow rate 1.2 ml/min. Operating conditions were as follows: column temperature, 40°C, injection volume, 20 µl, UV-Diode Array detection at 280 nm (hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds and were detected using an external standard method.

2.3 Extraction and determination of γ-oryzanol and α-tocopherol contents

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γ-Oryzanol and α-tocopherol were extracted according to the method of Butsat and Siriamornpun (2010) with some modifications. Each sample (1 g) was extracted with acetone at a ratio of 1:10 w/v, vortexed at maximum speed for 1 min then centrifuged at 2500 rpm for 20 min, after which the solvent was removed. The residue was further extracted twice, and the supernatants were combined before evaporating them to dryness under nitrogen gas. The determinations were made in triplicate.

The contents of γ-oryzanol and α-tocopherol were determined using HPLC. The crude extracts were dissolved in the mobile phase and filtered through a 0.45 μm pore size syringe-driven filter. The RP-HPLC system (Shimadzu) consisted of an auto sampler and column oven equipped with Inertsil ODS (4.6 mm × 250 mm, 5 μm) with mobile phase of acetonitrile/methanol(20:80, v/v), flow rate 1.5 ml/min and photodiode-array detector at 292 nm for the analysis of α-tocopherol and at 325 nm for the analysis of γ-oryzanol. To construct the calibration curves, standard solutions of γ-oryzanol and α-tocopherol, were used.

2.4 Analysis of fatty acid composition

Lipids were extracted according to the method of Bligh and Dyer (1959). Approximately 5 g of well-ground samples was extracted with 50 ml of chloroform–methanol (2:1 v/v) containing10 mg/l of BHT. Then, the samples were stored in a fume hood overnight. Each sample was filtered and transferred into a separate funnel and to this was added 15 ml of physiological saline solution. The samples were shaken well to allow the phases to separate; the lower phase was then evaporated. The fatty acids were esterified into methyl esters using the acid catalyzed methylation method. In detail, 3 ml HCl–methanol reagent and 1 ml toluene reagent were added to the extracted lipid samples of 100 mg. They were then heated at 70°C for 2 h. Fatty acid methyl esters were extracted in 2 ml hexane and stored at -25°C before chemical analysis. The fatty acid methyl esters were analyzed on a SHIMADZU (GC-2014) gas chromatograph with a flame ionization detector (FID). The esters were separated on a 60 m × 0.25 mm i.d. wall-coated open tubular fused silica capillary column coated with DB-WAX. Column injector and detector temperature were 250 and 270°C, respectively. The carrier gas used was nitrogen operated at 1.27 ml/min. The temperature program was set to 150–180°C at 20°C/min, then from 180°C to 220°C at

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2.5^{o}C/min, held at 220°C for 3 min, then from 220°C to 230°C at 10°C/min, held at 230°C for 3 min, and from 230°C to 235°C at 5.0^{o}C/min and held at 235°C for 10 min (Thammapat et al., 2010). Individual methyl esters were identified against the retention time of standard methyl esters (Sigma; St. Louis, USA).

3. Experiment three: determine physical, chemical and nutritional qualities of parboiled glutinous rice.

3.1 Extraction and determination of phenolic compounds

3.1.1 Determination of total phenolic content

The phenolic compounds present in rice fractions were analyzed by the method of Butsat and Siriamornpun (2010). Briefly, samples were extracted with 80% aqueous methanol (1:10, w/v) at 25°C in a shaking incubator at 150 rpm for 16 h. The mixtures were centrifuged at 2500 rpm for 20 min and the supernatants were collected. The residues were re-extracted under the same conditions, and supernatants from both extractions were combined. The total phenolic content (TPC) of rice extracts was determined using the Folin-Ciocalteu method with some modifications (Kubola et al., 2011; Butsat and Siriamornpun, 2010). Briefly, 200 µl of diluted extract solution was mixed with 800 µl of freshly diluted Folin-Ciocalteu reagent. The mixture was shaken for 1 min, then 2 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the mixture was shaken once again for 30 sec. The final mixture was increased to 5 ml with deionized water and then allowed to stand for 2 h at ambient temperature. The absorbance at 760 nm was measured. Using gallic acid as a standard, TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

3.1.2 Phenolic acids composition by HPLC-DAD

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(hydroxybenzoic acids) and 320 nm (hydroxycinnamic acids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds and were detected using an external standard method.

3.2 Extraction and determination of γ-oryzanol and α-tocopherol contents

γ-Oryzanol and α-tocopherol were extracted according to the method of Butsat and Siriamornpun (2010) with some modifications. Each sample (1 g) was extracted with acetone at a ratio of 1:10 w/v, vortexed at maximum speed for 1 min then centrifuged at 2500 rpm for 20 min, after which the solvent was removed. The residue was further extracted twice, and the supernatants were combined before evaporating them to dryness under nitrogen gas. The determinations were made in triplicate.

The contents of γ-oryzanol and α-tocopherol were determined using HPLC. The crude extracts were dissolved in the mobile phase and filtered through a 0.45 μm pore size syringe-driven filter. The RP-HPLC system (Shimadzu) consisted of an auto sampler and column oven equipped with Inertsil ODS (4.6 mm × 250 mm, 5 μm) with mobile phase of acetonitrile/methanol(20:80, v/v), flow rate 1.5 ml/min and photodiode-array detector at 292 nm for the analysis of α-tocopherol and at 325 nm for the analysis of γ-oryzanol. To construct the calibration curves, standard solutions of γ-oryzanol and α-tocopherol, were used.

3.3 Analysis of fatty acid composition

Lipids were extracted according to the method of Bligh and Dyer (1959). Approximately 5 g of well-ground samples was extracted with 50 ml of chloroform–methanol (2:1 v/v) containing10 mg/l of BHT. Then, the samples were stored in a fume hood overnight. Each sample was filtered and transferred into a separate funnel and to this was added 15 ml of physiological saline solution. The samples were shaken well to allow the phases to separate; the lower phase was then evaporated. The fatty acids were esterified into methyl esters using the acid catalyzed methylation method. In detail, 3 ml HCl–methanol reagent and 1 ml toluene reagent were added to the extracted lipid samples of 100 mg. They were then heated at 70°C for 2 h. Fatty acid methyl esters were extracted in 2 ml hexane and stored at -25°C before chemical analysis. The fatty acid methyl esters were analyzed on a SHIMADZU (GC-2014) gas chromatograph with a flame ionization detector (FID). The esters were separated on a 60 m × 0.25 mm i.d. wall-coated open tubular fused silica capillary

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column coated with DB-WAX. Column injector and detector temperature were 250 and 270°C, respectively. The carrier gas used was nitrogen operated at 1.27 ml/min. The temperature program was set to 150–180°C at 20°C/min, then from 180°C to 220°C at 2.5^{o}C/min, held at 220°C for 3 min, then from 220°C to 230°C at 10°C/min, held at 230°C for 3 min, and from 230°C to 235°C at 5.0^{o}C/min and held at 235°C for 10 min (Thammapat et al., 2010). Individual methyl esters were identified against the retention time of standard methyl esters (Sigma; St. Louis, USA).

. 4. Experiment four: develop product prototypes from parboiled rice bran oil as functional food.

4.1 Sample preparation

The extraction of the oil was carried out according to recommended procedures for commercial oil (Sunarya et al., 1996; Bimbo, 1998). The parboiled rice bran oil was stored under nitrogen at -25°C in amber glass container until used.

4.2 Preparation of free fatty acids from parboiled rice bran oil

The preparation of free fatty acids from parboiled rice bran oil took place according to the following procedure. A quantity of parboiled rice bran oil of 175 g was treated with 200 ppm butylated hydroxytoluene (BHT) before saponification with a mixture of 40.25 g KOH, 77 ml distillated water and 462 ml of 95% (v/v) aqueous ethanol. The saponification was operated at 62±2°C for 1 h under nitrogen. Distilled water of 350 ml was added to the saponified mixture and the unsaponifiable mixture was extracted into hexane (2×200 ml) and discarded. The aqueous layer containing the saponified matter was acidified to pH=1.0 with 3 N HCl. The mixture was transferred to a separating funnel and the liberated fatty acids were extracted into 350 ml hexane. The hexane layer containing free fatty acids was then dried over anhydrous sodium sulfate, and the solvent was removed in a rotator evaporator at 40°C under vacuum to recover free fatty acids which were then stored under nitrogen at -25°C in dark amber glass containers until used in the urea complexation.

4.3 Preparation of polyunsaturated fatty acid concentrates from parboiled rice bran oil by urea complexation

The separation of polyunsaturated fatty acid from the hydrolyzed fatty acid mixture of parboiled rice bran oil was carried out by urea-fatty acid adduct formation according to the following procedure. Free fatty acids of 300 g were mixed

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with 20% (w/v) urea in 95% aqueous ethanol and heated at 60-70°C, with stirring, until the whole mixture turned into a clear homogeneous solution. The urea-to-fatty acid ratio was changed by using different amounts of urea (1:1, 2:1 and 3:1 w/w). Initially, the urea-fatty acid adduct was allowed to crystallize at -10°C - 10°C for 8 h. The crystals formed were separated from the liquid by filtration under suction using a buchner funnel lined with a No.1 Watchman filter paper. The filtrate was diluted with an equal volume of water and was acidified to pH4-5 with 6 N HCl; an equal volume of hexane was subsequently added. The mixture was stirred thoroughly for 1 h and then transferred to a separating funnel. The hexane layer, containing liberated fatty acids, was separated from the aqueous phase. The hexane phase was washed out with distilled water (2×150 ml) to remove any remaining urea and then dried over anhydrous sodium sulphate, and the solvent was removed in a rotator evaporator at 40°C under vacuum. The percentage recovery was calculated.

4.4 Gas chromatography (GC) analysis

Free fatty acids were transformed into the corresponding methyl esters. In detail, 3 ml of HCl-methanol reagent and 1 ml of toluene reagent were added to the 100 mg of extracted lipid samples and they were then heated at 70°C for 2 h. Fatty acid methyl esters were extracted in 2 ml of hexane, and stored at -25oC before chemical analysis. The fatty acid methyl esters were analyzed by a SHIMADZU (GC-2014) gas chromatography with a flame ionization detector (FID). The esters were separated on a 30 m × 0.25 mm i.d. wall-coated open tubular fused silica capillary column coated with DB-WAX. Column injector and detector temperatures were 250 and 270°C, respectively. The carrier gas was nitrogen flowing at 1.27 ml/min. The temperature program was 150-180°C at 20°C/min, then from 180°C to 220°C at 2.5^{o}C/min, held at 220°C for 3 min, then from 220°C to 230oC at 10°C/min, held at 230°C for 3 min and from 230°C to 235°C at 5.0^{o}C/min, held at 235°C for 10 min (Thammapat et al., 2010). Individual methyl esters were identified against the retention time of standard methyl esters

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3.6 Statistical analysis

1. Resulting data were expressed as means and standard deviation of three replicates.

2. Analysis results were presented in tables, bar and line graphs.

3. The F-test was used to test hypothesis of data results of dependence variables.

4. Analysis of variance (ANOVA) and least significant difference tests were conducted to identify differences among means using one-way analysis of variance and comparison of paired samples mean was conducted according to Duncan's multiple range test method. The p values of < 0.05 were regarded as significant.

5. For mathematic model, a quadratic polynomial regression model was assumed for predicting individual Y variables. The model proposed for each response of Y is:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum\sum \beta_{ij} X_i X_j$$

In this model, β_0, β_i, β_{ii} and β_{ij} were intercept, linear, quadratic and interaction regression coefficient terms, respectively, and X_i and X_j were independent variables. The Design-Expert was used for multiple regression analysis, analysis of variance (ANOVA) and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. Response surfaces were developed using the fitted quadratic polynomial equations obtained from RSREG analysis by holding the independent variables with the least effect on the response at a constant value and by changing the levels of the other variables.

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CHAPTER 4

RESULTS AND DISCUSSION

The results of data analysis and describing were sequentially expressed as follows:

4.1 Symbols used for data resulting expression

4.2 Sequence of resulting expression

4.3 Results and discussions

4.1 Symbols used for resulting data expression

In this study, expression of data analysis results was conducted as various symbols.

SD = Standard deviation

$\bar{X}$ = Means

df = Degrees of freedom

Y = Response value

4.2 Sequence of resulting expression

The results were sequentially expressed as follows:

1. Experiment 1 was to develop soaking process for improvement of chemical and nutritional qualities

1.1 Total phenolic content of glutinous rice soaked at different soaking conditions.

1.2 Phenolic acids composition of glutinous rice soaked at different soaking conditions.

1.3 The α-tocopherol content of glutinous rice soaked at different soaking conditions.

1.4 The γ-oryzanol content of glutinous rice soaked at different soaking conditions.

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1.5 Fatty acid composition of contents of glutinous rice soaked at different soaking conditions.

1.6 Summary of experiment 1

2. Experiment 2 was to develop steaming process for improvement of chemical and nutritional qualities.

2.1 Total phenolic content of glutinous rice steamed at different steaming conditions.

2.2 Phenolic acids composition of glutinous rice steamed at different steaming conditions.

2.3 The α-tocopherol content of glutinous rice steamed at different steaming conditions.

2.4 Fatty acid composition of contents of glutinous rice steamed at different steaming conditions.

2.5 The γ-oryzanol content of glutinous rice steamed at different steaming conditions.

2.6 Summary of experiment 2

3. Experiment 3 was to determine physical, chemical and nutritional qualities of parboiled glutinous rice.

3.1 Total phenolic content and phenolic acids composition of parboiled glutinous rice.

3.2 The α-tocopherol, γ-oryzanol and GI of parboiled glutinous rice.

3.3 Fatty acid composition of parboiled glutinous rice..

3.4 Summary of experiment 3

4. Experiment 4 was to develop product prototypes from parboiled rice bran oil as functional food.

4.1 Optimization of process conditions to maximize contents of the total polyunsaturated fatty acids of parboiled glutinous rice oil concentrate.

4.2 Summary of experiment 4

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4.3 Results and discussion of experiment 1

1. Total phenolic content of glutinous rice soaked at different soaking conditions.

In general, after soaking, TPC increased (Figure 4.1). After soaking with different NaCl content and soaking temperature, TPC of soaked rice was significantly ($p < 0.05$) higher than that of the control (unsoaked). TPC of the glutinous rice showed an increasing trend as NaCl content and soaking temperature increased. Of all the soaking treatments, the highest TPC content was for the rice soaked in 3.0% NaCl and at 60°C. These data suggest the possibility of increasing TPC content as a result of modifying the soaking process. The increase in extractable phenolic compounds in soaked rice could be due to dismantling the cell wall during germination (Tian et al., 2004). These results were in agreement with previously published data on Thai rice (*Oryza sativa* L.) cv. Kum Doi Saket (Umnajkitikorn et al., 2013). Other studies have reported that Koshihikari brown rice increased in extractable total phenolics as a result of soaking and germination (Tian et al., 2004). Cereal grains contain phenolic acids and glycosides, in both soluble and insoluble forms, which are bound with polysaccharides at the cell wall. Most of the phenolic compounds are in an insoluble form (Miller et al., 2000). Hydrolysis of insoluble phenolics during soaking and germination facilitate an increase in the TPC (Adom and Liu, 2002). Besides ionic and osmotic effects, which are the primary stresses of salinity, high salt concentrations additionally induce secondary stresses such as oxidative stress due to the excessive generation of scavenging Reactive Oxygen Species (ROS). To prevent oxidative damage, plants utilize both enzymatic and non-enzymatic mechanisms for ROS scavenging (Ashraf, 2009). This study showed that the TPC of glutinous rice significantly increased after being soaked in various NaCl solutions for 6 hours as compared to that of the control (unsoaked) (Figure 4.1A). This suggests that salinity stress activates the antioxidative systems after the seeds had absorbed the NaCl solutions. Glutinous rice seeds exposed to 1.5 and 3.0% NaCl showed higher TPC than that of the control (unsoaked). This indicates that salinity stress increases the TPC in soaked rice seeds. Similar results were found in pigmented rice (Kum Doi Saket), where TPC increased after soaking in 25, 75, 150, and 300 mM NaCl for 12 hours (Umnajkitikorn et al., 2013). However, using NaCl in the production

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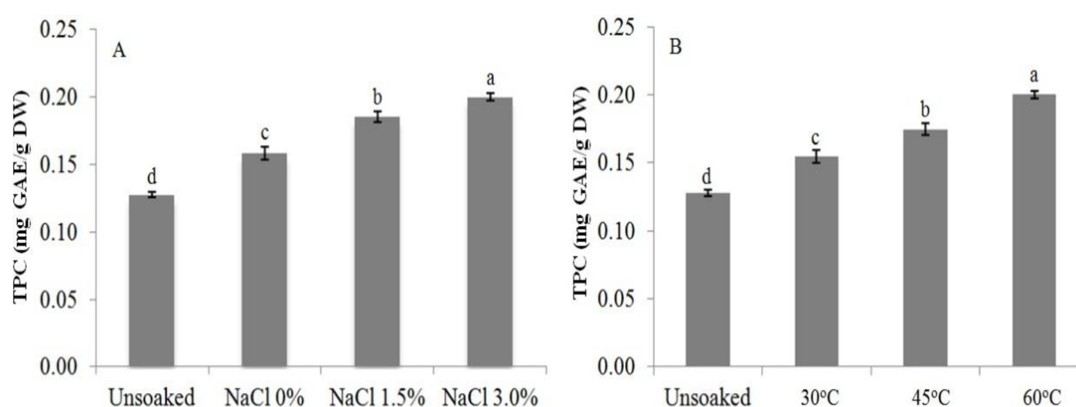
of food has been controversial since an increased sodium intake is associated with hypertension. Therefore alternatives to the use of NaCl are desired. In our present study we also found that increasing the content of NaCl above 3% may cause unacceptable salty taste in the rice product.

Figure 4.1 Total phenolic content of glutinous rice at different salinity and soaking temperatures. (A) Effect of salinity on total phenolic content and (B) Effect of soaking temperature on total phenolic content. Different letters above the bars indicate significant differences ($P < 0.05$).



2. Phenolic acids composition of glutinous rice soaked at different soaking conditions.

Phenolic compounds are mostly found in the outer layers of plants, such as the peel, shell and hull. They contain large amounts of polyphenolic compounds to protect the inner components (Bors et al., 2001). There are two classes of phenolic acids: hydroxybenzoic acids and hydroxycinnamic acids. A number of phenolic acids are linked to various cell-wall components, such as arabinoxylans and proteins (Hartley et al., 1990). They are known to be good natural antioxidants and exert various physiological effects in humans, such as preventing oxidative damage of lipid and low-density lipoproteins and reducing the risk of coronary heart disease (Morton et al.,

2000). RP-HPLC analysis was used to identify the phenolic compounds of glutinous rice extracts, by comparison with standard compounds. In the rice samples analyzed, it was possible to identify 4 hydroxybenzoic acid (HBA): gallic acid, protocatechuic acid, *p*-hydroxybenzoicacid, vanilic acid and 6 hydroxycinnamic acid (HCA): chorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid. The distribution of phenolic acids in all samples is presented in Table 4.1. The most abundant phenolic acids found in all samples were protocatechuic, gallic and ferulic acids. Comparing the phenolic acids of all samples, the content of total phenolic acids (TPA) in soaked rice (55.15-63.61 μg/g) was found to be significantly higher than that in the control (45.61μg/g). Results showed that a similar trend with TPC. The TPA of the glutinous rice showed an increasing trend as NaCl content and soaking temperature increased. The results showed that the rice soaked at 3.0% NaCl had the highest TPA (63.61 μg/g) among soaking treatments. Our findings have shown that the concentration of NaCl and the levels of soaking temperatures affected the TPA of HCA and HBA. The results indicated that the rice soaked with NaCl and high temperature had the greatest contents. In all samples studied, the total content of the HBA group was higher than the total content of the HCA group. The results for the HCA group ranged from 13.64 (unsoaked) to 16.90 μg/g in soaked rice, while the HBA group ranged from 31.97 (unsoaked) to 50.32 μg/g in soaked rice. Phenolic compounds are not uniformly distributed in plants at the tissue, cellular and subcellular levels. Insoluble phenolics are the components of cell walls, particularly hydroxycinnamates; they are ester linked to insoluble fiber, polysaccharides, and lignin components, while soluble phenolics are compartmentalized within the plant cell vacuoles (Faulds and Williamson, 1999). The increase in phenolic acids in soaked rice could be explained as an increase in the free forms with hydrolysis, due to dismantling of the cell wall during soaking from salinity stress. In glutinous rice soaked at 1.5-3.0% NaCl, no *p*-hydroxybenzoic acid was detected. Greater amounts of protocatechuic acid, *p*-coumaric acid and ferulic acid were found in soaked rice with NaCl and high temperature compared to control, except for gallic acids and caffeic acid (4.14-5.22 and 1.32-1.84 μg/g, respectively), which were found in lower amounts than the control. In case of caffeic acid soaking reduce the level by approximately 50%. These results are in agreement with previously published data on the stability of plant phenolics (Friedman and Jürgens, 2000; Chang et al., 2006).

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These studies consistently showed that the phenolic compounds, such as chlorogenic acid, caffeic acid and gallic acid, are temperature and pH sensitive.

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Table 4.1 Phenolic acids (μg/g) of glutinous rice at different salinities and soaking temperatures

Treatment	Hydrobenzoic acids				Hydrocinnamic acids						Total
	GA	PCCA	*p*-OH	VA	ChA	CFA	SyA	*p*-CA	FA	SNA	
Control (Unsoaked)	5.72±0.09^{a}	24.34±0.18^{e}	0.87±0.04^{c}	1.04±0.02	1.52±0.06	3.32±0.08^{a}	1.69±0.04^{a}	1.40±0.10^{d}	4.41±0.04^{e}	1.30±0.02	45.61±0.36^{d}
NaCl content (%w/v)											
0.0	5.09±0.06^{bc}	32.20±0.23^{d}	0.85±0.08^{c}	1.04±0.03	1.51±0.07	1.79±0.04^{bc}	1.65±0.03^{a}	2.79±0.17^{b}	6.91±0.08^{b}	1.31±0.02	55.15±0.20^{c}
1.5	5.09±0.08^{bc}	37.58±0.41^{b}	ND	1.04±0.02	1.54±0.04	1.71±0.06^{c}	1.46±0.04^{b}	2.00±0.14^{c}	6.08±0.07^{c}	1.32±0.03	57.82±0.57^{b}
3.0	5.08±0.09^{bc}	44.19±0.18^{a}	ND	1.05±0.02	1.51±0.08	1.32±0.06^{d}	1.45±0.04^{b}	2.12±0.04^{c}	5.57±0.10^{d}	1.32±0.03	63.61±0.23^{a}
Soaking temperature (^{o}C)											
30	5.22±0.08^{b}	32.15±0.88^{d}	0.88±0.07^{c}	1.05±0.02	1.50±0.08	1.84±0.05^{b}	1.68±0.02^{a}	2.86±0.12^{b}	6.92±0.10^{b}	1.32±0.03	55.43±1.20^{c}
45	5.00±0.14^{c}	33.29±0.22^{c}	1.30±0.09^{b}	1.04±0.03	1.54±0.05	1.80±0.05^{bc}	1.68±0.04^{a}	3.12±0.10^{a}	7.15±0.12^{a}	1.33±0.05	57.26±0.49^{b}
60	4.14±0.15^{d}	33.83±0.19^{c}	1.86±0.07^{a}	1.06±0.05	1.58±0.12	1.83±0.06^{b}	1.66±0.07^{a}	3.17±0.14^{a}	7.31±0.14^{a}	1.35±0.06	57.79±0.76^{b}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each column were significantly different ($p < 0.05$).

GA - gallic acid; PCCA- protocatechuic acid; *p*-OH - *p*-hydroxybenzoic acid; VA - vanilic acid; ChA - chorogenic acid; CFA-caffeic acid; SyA - syringic acid; *p*-CA - *p*-coumaric acid; FA - ferulic acid and SNA - sinapic acid.

ND - not detected

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3. The α-tocopherol content of glutinous rice soaked at different soaking conditions.

The concentration of α-tocopherol was also affected by the NaCl and soaking temperature (Figure 3.2A,B). The α-tocopherol of the glutinous rice showed a decreasing trend as NaCl content and soaking temperature increased. The results showed that the extract of the control (unsoaked) had the highest α-tocopherol content (18.30 mg/100g) among soaking treatment. The salinity of soaking at 3.0% NaCl and soaking temperature of 60°C induced the level of α-tocopherol by 50% lower (8.23 and 8.10 mg/100g, respectively) than that in the control (18.3 mg/100g). These data suggest the possibility that there is a loss of α-tocopherol during the soaking process. Salinity is a harsh environmental factor that has the major effect on plant quantity and quality (Zhu, 2002). In order to survive in salt-stress conditions, plants develop the network responses of physiological and biochemical defense mechanisms to protect themselves against stress. In addition, the high level of Na^{+} also causes secondary responses in plants; consequently oxidative stress occurs leading to cellular damage in the plant cells. To prevent the potential cytotoxic effects of ROS, plant used α-tocopherol to protecting from oxidative stress, which regularly maintain ROS balances within the cell (Mandhania et al., 2006).

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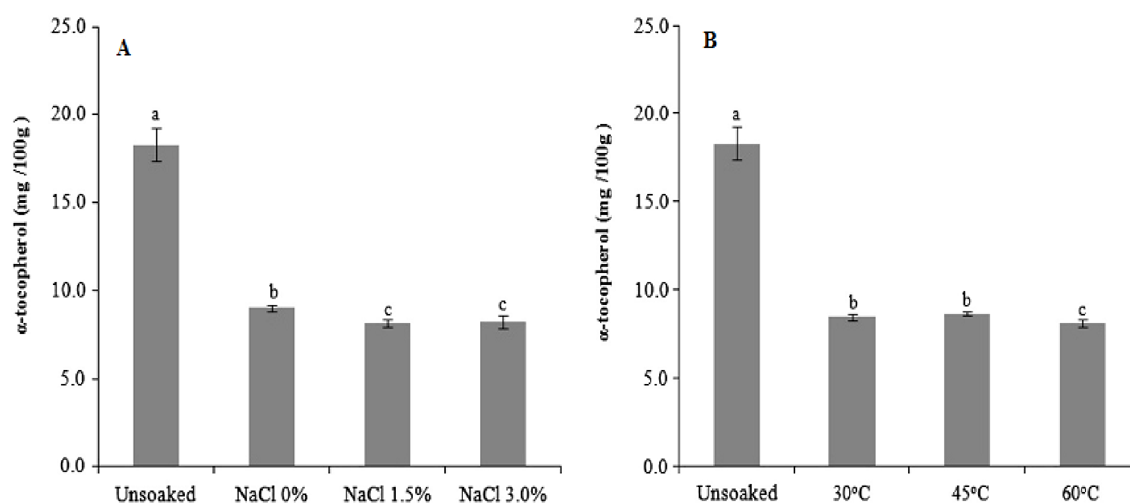
Figure 4.2 The α-tocopherol content (mg/100g) of glutinous rice at different salinity and soaking temperatures. (A) Effect of salinity on α-tocopherol content and (B) Effect of temperature on α-tocopherol content. Different letters above the bars indicate significant differences ($P < 0.05$).



4. Fatty acid composition of contents of glutinous rice soaked at different soaking conditions.

The percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in soaked and unsoaked glutinous rice are as shown in Table 4.2 and 4.3. MUFA was the predominant fatty acid in soaked and unsoaked glutinous rice, followed by PUFA and SFA. These results were in agreement with previous published data on soaking and germination of red, black and bario rice from India (Megatrusydi et al., 2011). The variation of salinity also induced many changes in the fatty acids of glutinous rice as shown in Table 4.2. After soaking with different NaCl content, PUFA significantly ($p < 0.05$) decreased, while the level of SFA and MUFA in soaking samples increased. Megatrusydi et al. (2011) have reported that more SFA and MUFA could be extracted from legumes and rice after soaking and germination. The long-chain fatty acid profile of glutinous rice after soaking showed an intriguing shift. While the relative percentage of linolenic acid (C18:3), dihomo-γ-linolenic acid (C20:3) and arachidonic acid (C20:4) decreased in all samples, a noticeable increase of palmitic

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acid and palmitoleic acid was observed alongside a decrease in linoleic acid and linolenic acid content after soaking with NaCl. All treatments studied had appreciable amounts of fatty acid content, which indicates that soaking with a high content of NaCl, favors the extraction of SFA and MUFA in this glutinous rice. During the soaking process, the fatty acid composition of newly differentiated tissues was significantly different from that of the original seeds. The authors considered that these results were probably due to the desaturation of fatty acids that took place during the β-oxidation process (Dutton and Mounts, 1966).

The fatty acid composition of the glutinous rice showed some variations when the temperature changed (Table 4.3). The amounts of linoleic acid (18:2) and linolenic acid (18:3) decreased respectively from 35.48 to 31.75% and 1.96 to 1.77% of the total fatty acids when the temperature increased from 30°C to 60°C. In addition, the traced SFA, such as myristic acid, decreased slightly when the soaking temperature increased from 30°C to 60°C. However, dihomo-γ-linolenic acid (C20:3) and arachidonic acid (C20:4) showed decreased contents while palmitic acid (C16:0) and palmitoleic acid (C16:1) showed increased contents with the temperature increasing from 30°C to 60°C. The change of temperature also affected PUFA in the rice. After soaking with different soaking temperatures, PUFA significantly ($p < 0.05$) decreased, while the level of SFA and MUFA in soaking samples increased. Other studies have reported that soaking and germination increased the level of SFA and MUFA in legumes and rice. (Megatrusydi et al, 2011). Mostafa et al. (1987) also reported that palmitic and linoleic fatty acids were highly affected by soaking and germination. It is noteworthy to mention a possible stimulation effect of these NaCl concentrations on the eicosanoyl desaturase activity (Zhu et al, 2007). Besides, it is worth highlighting the relatively opposite directions in the percentages of oleic and linoleic acids at all NaCl levels in comparison to the control. This fact could be explained by a possible reduction of the desaturase activity which appeared as an adaptive feature to salinity, since some plants could be protected against the oxidative effects of salt ions through restructuring membranes with less polyunsaturated fatty acids. Moreover, this low unsaturation degree limited the membrane fluidity and restricted permeability to Na^{+} and Cl^{-} ions (Azachi et al., 2002).

Polyunsaturated fatty acids, such as linoleic and linolenic, are called essential fatty acids (EFAs); they are fatty acids that humans and other animals must ingest because

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the body requires them for good health but cannot synthesize them. Although humans and other mammals can synthesize saturated fatty acids and some monounsaturated fatty acids from carbon groups in carbohydrates and proteins, they lack the enzymes necessary to insert a cis double bond at the ω-6 or the ω-3 position of a fatty acid (FAO, 2010). Only two EFAs are known in humans: α-linolenic acid as an ω-3 fatty acid, and linoleic acid as an ω-6 fatty acid. Linolenic acid is a known enhancer of the transport of bioactive compounds into the skin, and is converted to arachidonic acid which serves as a precursor for powerful hormone-like compounds (Benatti et al., 2004). The results of the present study show that most fatty acids in soaked and unsoaked glutinous rice are unsaturated, with linoleic acid being the major fatty acid species. These results indicate that glutinous rice is a good source of EFAs for healthy dietary supplements.

Table 4.2 Fatty acid composition (% total fatty acids) of glutinous rice soaked at different salinities

Fatty acids (%)	NaCl content (%)			
	Unsoaked	0.0	1.5	3.0
C14:0	0.21±0.02	0.22±0.02	0.24±0.03	0.24±0.02
C16:0	0.33±0.03	0.33±0.04	0.35±0.03	0.35±0.04
C18:0	0.06±0.02	0.07±0.03	0.07±0.03	0.08±0.06
Total SFA	$0.60±0.03^{c}$	$0.62±0.03^{bc}$	$0.65±0.01^{ab}$	$0.66±0.02^{a}$
C16:1 n-9	$25.21±0.18^{c}$	$25.50±0.17^{c}$	$26.61±0.49^{b}$	$27.22±0.28^{a}$
C18:1 n-9	32.67±0.50	32.88±0.47	32.81±0.33	32.90±0.32
Total MUFA	$57.88±0.32^{b}$	$58.38±0.64^{b}$	$59.42±0.16^{a}$	$60.12±0.60^{a}$
C18:2 n-6	$32.31±0.36^{a}$	$35.87±0.11^{a}$	$35.78±0.14^{a}$	$34.75±0.79^{b}$
C18:3 n-3	$2.11±0.21^{a}$	$1.94±0.08^{ab}$	$1.64±0.15^{bc}$	$1.57±0.20^{c}$
C20:3 n-6	$0.58±0.18^{a}$	$0.46±0.11^{ab}$	$0.38±0.07^{ab}$	$0.28±0.05^{b}$
C20:4 n-6	$0.74±0.11^{a}$	$0.59±0.07^{b}$	$0.51±0.03^{b}$	$0.51±0.08^{b}$
Total PUFA	$39.74±0.72^{a}$	$38.86±0.10^{ab}$	$38.31±0.07^{b}$	$37.11±1.04^{c}$
Unknown	1.77±0.65	2.14±0.74	1.62±0.15	2.11±0.81

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each row were significantly different ($p < 0.05$).

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Table 4.3 Fatty acid composition (% total fatty acids) of glutinous rice at different soaking temperatures

Fatty acids (%)	Soaking temperature			
	Unsoaked	30°C	45°C	60°C
C14:0	0.21±0.02[a]	0.18±0.02[b]	0.16±0.03[bc]	0.15±0.02[c]
C16:0	0.33±0.03[b]	0.34±0.02[b]	0.35±0.02[b]	0.39±0.01[a]
C18:0	0.06±0.02	0.07±0.01	0.07±0.01	0.09±0.02
Total SFA	0.60±0.03	0.61±0.02	0.59±0.04	0.63±0.03
C16:1 n-9	25.21±0.18[d]	25.74±0.22[c]	27.73±0.30[b]	28.96±0.11[a]
C18:1 n-9	32.67±0.50[c]	32.77±0.18[c]	34.75±0.22[a]	33.86±0.11[b]
Total MUFA	57.88±0.32[c]	58.52±0.25[b]	62.48±0.48[a]	62.82±0.20[a]
C18:2 n-6	32.31±0.36[a]	35.48±0.26[b]	32.35±0.43[c]	31.75±0.17[c]
C18:3 n-3	2.11±0.21[a]	1.96±0.11[ab]	1.84±0.09[b]	1.77±0.10[b]
C20:3 n-6	0.58±0.18[a]	0.49±0.16[ab]	0.33±0.05[b]	0.29±0.05[b]
C20:4 n-6	0.74±0.11[a]	0.59±0.05[ab]	0.54±0.03[b]	0.54±0.10[b]
Total PUFA	39.74±0.72[a]	38.54±0.15[b]	35.06±0.49[b]	34.34±0.28[b]
Unknown	1.77±0.65	2.34±0.34	1.87±0.97	2.20±0.10

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each row were significantly different ($p < 0.05$).

5. The γ-oryzanol content of glutinous rice soaked at different soaking conditions.

Phytosterols carry out functions in plants equivalent to those of cholesterol in animals, being thus required as necessary components of cell membranes and as precursors of important biomolecules, including sex hormones and vitamins (Lerma-García et al., 2009). γ-Oryzanol is a mixture of 10 esters of triterpene alcohols and can be used to reduce blood cholesterol, to treat nerve imbalances as an antioxidant or preservative (Rong et al., 1997; Akihisa et al., 2000). Changes in the γ-oryzanol content is enhanced in the germination state, so allowing temperature and salt for soaking during processing can help improve rice quality. Experimental values obtained for response. The total content of γ-oryzanol for ten design points are given in Table 4.4.

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The γ-oryzanol content of raw glutinous rice grain was 32.48 mg/100g and it ranged from 130.09 to 149.66 mg/100g after soaking at different conditions. This result indicated that soaking condition contributes to the increase in γ-oryzanol content. The maximum increase of γ-oryzanol content was 149.66 mg/100g, which was obtained at soaking temperature of 60°C and NaCl content of 1.50%.

Table 4.4 Hexagonal rotatable design arrangement and response for γ-oryzanol content of glutinous rice

Variable levels		Response (Y)
X_1 (Soaking temperature, ^{o}C)	X_2 (NaCl content, %)	γ-oryzanol content (mg/100g)
Raw		32.48
60.00	1.50	149.66
42.50	2.79	148.54
37.50	2.79	147.51
30.00	1.50	142.04
37.50	0.21	130.09
42.50	0.21	134.48
45.00	1.50	146.67
45.00	1.50	145.89
45.00	1.50	146.06
45.00	1.50	145.95

The soaking temperature and salt content were the most influential variables affecting the γ-oryzanol content. Generally, enrichment of γ-oryzanol content in glutinous rice soaked increased with the increasing soaking temperature as well as with the NaCl content. Banchuen et al. (2009) have reported that the nutritional and bioactive compounds of the germination process of brown rice is influenced by the nature of the raw material, variety, soaking and germination conditions. Other studies have reported that the γ-oryzanol content was affected by the temperature. Its levels increased 35–57% at elevated temperature, thus suggesting that the physiological action of individual

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ferulated phytosterols should be investigated, because their relative proportions in γ-oryzanol can change with temperature during the growing season (Britz et al., 2007; Lerma-García et al., 2009). Additionally, Guo et al. (1995) have reported that the sterol synthesis occurs following the germination process of the seed and then gradually decreases as the seed matures. The typical plant sterols act in membranes to restrict the motion of fatty acyl chains and also play a role in cellular differentiation and proliferation (Piironen et al., 2000).

5.1 Analysis of model

Optimization of processing conditions, such as soaking temperature (X_1) and NaCl content (X_2), to maximize the γ-oryzanol content in glutinous rice by soaking was determined. The multiple regression coefficients obtained by employing a least square technique to predict a quadratic polynomial model for the γ-oryzanol content (Y) is summarized in Table 4.5.

Examination of these coefficients with the t-test, for the γ-oryzanol content, indicated that linear term of soaking temperature and NaCl content were highly significant (P < 0.01) as well as quadratic term of NaCl content, whereas the interaction term of soaking temperature and NaCl content were also significant (P < 0.05), but the quadratic term of soaking temperature was not significant (P > 0.05). These results suggested that the linear of soaking temperature and NaCl content and/or the quadratic effect of NaCl content may be the primarily determining factors for γ-oryzanol content. The coefficients of independent variables determined for the quadratic polynomial models (Table 4.5) for the γ-oryzanol content (Y) of glutinous rice is given below:

$$Y = 146.14 - 3.34Z_1 + 9.05Z_2 - 0.29Z_1^2 - 7.81Z_2^2 - 1.93Z_{12}$$

These results show that the models predicted for Y were adequate as indicated by error analysis that showed non-significant lack-of-fit ($P > 0.05$). The adjusted correlation coefficients for the determination (R^2) of the γ-oryzanol content was 0.99

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Table 4.5 Regression coefficients of predicted quadratic polynomial model for response Variable

Variables	Coefficients (β)
	Y (mg/100g)
Intercept	146.14
Linear	
Z_1	3.34**
Z_2	9.05**
Quadratic	
Z_{11}	-0.29
Z_{22}	-7.81**
Interaction	
Z_{12}	-1.93*
R^2	0.99

Y: the γ-oryzanol content

**$P < 0.01$ highly significant, *$P < 0.05$ significant, no asterisk $P > 0.05$ not significant

5.2 Optimization of soaking conditions to maximize content of the γ-oryzanol

Two variables given the fitted model were chosen as the axes for the response surface plots. The linear, quadratic and interaction terms in second order polynomial were used to generate a three-dimensional response surface graph. The response surface for the γ-oryzanol content is given in Figure 4.3. The results of canonical response surfaces were given in Table 4.6. The stationary points for the γ-oryzanol content of glutinous rice by soaking predicted a maximum increase of 150.82 mg/100g at the soaking temperature of 60°C and NaCl content of 2.34%. The result of the three-dimensional response surface indicated that the γ-oryzanol content would be increased with high temperature and salt content. The γ-oryzanol is important components of plant membranes; regulate the fluidity of membranes and probably play a role in the adaptation of membranes to temperature (Moreau et al., 2002). That moderate increases in temperature induce increases in this compound suggests they are

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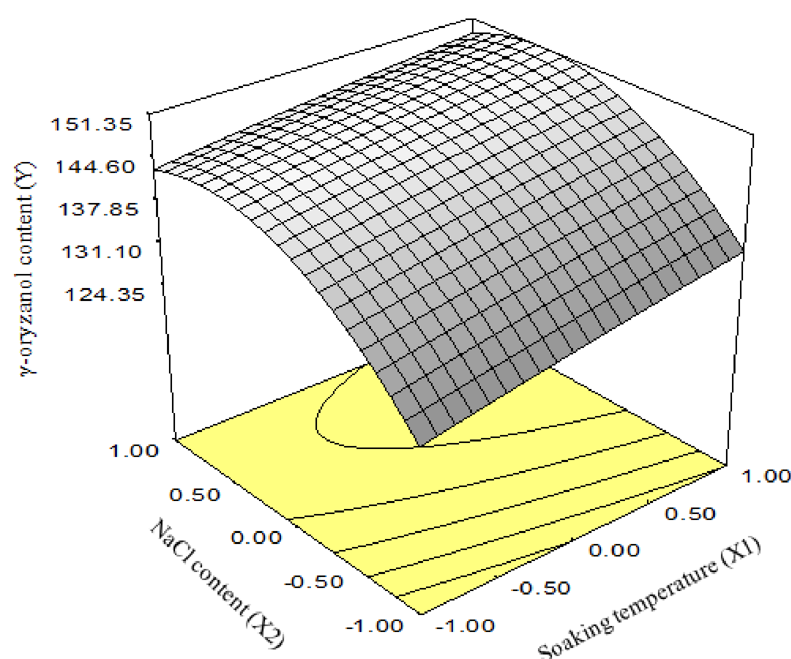
involved in plant stress responses (Britz et al., 2007). During the process of soaking, hydrolytic enzymes are thus activated, thereby increasing the amount of digestible oligosaccharides, amino acids, minerals and vitamins. The decomposition of the high molecular weight polymers during soaking leads to the generation of bio-functional substances (Ohtsubo et al., 2005).

Figure 4.3 Response surface for the effect of soaking temperature and NaCl content on the γ-oryzanol content of glutinous rice.



Under saline conditions, the plant cell is confronted by osmotic stress, ionic toxicity and nutritional disorder (Patade et al., 2008). This is the harsh environmental factor that has the major effect on plant quantity and quality. In order to survive in salt and temperature stress condition, Rice adapted in response to these stress by dangerously increasing reactive oxygen species (ROS) production, and this response affected rice biomass (Wanichananan et al., 2003; Cha-um et al., 2004). The processes leading to plant adaption to salt stress include control of water loss through stomata, metabolic adjustment, toxic ion homeostasis, and osmotic adjustment (Hasegawa et al., 2000; Munns and Tester, 2008). However, the significance of an additional process that is detoxification of ROS is still a matter of debate. ROS derived from molecular oxygen

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can accumulate in the plant cell and cause oxidative damages in cellular components, including chlorophylls, proteins and lipids by lipid peroxidation. Reaction of lipid peroxidation can degrade polyunsaturated fatty acids in cell membranes, which impart the membrane functions. Therefore this system is easily approved to oxidative damage (Lin and Kao, 2000). In plant cells, an antioxidant defense mechanism has been developed for protection against ROS (Bowler et al., 1992). Scavenging of ROS depends on both enzymatic and non-enzymatic component. Plants have developed antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, peroxidase and non-enzymatic scavengers like glutathione, ascorbic acid, carotenoids, flavonoids and other bioactive compound, which regularly maintain ROS balances within the cell (Vranová et al., 2002; Mandhania et al., 2006). Accumulate of γ-oryzanol in glutinous rice soaked at different conditions suggested role of γ-oryzanol in temperature and salt stress, as it might be involved in free radical scavenging and membrane stabilization which is manifestations associated with stress. Both natural and man-made stress situations provoke increase production of toxic oxygen species. In response, the capacity of the antioxidative defence system is increased (Keleş and Őncel, 2002).

The adequacy of the models predicted was examined by performing independent experiments at the optimal conditions for improved γ-oryzanol content by soaking conditions. Verification results revealed that the predicted values from this model was reasonably close to the observed one (Table 4.6), indicating that the modelis suitable for the prediction of the study response. This process indicates the importance for improved of γ-oryzanol by soaking glutinous rice for adding nutritional value to functional food products.

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Table 4.6 Predicted and observed values for optimize of soaking conditions to maximize contents of the γ-oryzanol

Response variable	Critical values of independent variables		Stationary point	Predicted value (mg/100g)	Observed value[a] (mg/100g)
	Soaking temperature (^{o}C)	NaCl content (%)			
γ-oryzanol content	60.00	2.34	Maximum	150.82	149.69±1.35

[a]Mean values ± standard deviation of determinations for triplicate samples

7. Summary of experiment 1

This study reveals that soaking temperature and NaCl content are the most important factors of rice soaking. The experiment findings have demonstrated that significant differences were found in total phenolic content, phenolic acid, α-tocopherol, fatty acids and γ-oryzanol content of glutinous rice soaked compared to unsoaked. The amounts of TPC, phenolic acid, γ-oryzanol, SFA and MUFA content of the glutinous rice increased as NaCl content and soaking temperature increased, while the amounts of α-tocopherol and PUFA in soaked samples decreased. Since soaking temperature and NaCl content also resulted in chemical and physicochemical changes, optimum combinations of soaking temperature and NaCl content need to be determined to achieve processing conditions to achieve consumer acceptability for parboiled paddy rice. The processing parameters were found to be: a soaking temperature of 60°C and NaCl content of 2.34%. Under these conditions, the γ-oryzanol content could be increased by up to 149.69 mg/100g. The soaking process at high temperature and salinity were demonstrated to be a feasible method for the improved of γ-oryzanol content of glutinous rice in parboiled rice industry.

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4.4 Results and discussion of experiment 2

1. Total phenolic content of glutinous rice steamed at different steaming conditions.

In general, after steaming, TPC decreased (Table 4.7). After steaming with different steaming temperature and steaming time, TPC of steamed rice was significantly ($P < 0.05$) lower than that of the control (un-steamed). TPC of the glutinous rice showed a decreasing trend as steaming temperature and steaming time increased. Of all the soaking treatments, the highest TPC content was for the rice steamed at 110°C for 10 min.

Table 4.7 Total phenolic content of glutinous rice at different steaming temperatures and steaming times

Soaking temperature (^{o}C)	Soaking time (min)	TPC (mg GAE/g DW)
	10	0.18 ± 0.01^{a}
110	15	0.17 ± 0.01^{a}
	20	0.17 ± 0.00^{a}
	10	0.17 ± 0.00^{a}
115	15	0.16 ± 0.01^{b}
	20	0.15 ± 0.00^{c}
	10	0.16 ± 0.00^{b}
120	15	0.15 ± 0.01^{c}
	20	0.14 ± 0.01^{d}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each column were significantly different ($p < 0.05$).

2. Phenolic acids composition of glutinous rice steamed at different steaming conditions.

RP-HPLC analysis was used to identify the phenolic compounds of glutinous rice extracts, by comparison with standard compounds. In the rice samples analyzed, it was possible to identify 4 hydroxybenzoic acid (HBA): gallic acid,

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protocatechuic acid, *p*-hydroxybenzoicacid, vanilic acid and 6 hydroxycinnamic acid (HCA): chorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid. The distribution of phenolic acids in all samples is presented in Table 4.8. Comparing the phenolic acids of all samples, the content of total phenolic acids (TPA) in steamed rice (60.67-63.08 μg/g) was found to be significantly lower than that in the control (un-steamed). Results showed that a similar trend with TPC. The TPA of the glutinous rice showed a decreasing trend as steaming temperature and steaming time increased. The results showed that the rice steamed at 100°C for 10 min had the highest TPA (63.08 μg/g) among steaming treatments. The data has shown that the steaming temperature and steaming time affected the TPA of HCA and HBA. The results indicated that the rice steamed at low temperature and short time had the greatest contents.

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Table 4.8 Phenolic acids (μg/g) of glutinous rice at different steaming temperatures and steaming times

Treatment		Hydrobenzoic acids				Hydrocinnamic acids						Total
Steaming temperature (^{o}C)	Steaming time (Min)	GA	PCCA	p-OH	VA	ChA	CFA	SyA	p-CA	FA	SNA	
110	10	5.08±0.16^{a}	43.97±0.53^{a}	ND	1.04±0.03	1.44±0.07	1.29±0.10	1.42±0.07	2.09±0.08	5.47±0.09	1.28±0.06	63.08±0.35^{a}
	15	5.03±0.10^{a}	43.71±0.45^{ab}	ND	1.05±0.03	1.43±0.06	1.26±0.08	1.37±0.08	2.14±0.10	5.49±0.08	1.27±0.08	62.76±0.43^{ab}
	20	4.98±0.12^{ab}	43.79±0.25^{ab}	ND	1.03±0.02	1.44±0.05	1.28±0.09	1.37±0.10	2.13±0.05	5.45±0.07	1.26±0.07	62.73±0.34^{ab}
115	10	5.07±0.07^{a}	43.26±0.30^{bc}	ND	1.05±0.03	1.40±0.08	1.21±0.07	1.31±0.09	2.09±0.07	5.50±0.10	1.28±0.09	62.15±0.33^{abc}
	15	5.02±0.14^{a}	43.12±0.27^{cd}	ND	1.04±0.03	1.37±0.10	1.20±0.11	1.26±0.08	2.15±0.10	5.40±0.07	1.27±0.09	61.82±0.51^{abcd}
	20	4.94±0.19^{abc}	42.98±0.22^{cd}	ND	1.04±0.05	1.34±0.09	1.19±0.09	1.26±0.08	2.08±0.09	5.42±0.07	1.25±0.12	61.51±0.48^{bcd}
120	10	4.92±0.08^{abc}	42.84±0.27^{cd}	ND	1.07±0.02	1.38±0.07	1.17±0.16	1.31±0.14	2.14±0.07	5.55±0.11	1.23±0.15	61.62±0.35^{bcd}
	15	4.77±0.11^{bc}	42.73±0.27^{cd}	ND	1.03±0.03	1.34±0.10	1.15±0.12	1.27±0.04	2.06±0.07	5.40±0.10	1.20±0.12	60.96±0.35^{cd}
	20	4.71±0.18^{c}	42.56±0.13^{d}	ND	1.03±0.05	1.30±0.10	1.12±0.11	1.28±0.07	2.03±0.10	5.40±0.15	1.22v0.15	60.67±0.31^{d}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each column were significantly different ($p < 0.05$).

GA - gallic acid; PCCA- protocatechuic acid; *p*-OH - *p*-hydroxybenzoic acid; VA - vanilic acid; ChA - chorogenic acid; CFA-caffeic acid; SyA - syringic acid; *p*-CA - *p*-coumaric acid; FA - ferulic acid and SNA - sinapic acid.

ND - not detected

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2.3 The α-tocopherol content of glutinous rice steamed at different steaming conditions.

The concentration of α-tocopherol was also affected by the steaming conditions (Table 4.9). The α-tocopherol of the glutinous rice showed a decreasing trend as steaming temperature and steaming time increased. The results showed that the extract of the low steaming temperature and shot time (110°C, 10 min) had the highest α-tocopherol content (7.71 mg/100g) among steaming treatment. These data suggest the possibility that there is a loss of α-tocopherol during the steaming process.

Table 4.9 The α-Tocopherol content of glutinous rice at different steaming conditions

Steaming temperature (^{o}C)	Steaming time (min)	α-tocopherol content (mg/100g)
	10	7.71 ± 0.29^{a}
110	15	7.62 ± 0.23^{a}
	20	7.50 ± 0.32^{ab}
	10	7.25 ± 0.12^{bc}
115	15	7.19 ± 0.17^{bcd}
	20	6.99 ± 0.06^{cde}
	10	6.97 ± 0.12^{cde}
120	15	6.86 ± 0.12^{cde}
	20	6.80 ± 0.19^{d}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each column were significantly different ($p < 0.05$).

2.4 Fatty acid composition of contents of glutinous rice steamed at different steaming conditions.

The percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in steamed glutinous rice is shown in Table 4.10. The variation of steaming induced many changes in the fatty acids of glutinous rice. After steaming at high temperature and long time, PUFA significantly ($p < 0.05$) decreased, while the level of SFA and MUFA in steaming samples increased. All

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treatments studied had appreciable amounts of fatty acid content, which indicates that steaming with a high temperature and long time, favors the extraction of SFA and MUFA in this glutinous rice. During the steaming process, the fatty acid composition of newly differentiated tissues was significantly different from that of the original seeds. The authors considered that these results were probably due to disintegrate of fatty acids.

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Table 4.10 Fatty acid composition (% total fatty acids) of glutinous rice at different steaming conditions

Treatments		Fatty acids (%)												
Steaming temperature (°C)	Steaming time (min)	C14:0	C16:0	C18:0	Total SFA	C16:1 n-9	C18:1 n-9	Total MUFA	C18:2 n-6	C18:3 n-3	C20:3 n-6	C20:4 n-6	Total PUFA	Unknown
	10	0.23 ± 0.01^{d}	0.35 ± 0.02^{d}	0.07 ± 0.01^{c}	0.65 ± 0.01^{f}	28.00 ± 0.09^{e}	33.57 ± 0.16^{d}	61.58 ± 0.12^{e}	32.33 ± 0.06^{a}	1.75 ± 0.01^{a}	0.31 ± 0.02^{a}	0.59 ± 0.03^{a}	34.98 ± 0.11^{a}	2.80 ± 0.07^{a}
110	15	0.25 ± 0.01^{c}	0.36 ± 0.01^{d}	0.08 ± 0.01^{bc}	0.69 ± 0.02^{e}	28.09 ± 0.05^{e}	33.56 ± 0.13^{d}	61.65 ± 0.08^{e}	32.20 ± 0.07^{a}	1.75 ± 0.02^{a}	0.30 ± 0.01^{ab}	0.58 ± 0.04^{ab}	34.82 ± 0.09^{ab}	2.84 ± 0.17^{a}
	20	0.26 ± 0.01^{bc}	0.36 ± 0.01^{d}	0.08 ± 0.01^{bc}	0.70 ± 0.01^{de}	28.31 ± 0.07^{d}	33.71 ± 0.07^{cd}	62.02 ± 0.14^{d}	32.21 ± 0.10^{a}	1.73 ± 0.02^{ab}	0.29 ± 0.01^{ab}	0.56 ± 0.03^{abc}	34.79 ± 0.18^{abc}	2.48 ± 0.05^{bc}
	10	0.25 ± 0.02^{c}	0.36 ± 0.01^{d}	0.07 ± 0.01^{c}	0.68 ± 0.03^{e}	28.29 ± 0.07^{d}	33.77 ± 0.07^{bc}	62.06 ± 0.13^{d}	32.00 ± 0.08^{b}	1.72 ± 0.07^{abc}	0.28 ± 0.02^{abc}	0.57 ± 0.02^{abc}	34.58 ± 0.15^{bcd}	$2.68 \pm 0.05a^{b}$
115	15	0.26 ± 0.02^{bc}	0.38 ± 0.01^{bc}	0.08 ± 0.01^{bc}	0.73 ± 0.03^{cd}	28.37 ± 0.06^{d}	33.83 ± 0.05^{abc}	62.20 ± 0.11^{d}	31.96 ± 0.08^{b}	1.70 ± 0.09^{abc}	0.28 ± 0.02^{abc}	0.54 ± 0.02^{abcd}	34.49 ± 0.18^{cde}	2.59 ± 0.10^{abc}
	20	0.28 ± 0.01^{ab}	0.40 ± 0.01^{ab}	0.08 ± 0.01^{bc}	0.76 ± 0.03^{ab}	28.64 ± 0.12^{c}	33.84 ± 0.05^{abc}	62.48 ± 0.11^{c}	31.90 ± 0.09^{b}	1.67 ± 0.06^{abc}	0.27 ± 0.02^{bcd}	0.52 ± 0.02^{bcd}	34.35 ± 0.15^{de}	2.41 ± 0.19^{cd}
	10	0.28 ± 0.01^{ab}	0.38 ± 0.01^{bc}	0.09 ± 0.01^{a}	0.75 ± 0.02^{c}	28.94 ± 0.10^{b}	33.90 ± 0.05^{ab}	62.84 ± 0.07^{b}	31.85 ± 0.06^{b}	1.69 ± 0.09^{abc}	0.27 ± 0.03^{bcd}	0.52 ± 0.03^{bcd}	34.34 ± 0.20^{de}	2.07 ± 0.25^{e}
120	15	0.28 ± 0.01^{ab}	0.40 ± 0.01^{ab}	0.09 ± 0.01^{a}	0.77 ± 0.01^{ab}	29.07 ± 0.07^{ab}	33.85 ± 0.06^{abc}	62.92 ± 0.04^{ab}	31.83 ± 0.08^{b}	1.63 ± 0.06^{bc}	0.25 ± 0.03^{cd}	0.51 ± 0.02^{d}	34.22 ± 0.12^{e}	2.09 ± 0.09^{e}
	20	0.29 ± 0.02^{a}	0.41 ± 0.01^{a}	0.10 ± 0.01^{a}	0.80 ± 0.02^{a}	29.17 ± 0.18^{a}	33.95 ± 0.10^{a}	63.12 ± 0.22^{a}	31.53 ± 0.21^{c}	1.61 ± 0.07^{c}	0.24 ± 0.03^{d}	0.50 ± 0.04^{d}	33.88 ± 0.32^{f}	2.20 ± 0.15^{de}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each column were significantly different ($p < 0.05$).

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2.5 The γ-oryzanol content of glutinous rice steamed at different steaming conditions.

Experimental values obtained for response. The total content of γ-oryzanol for ten design points is given in Table 4.11. The γ-oryzanol content of un-steamed rice grain was 149.69 mg/100g and it ranged from 149.67 to 151.40 mg/100g after steaming at different conditions. This result indicated that steaming condition contributes to the increase in γ-oryzanol content. The maximum increase of γ-oryzanol content was 151.40 mg/100g, which was obtained at steaming temperature of 115°C and steaming time of 17.40 min.

Table 4.11 Hexagonal rotatable design arrangement and responses for γ-oryzanol content of glutinous rice at different steaming conditions

Variable levels		Response (Y)
X_1 (Steaming temperature, °C)	X_2 (Steaming time, min)	γ-oryzanol content (mg/100g)
Un-steamed		149.69
120.00	10.00	151.24
115.00	17.40	151.40
112.50	17.40	150.35
110.00	10.00	149.98
112.50	12.60	149.67
115.00	12.60	150.07
115.00	15.00	150.12
115.00	15.00	150.09
115.00	15.00	150.17
115.00	15.00	150.04

The steaming temperature and steaming time were the most influential variables affecting the γ-oryzanol content. Generally, enrichment of γ-oryzanol content in glutinous rice steamed increased with the increasing steaming temperature as well as with the steaming time.

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2.5.1 Analysis of model

Optimization of processing conditions, such as steaming temperature (X_1) and steaming time (X_2), to maximize the γ-oryzanol content in glutinous rice by steaming was determined. The multiple regression coefficients obtained by employing a least square technique to predict a quadratic polynomial model for the γ-oryzanol content (Y) is summarized in Table 4.12.

Examination of these coefficients with the t-test, for the γ-oryzanol content, indicated that linear term of steaming temperature and steaming time were highly significant ($P < 0.01$) as well as quadratic term of steaming temperature and interaction term, whereas the quadratic term of steaming time were also significant ($P < 0.05$). These results suggested that the linear term of steaming temperature, steaming time and an interaction term and/or the quadratic effect of steaming temperature may be the primarily determining factors for γ-oryzanol content. The coefficients of independent variables determined for the quadratic polynomial models (Table 4.12) for the γ-oryzanol content (Y) of glutinous rice is given below:

$$Y = 150.11 + 0.66Z_1 + 0.58Z_2 + 0.51Z_1^2 + 0.19Z_2^2 + 0.37Z_{12}$$

These results show that the models predicted for Y were adequate as indicated by error analysis that showed non-significant lack-of-fit ($P > 0.05$). The adjusted correlation coefficients for the determination (R^2) of the γ-oryzanol content was 0.99

Table 4.12 Regression coefficients of predicted quadratic polynomial model for response variable at different steaming conditions

Variables	Coefficients (β)
	Y (mg/100g)
Intercept	150.11
Linear	
Z_1	0.66**
Z_2	0.58**
Quadratic	
Z_{11}	0.51**
Z_{22}	0.19*
Interaction	
Z_{12}	0.37**
R^2	0.99

Y: the γ-oryzanol content

**P < 0.01 highly significant, *P < 0.05 significant

2.5.2 Optimization of steaming conditions to maximize content of the γ-oryzanol

Two variables given the fitted model were chosen as the axes for the response surface plots. The linear, quadratic and interaction terms in second order polynomial were used to generate a three-dimensional response surface graph. The response surface for the γ-oryzanol content is given in Figure 4.4. The results of canonical response surfaces were given in Table 4.13. The stationary points for the γ-oryzanol content of glutinous rice by steaming predicted a maximum increase of 151.86 mg/100g at the steaming temperature of 120°C and steaming time 17.80 min. The result of the three-dimensional response surface indicated that the γ-oryzanol content would be increased with high steaming temperature and steaming time. The decomposition of the cell walls during steaming leads to increase γ-oryzanol content.

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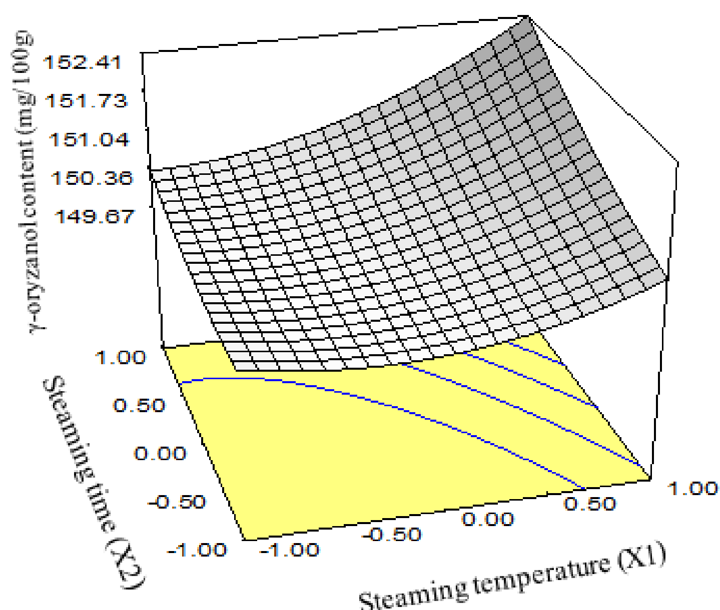
Figure 4.4 Response surface for the effect of steaming temperature and steaming time on the γ-oryzanol content of glutinous rice.



The adequacy of the models predicted was examined by performing independent experiments at the optimal conditions for improved γ-oryzanol content by steaming conditions. Verification results revealed that the predicted values from this model was reasonably close to the observed one (Table 4.13), indicating that the modelis suitable for the prediction of the study response. This process indicates the importance for improved of γ-oryzanol by steaming glutinous rice for adding nutritional value to functional food products.

Table 4.13 Predicted and observed values for optimize of soaking conditions to maximize contents of the γ-oryzanol

Response variable	Critical values of independent variables		Stationary point	Predicted value (mg/100g)	Observed value[a] (mg/100g)
	Steaming temperature (°C)	Steaming temperature (min)			
γ-oryzanol content	120.00	17.80	Maximum	151.86	152.04±0.92

[a]Mean values ± standard deviation of determinations for triplicate samples

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2.6 Summary of experiment 2

This study reveals that steaming temperature and steaming time are the most important factors of rice steaming. The experiment findings have demonstrated that significant differences were found in total phenolic content, phenolic acid, α-tocopherol, fatty acids, and γ-oryzanol content of glutinous rice steamed compared to un-steamed. The amounts of γ-oryzanol, SFA and MUFA content of the glutinous rice increased as steaming temperature and steaming time increased, while the amounts of TPC, phenolic acid, α-tocopherol and PUFA in steamed samples decreased. The processing parameters were found to be: a steaming temperature of 120°C and steaming time of 17.80 min. Under these conditions, the γ-oryzanol content could be increased by up to 152.04 mg/100g. The steaming process at high temperature and long-time were demonstrated to be a feasible method for the improved of γ-oryzanol content of glutinous rice in parboiled rice industry.

4.6 Results and discussion of experiment 3

1. Total phenolic content and phenolic acids composition of parboiled glutinous rice.

Phenolic compounds are the most active antioxidant derivatives in plants which mostly found in the outer layers of plants, such as the peel, shell and hull, contain large amounts of polyphenolic compounds to protect the inner components (Bors et al., 2001). There are two classes of phenolic acids: hydroxybenzoic acids and hydrocynamic acids. In the raw rice and parboiled rice samples analyzed, it was possible to identify 4 hydroxybenzoic acid (HBA): gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanilic acid and 6 hydrocinnamic acid (HCA): chorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid. To achieve the maximum benefits from diet it is critical to understand the bioactive compounds and their distributions in food ingredients, as well as the effects of food processing on the amount of these beneficial components. So, this research has investigated the effect of parboiling process on the phenolic acid of glutinous rice, a common Thai rice, to elucidate its nutritional value as a potential contributor of phenolic acid in the diet. The effect of parboiling process on the distributions and concentrations of phenolic acids

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were determined using a reverse phase HPLC (Table 4.14). In general, parboiling process increased the total phenolic acids (TPA) and the percent increases were positively correlated with the parboiling method, with the highest observed in parboiled glutinous (62.39 μg/g). As compared to raw rice, parboiling method significantly increased TPA in the glutinous rice by 36%. This result is consistent with Min et al. (2014), which showed significant increase of total simple phenolic acids in parboiled purple rice (IAC600) by 48%. The changes in the concentration of phenolic acids in glutinous rice by parboiling process are shown in Table 4.14. The most abundant phenolic acids found in raw and parboiled rice were protocatechuic, gallic,ferulicand *p*-coumaric acids, no *p*-hydroxybenzoic acid was detected in parboiled rice. Similar results were also presented by Min et al. (2014), who reported protocatechuic acid as predominate in purple rice followed by vanillic and p-coumaric acids, respectively. Sompong et al. (2011) also reported that the ferulic acid was the most abundant in black rice varieties, followed by vanillic and *p*-coumaric acids. Parboiling process significantly increased the concentrations of the protocatechuic acid, syringic acid, ferulic acid and *p*-coumaric acid, but slightly decreased the gallic acid and ceffeic caffeic acid as compared to raw rice ($p < 0.05$). This finding has shown that the parboiling process affected TPA of HCA and HBA. The results indicated that the parboiled rice had the greatest content. In all samples studied, the total content of the HCA group was higher than the total content of the HBA group. These result found that the parboiling process had the highest HCA and HBA (35.77and 26.61 μg/g, respectively). Cereal grains contain phenolic acids and glycosides, in both soluble and insoluble form, which are bound with polysaccharides at the cell wall. Most of the phenolic compounds are in an insoluble form (Miller et al., 2000). These changes in phenolic acid may imply that heat treatment causes the instability of cell-wall structure and binding properties, resulting in an increase in the extractability and/or release of bound phenolics. The data obtained indicate that parboiling process of rice grains may liberate phenolic acids and their derivatives from the wall cells. Phenolic acids, especially ferulic acid, are known to be the major phenolic compounds bound to the cell wall in grains (Adom & Liu, 2002). The effect of parboiling process on polyphenols should be considered because parboiling is a thermal process. Several studies have reported various effects of thermal processing on soluble phenolic concentrations: an

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increase (Zieliński et al., 2001; Min et al., 2014) or a decrease (Finocchiaro et al., 2007; Walter et al., 2013)

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Table 4.14 Total phenolic content (mg GAE/g) and phenolic acids (µg/g dry weight) of raw and parboiled glutinous rice

Sample	Hydrobenzoic acids					Hydrocinnamic acids					Total	TPC
	GA	PCCA	*p*-OH	VA	ChA	CFA	SyA	*p*-CA	FA	SNA		
Raw	5.66±0.05^{a}	24.20±0.07^{a}	0.85±0.06	1.03±0.11	2.00±0.05	3.35±0.10^{a}	1.68±0.10^{b}	1.38±0.07^{b}	4.55±0.18^{b}	1.25±0.02	45.94±0.22^{b}	0.13±0.02
Parboiled	4.44±0.08^{b}	30.34±0.18^{a}	ND	0.99±0.06	2.12±0.14	2.57±0.08^{b}	4.01±0.08^{a}	10.14±0.14^{a}	6.50±0.10^{a}	1.28±0.03	62.39±0.12^{a}	0.14±0.01

Mean values ± standard deviation of determinations for triplicate samples. Values with the different superscript in each column were significantly different ($p < 0.05$).

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2. The α-tocopherol and γ-oryzanol of parboiled glutinous rice.

The changes in the γ-oryzanol and α-tocopherol by parboiling processes are shown in table 4.15. γ-Oryzanol is an antioxidant compound and is associated with decreasing plasma cholesterol, decreasing cholesterol absorption, lowering serum cholesterol, decreasing platelet aggregation (Wennermark et al., 1994). That acts as a strong antioxidant both in vivo (Islam et al., 2011) and in vitro (Patel and Naik, 2004) and is a typical component of glutinous rice. γ-oryzanol is a mixture of steryl and other triterpenyl esters of ferulic acids and the content depends upon rice varieties (Pereira-Caro et al., 2013). The γ-oryzanol content was significantly increased after parboiling process, while α-tocopherol content was decreased ($p < 0.05$). Parboiling method had the higher γ-oryzanol content than raw rice. The γ-oryzanol content was increased by increasing salinity and soaking temperature in soaking process prior parboiling. Therefore, parboiling process resulted in amount of γ-oryzanol higher than raw rice. Increase in γ-oryzanol in brown rice on parboiling has also been reported by Min et al. (2014). This phenomena was attributed to the synthesis of γ-oryzanol as a physiological metabolic component before the sprouting of rough rice and its use as a growth accelerator and synergist in the rice plants. Tocopherols are naturally occurring phytochemicals present in rice grains and their health benefit are well documented including prevention of chronic diseases (Tiwari & Cummins, 2009). Rice processing include milling, hydrothermal, extrusion, parboiling and cooking are also shown to have major influence on the tocol content (Zieliński et al., 2001; Tiwari & Cummins, 2009; Pascual et al., 2013; Min et al., 2014). The levels of α-tocopherol significantly decrease from raw rice ($p< 0.05$), indicating that the parboiling process cause a significant loss of α-tocopherol, as had been observed previously by Pascual et al. (2013). Which α-tocopherol was the most sensitive and parboiling caused a loss of 93% in respect to its original content. Khatoon and Gopalakrishna (2004) also reported high losses of tocols after parboiling a single Indian commercial brown rice sample. Very low amounts of tocopherols (α- and γ-) were found (2.18 mg/kg) while tocotrienols were not detected. In general, tocols are not severely affected by processing as they are water soluble and reported losses are closely associated with lipid degradation (Hakannson & Jagerstad, 1990). The factors that can affect this degradation include cooking temperatures, time and exposure to light and oxidative conditions (Wyatt et al., 1998). Lipid peroxidation

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resulted in losses of tocopherols, but the γ-oryzanol contents did not decrease (Lerma-García et al., 2009).

Table 4.15 The α-tocopherol and γ-oryzanol contents of raw and parboiled glutinous Rice

Sample	α-tocopherol (mg/100g)	γ-oryzanol (mg/100g)
Raw	17.58 ± 0.79^{a}	31.46 ± 1.25^{b}
Parboiled	6.81 ± 0.24^{b}	151.34 ± 0.92^{a}

Mean values ± standard deviation of determinations for triplicate samples. Values with the different superscript in each column were significantly different ($p < 0.05$).

3. Fatty acid composition of parboiled glutinous rice.

The compositions of fatty acids of raw and parboiled rice are shown in Table 4.16. Since unidentified fatty acids were not included, the sum of the fatty acids does not add up to 100%. Monounsaturated fatty acids (MUFA) was the predominant in raw and parboiled rice followed by polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA).These results were in agreement with previous published data on Indian medicinal rice. The fatty acid composition was in order: MUFA > PUFA > SFA (Deepa et al., 2008). The main saturated fatty acids (SFA) found all samples were palmitic acid (16:0) and myristic acid (14:0). The oleic acid (18:1) was the major MUFA in all samples. PUFAs found in the analyzed samples included linoleic acid (18:2), α-linolenic acid (18:3), eicosatrienoic acid (20:3) and arachidonic (20:4). The major contributors to n-3 PUFA was 18:3 while 18:2 was found to be the major n-6 PUFA for all samples examined and raw had the highest whereas parboiled rice has the lowest level of n-3 and n-6 PUFAs. Similar results were also presented by Kitta et al. (2005), who reported α-linolenic acid and linoleic acid as the main n-3 and n-6 PUFAs presents in non-glutinous brown rice, respectively. The parboiling process to the rice caused only marginal changes in its fatty acid contents. A slight increase in the SFA and MUFA in parboiled rice ($p < 0.05$). On the other hand, the level of PUFA in parboiled rice was decreased. The long-chain fatty acid profile of parboiled rice showed an intriguing shift. While the changes in

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relative percentage of linoleic acid (C18:2), α-linolenic acid (C18:3) and arachidonic acid (C20:4) were decreased in parboiling processes. This indicates that parboiled rice, favors formation of SFA and MUFA more than raw rice. The decrease in the PUFA contents as a result of parboiling process to the rice may be due to complexing of amylose specifically with linoleic acid (Karkalas et al., 1995). Dharmaraj and Malleshi (2011) also reported decrease in the PUFA and increase SFA in hydrothermally processed millet when compared to its raw counterpart. This leads to a slight (*per se*) increase in the SFA and MUFA of the rice.

Table 4.16 Fatty acid composition (% total fatty acids) of raw and parboiled glutinous rice

Fatty acids	Fatty acid content (%)	
	Raw	Parboiled
C14:0	0.24±0.01	0.26±0.01
C16:0	0.31±0.02	0.32±0.02
C16:1 n-9	23.54±0.16^{b}	26.24±0.11^{a}
C18:0	0.10±0.02^{b}	0.15±0.01^{a}
C18:1 n-9	33.69±0.13^{b}	34.22±0.10^{a}
C18:2 n-6	37.43±0.18^{a}	34.72±0.12^{b}
C18:3 n-3	1.81±0.08^{a}	1.65±0.04^{b}
C20:3 n-6	0.35±0.03	0.32±0.02
C20:4 n-6	0.54±0.05	0.49±0.03
∑SFA	0.66±0.01^{b}	0.73±0.02^{a}
∑MUFA	57.24±0.28^{b}	60.46±0.10^{a}
∑PUFA	40.12±0.11^{a}	37.18±0.10^{b}

Mean values ± standard deviation of determinations for triplicate samples. Values with the different superscript in each row were significantly different ($p < 0.05$).

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4. Summary of experiment 3

This study has focused on the effect of parboiling processes on fatty acids and bioactive compounds of glutinous rice. Parboiling processes significantly increased the concentrations of γ-oryzanol and total phenolic acid in glutinous rice. In general, parboiling processes decreased the concentrations of α-tocopherol and PUFA, especially the linoleic acid (C18:2), an essential n-6 fatty acid. However, the SFA and MUFA (oleic acid) of parboiled glutinous rice were increased after hydrothermal processes. The parboiling process gave better results for glutinous parboiled rice in terms of improving γ-oryzanol and total phenolic acid contents. The results suggest that parboiling process can be excellent dietary source of bioactive compounds. These data will provide the nutritional fact of various parboiling process leading to fundamental importance for the application of different technological processes and provide more information to consumer.

4.7 Results and discussion of experiment 4

Experimental values obtained for responses: The total content of PUFA of parboiled glutinous rice bran oil for ten design points are given in Table 4.17. The maximum increase of total PUFA content was 61.27% in the non-urea complexed fraction, which was obtained at crystallization temperature of -5°C and urea-to-fatty acid ratio of 2.87.

The crystallization temperature and urea-to-fatty acid ratio were the most influential variables affecting the degree of PUFA concentration. Generally, enrichment of PUFA in concentrate varied inversely with the decreasing crystallization temperature as well as with the increasing urea-to-fatty acid ratio (Liu et al., 2006). At low temperatures, fatty acids had a greater tendency to form urea compounds than at high temperatures, and the urea-to-fatty acid ratio could be used to segregate the fatty acids by their degree of unsaturation. Liu et al. (2006) have reported similar results for the urea complexation experiment conducted on tuna oil. In addition, Wanasundara and Shahidi (1999) have reported that DHA was a major portion in the non-urea complexed

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fraction. Urea complexation of seal blubber oil resulted in an increase in the total PUFA content by up to 89.50% in the non-urea complexed fraction.

When urea crystallizes from the mixture, the saturated fatty and mono-unsaturated fatty acids were first included in the crystal while the PUFAs remained in the mixture. The long-chain mono-unsaturated fatty acids, especially those of the C20 and C22, form complexes with urea more readily than those of the shorter chain saturated fatty acids (C14 and C16) (Medina et al., 1998; Shahidi and Wanasundara, 1998). The tendency of fatty acids to combine with urea decreased with unsaturation increase and with chain-length decrease (Medina et al., 1998). Complete removal of saturated fatty acids by urea complexation could be quite impractical. This was because some saturated fatty acids did not form complex with urea during crystallization time (Liu et al., 2006).

Table 4.17 Hexaganal ratatable design arrangement and response for PUFA concentrate of parboiled glutinous rice bran oil

Variable levels		
X1 (Crystallization Temperature, ^{o}C)	X2 (Urea-to-fatty acid ratio, w/w)	Response, Y (Total PUFAs, %)
parboiled glutinous rice bran oil		37.56
10.00	2.00	48.27
0.00	2.87	55.38
-5.00	2.87	61.27
-10.00	2.00	60.53
-5.00	1.13	60.18
5.00	1.13	53.29
0.00	2.00	54.15
0.00	2.00	54.77
0.00	2.00	54.89
0.00	2.00	54.70

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1. Analysis of model

Optimization of processing conditions, such as crystallization temperature (X_1) and urea-to-fatty acid ratio (X_2), to maximize the total content of PUFA in the prepared concentrate was determined. The multiple regression coefficients obtained by employing a least square technique to predict a quadratic polynomial model for the total content of PUFA (Y) is summarized in Table 4.18.

Examination of these coefficients with the t-test, for the total content of PUFA, indicated that linear term of crystallization temperature and urea-to-fatty acid ratio were highly significant ($P < 0.01$), as well as quadratic term of urea-to-fatty acid ratio, but the quadratic term of crystallization temperature and interaction term of crystallization temperature and urea-to-fatty acid ratio were not significant ($P > 0.05$). These results suggested that the linear and/or the quadratic effect of urea-to-fatty acid ratio and linear term of crystallization temperature may be the primarily determining factors for the PUFA concentrate. The coefficients of independent variables determined for the quadratic polynomial models (Table 4.18) for the total content of PUFA (Y) of the prepared concentrate are given below:

$$Y = 54.63 - 6.22Z_1 + 0.91Z_2 - 0.231Z_1^2 + 3.91Z_2^2 + 0.57Z_{12}$$

These results show that the models predicted for Y were adequate as indicated by error analysis that showed non-significant lack-of-fit ($P > 0.05$). The adjusted correlation coefficients for the determination (R^2) of the total content of PUFA was 0.99

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Table 4.18 Regression coefficients of predicted quadratic polynomial model for response variable

Variables	Coefficients (β)
	Y (%)
Intercept	54.63
Linear	
Z_1	-6.22**
Z_2	0.91**
Quadratic	
Z_{11}	-0.23
Z_{22}	3.91**
Interaction	
Z_{12}	0.57
R^2	0.99

Y: the total PUFAs

**$P < 0.01$ highly significant, no asterisk $P > 0.05$ not significant

2. Optimization of soaking conditions to maximize content of the γ-oryzanol

Two-variables giving the fitted models were chosen as the axes for the response surface plots. The linear, quadratic and interaction terms in the second order polynomial were used to generate a three-dimensional response surface graph. The response surfaces for the total content of PUFA are given in Figure 4.5. The results of canonical response surfaces were given in Table 4.19. The stationary points for the total content of PUFA by urea complexation predicted a maximum increase of 56.81% at the crystallization temperature of -4.6^{o}C and the urea-to-fatty acid ratio of 3.00. Previous studies have reported different variable processing conditions for producing fish oil concentrates. For example, Wanasundara and Shahidi (1999) have reported that the content of total ω3 fatty acids was highest at the crystallization temperature of -10°C, urea-to-fatty acid ratio of 4.5 and crystallization time of 24 h. On the other hand, Liu et al. (2006) have reported that the total content of EPA and DHA was highest at the

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crystallization temperature of -3.88°C, urea-to-fatty acid ratio of 15.78 (mole ratio) and crystallization time of 15.86 h.

The adequacy of the models predicted was examined by performing independent experiments at the optimal conditions for the total PUFA content. Verification results revealed that the predicted values from these models were reasonably close to the observed ones (Table 4.19), indicating that the models are suitable for the prediction of the study responses.

Figure 4.5 Response surface for the effect of crystallization and urea-to-fatty acid ratio on the total content of PUFA of the concentrate of parboiled glutinous rice bran oil.

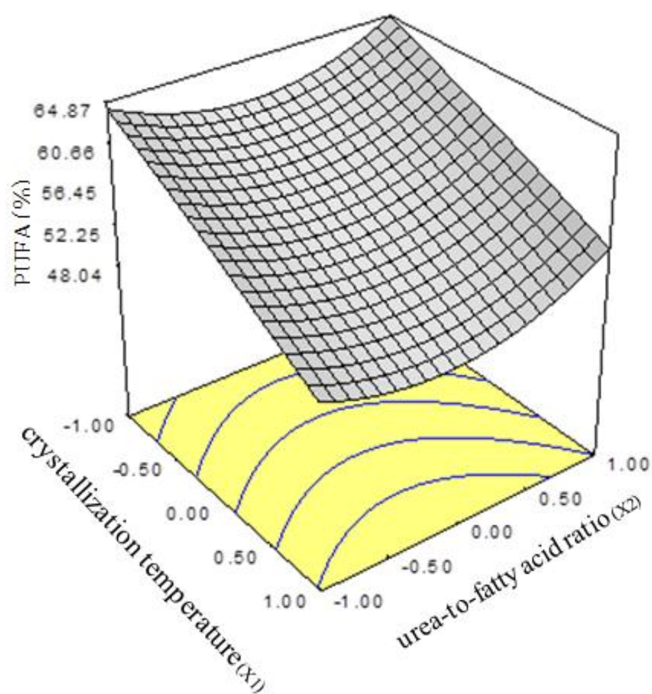

Table 4.19 Predicted and observed values for response variables in urea complexation experiment of parboiled glutinous rice bran oil

Response variable	Critical values of independent variables		Stationary point	Predicted value (%)	Observed value[a] (%)
	Crystallization Temperature (^{o}C)	Urea-to-fatty acid ratio (w/w)			
Total PUFA (%)	-4.6	3.00	Maximum	56.81	57.26±1.75

[a] Mean values ± standard deviation of determinations for triplicate samples

3. Summary of experiment 4

The processing parameters were found to be: a crystallization temperature of -4.6^{o}C and a urea-to-fatty acid ratio of 3 (w/w). Under these conditions, the total content of PUFA could be increased by up to 57.26%. The urea complexation was demonstrated to be a feasible method for the concentration of PUFA from parboiled glutinous rice bran oil in food industry.

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CHAPTER 5

CONCLUSION

This research was experimental research to develop parboiled glutinous rice process for improvement of physical, chemical and nutritional qualities, including soaking, steaming, cooling and drying processes. Moreover, this study includes model equations that predict the optimization conditions based on the process factors. Finally, application of parboiled rice bran as functional food prototype was examined.

The objectives of the present study included as follows:

1. To develop parboiled glutinous rice process for improvement of physical, chemical and nutritional qualities.

2. To optimize condition of parboiling process for improvement of physical, chemical and nutritional qualities.

3. To apply the parboiling process of glutinous rice on industrial scale as functional food.

4. To develop product prototypes from parboiled rice bran oil as functional food.

5.1Conclusion

The findings of the present thesis can be concluded as follows:

1. The present findings have demonstrated that significant differences were found in total phenolic content, phenolic acid, α-tocopherol, γ-oryzanol and fatty acids content of glutinous rice soaked compared to unsoaked. The amounts of TPC, phenolic acid, γ-oryzanol, SFA, and MUFA content of the glutinous rice increased as NaCl content and soaking temperature increased, while the amounts of α-tocopherol and PUFA in soaked samples decreased**.** The processing parameters were found to be: a soaking temperature of 60°C and NaCl content of 2.34%. Under these conditions, the γ-oryzanol content could be increased by up to 149.69 mg/100g.

2. The experiment findings have demonstrated that significant differences were found in total phenolic content, phenolic acid, α-tocopherol, fatty acids and γ-

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oryzanol content of glutinous rice steamed compared to un-steamed. The amounts of γ-oryzanol, SFA and MUFA content of the glutinous rice increased as steaming temperature and steaming time increased, while the amounts of TPC, phenolic acid, α-tocopherol and PUFA in steamed samples decreased. The processing parameters were found to be: a steaming temperature of 120°C and steaming time of 17.80 min. Under these conditions, the γ-oryzanol content could be increased by up to 152.04 mg/100g.

3. Parboiling processes significantly increased the contents of γ-oryzanol and total phenolic acid in glutinous rice. In general, parboiling processes decreased the contents of α-tocopherol and PUFA, especially the linoleic acid (C18:2), an essential n-6 fatty acid. However, the SFA and MUFA (oleic acid) of parboiled glutinous rice were increased after hydrothermal processes. The parboiling process gave better results for glutinous parboiled rice in terms of improving γ-oryzanol and total phenolic acid contents.

4. The processing parameters were found to be: a crystallization temperature of -4.6^{o}C and a urea-to-fatty acid ratio of 3 (w/w). Under these conditions, the total content of PUFA could be increased by up to 57.26%. The urea complexation was demonstrated to be a feasible method for the concentration of PUFA from parboiled glutinous rice bran oil in food industry.

5.2 Recommendations

Parboiling process resulted in chemical and physicochemical changes, optimum combinations of parboiling process need to be determined to achieve processing conditions to achieve consumer acceptability for parboiled paddy rice. According to present data, my specially recommend the parboiling process for improvement of bioactive compounds and cooking quality. Besides, the greater nutritional values of parboiled rice are to be considered as a functional food.

Furthermore, as regards the physical, chemical and nutritional properties of parboiled rice, more studies need to be performed to evaluate the physiological effects in humans of bioactive compound as well as the so far unidentified compounds, in order to know more of the actual value and importance of consuming these.

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APPENDICES

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Appendix A

HPLC Chromatograms of standard phenolic acids

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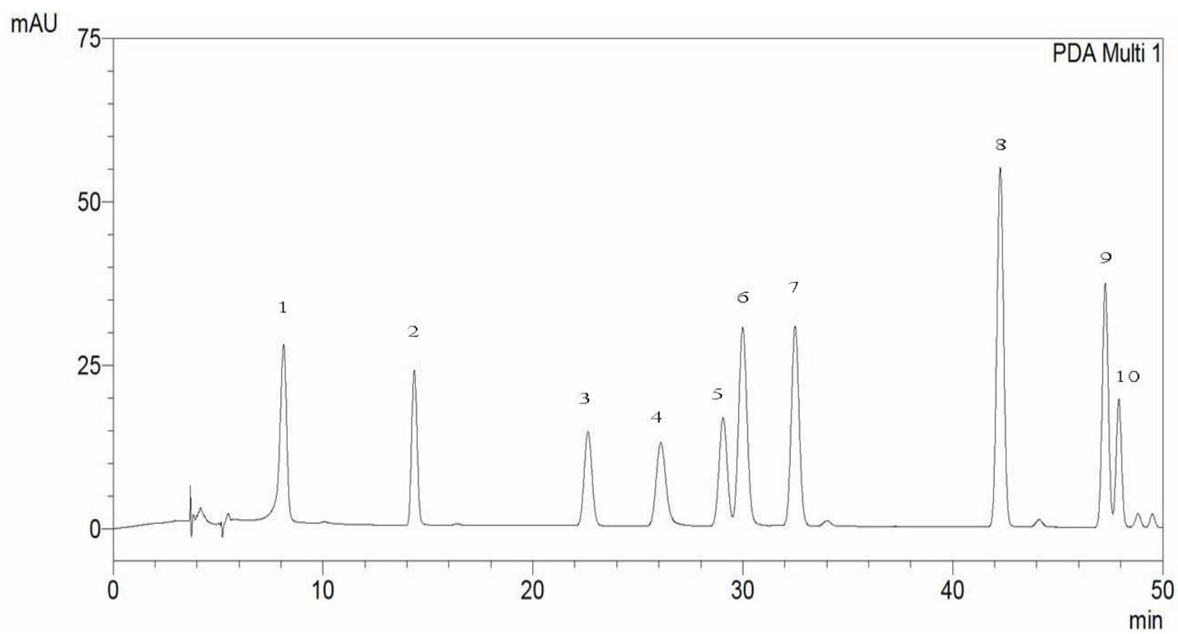
Figure A-1 HPLC chromatograms of standard phenolic acids
(1) gallic acid (2) protocatechuic acid (3) *p*-hydrozybenzoic acid (4) chrologenic acid (5) vanillic acid (6) caffeic acid (7) syringic acid (8) *p*-coumaric acid (9) ferulic acid (10) sinapic acid



Appendix B
Calibration curves of standard phenolic acids

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Figure B-1 Calibration curve of standard gallic acid

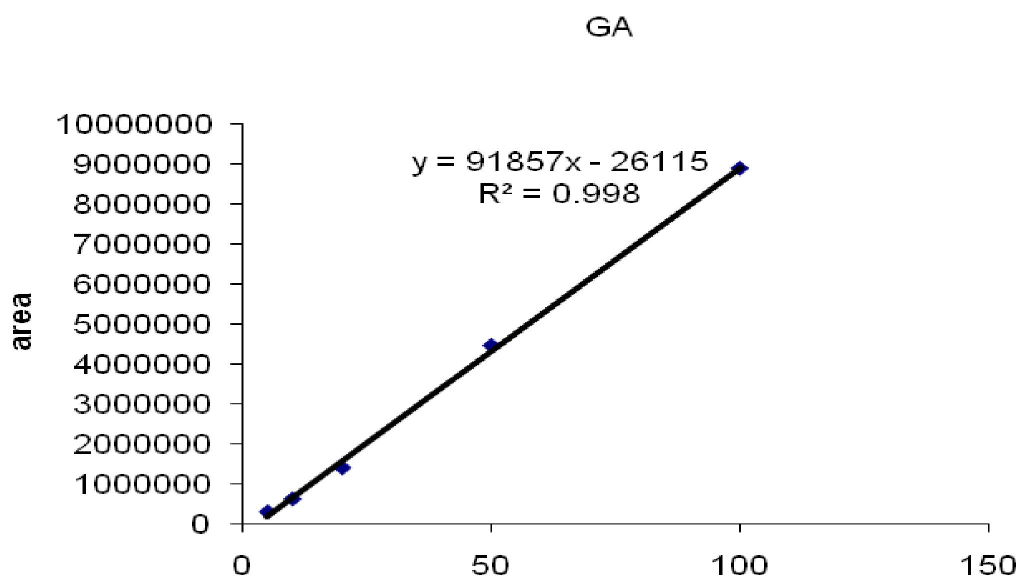

Figure B-2 Calibration curve of standard protocatechuic acid

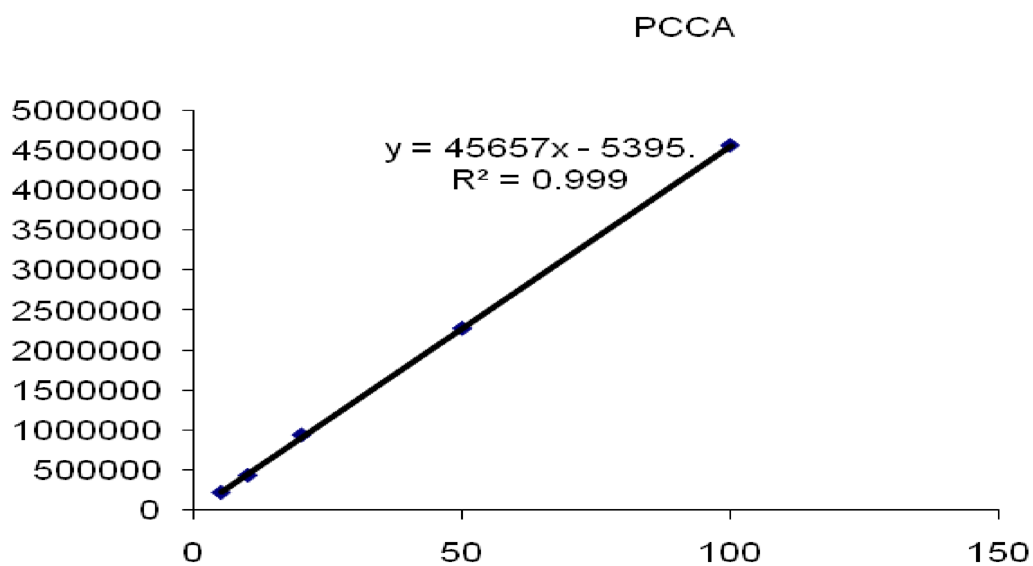

Figure B-3 Calibration curve of standard *p*-hydroxybenzoic acid

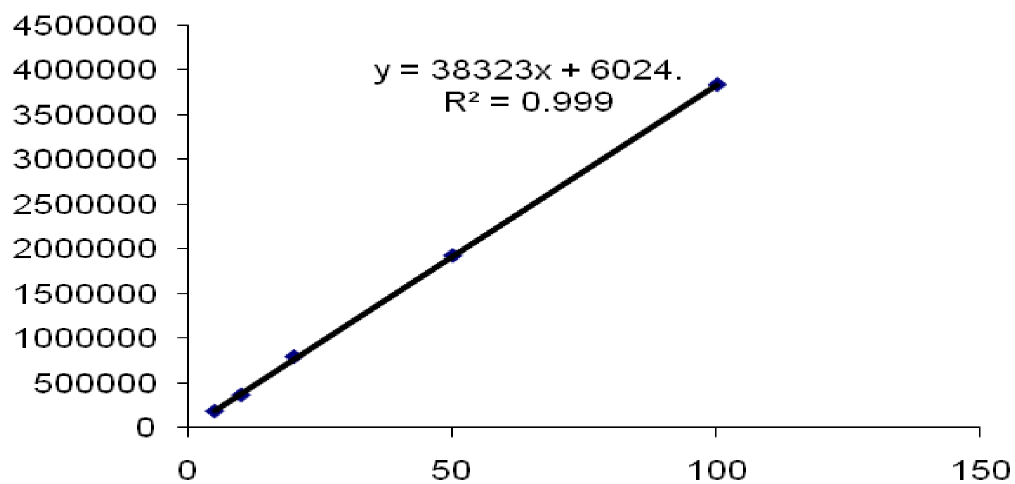

Figure B-4 Calibration curve of standard chlorogenic acid

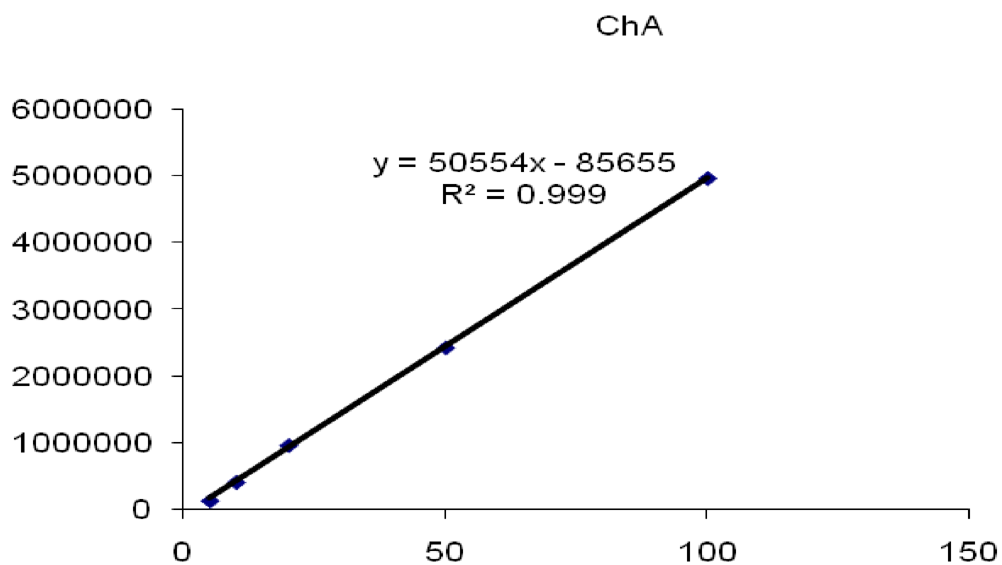

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Figure B-5 Calibration curve of standard vanillic acid

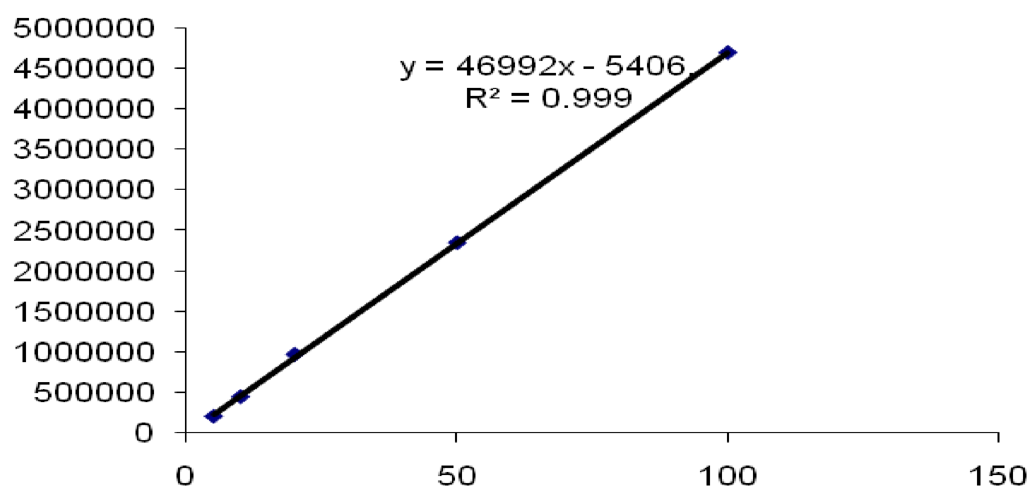

Figure B-6 Calibration curve of standard caffeic acid

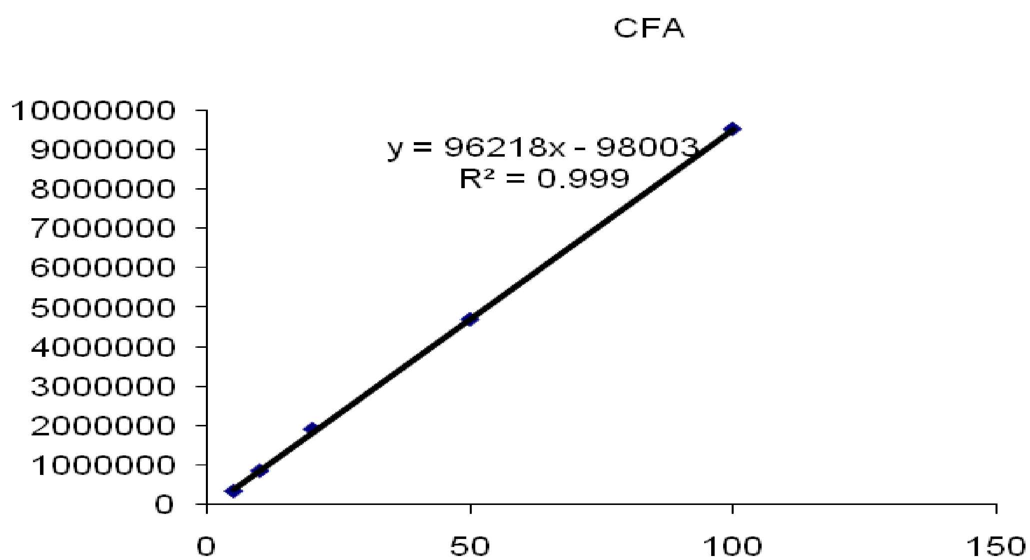

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Figure B-7 Calibration curve of standard syringic acid

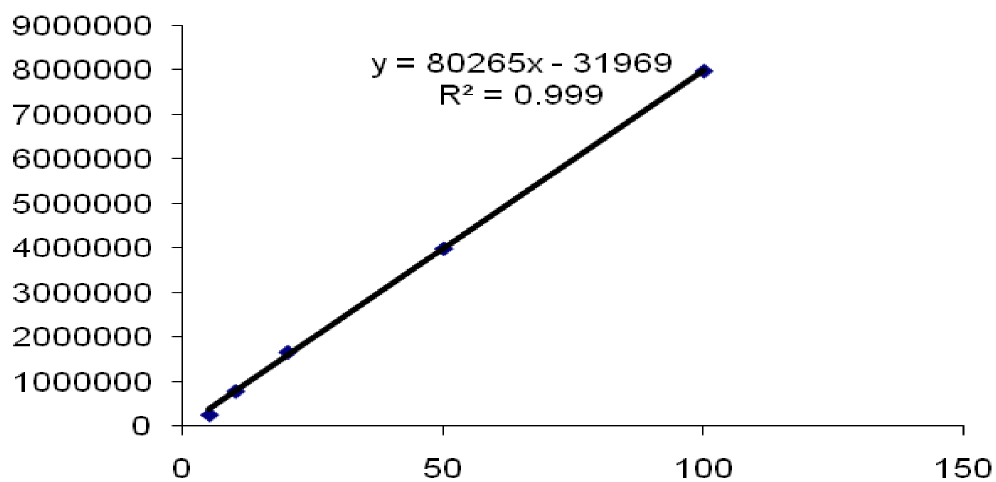


Figure B-8 Calibration curve of standard *p*-coumaric acid

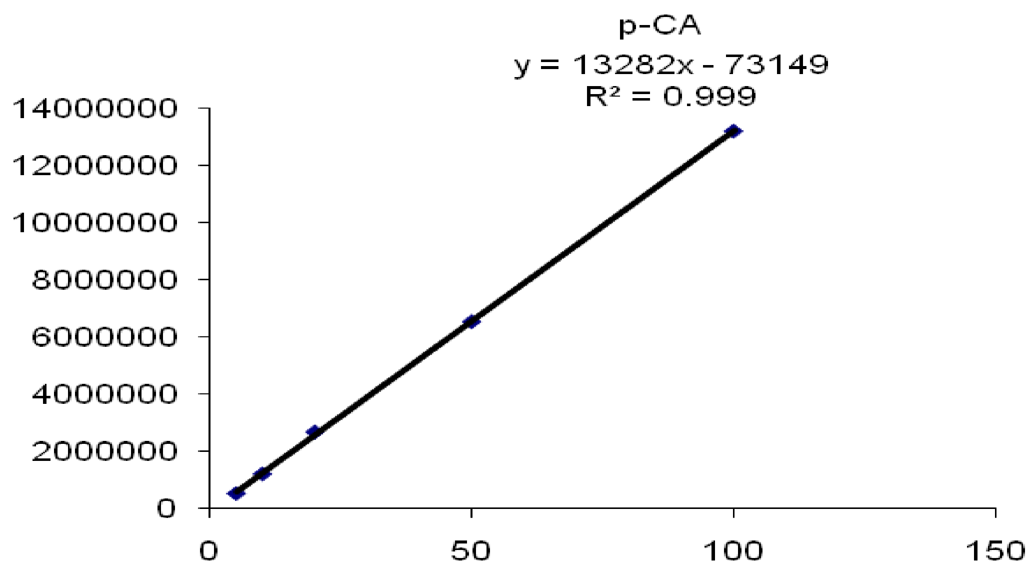


Figure B-9 Calibration curve of standard ferulic acid

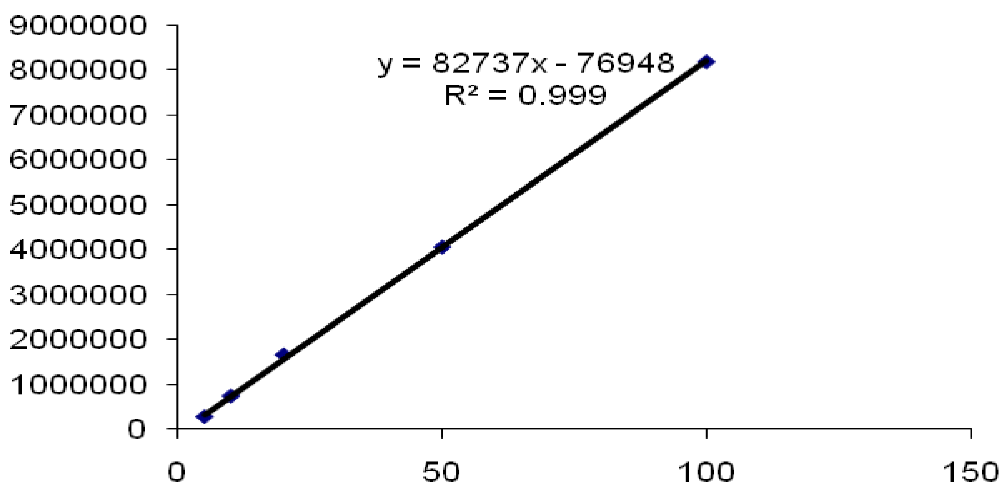
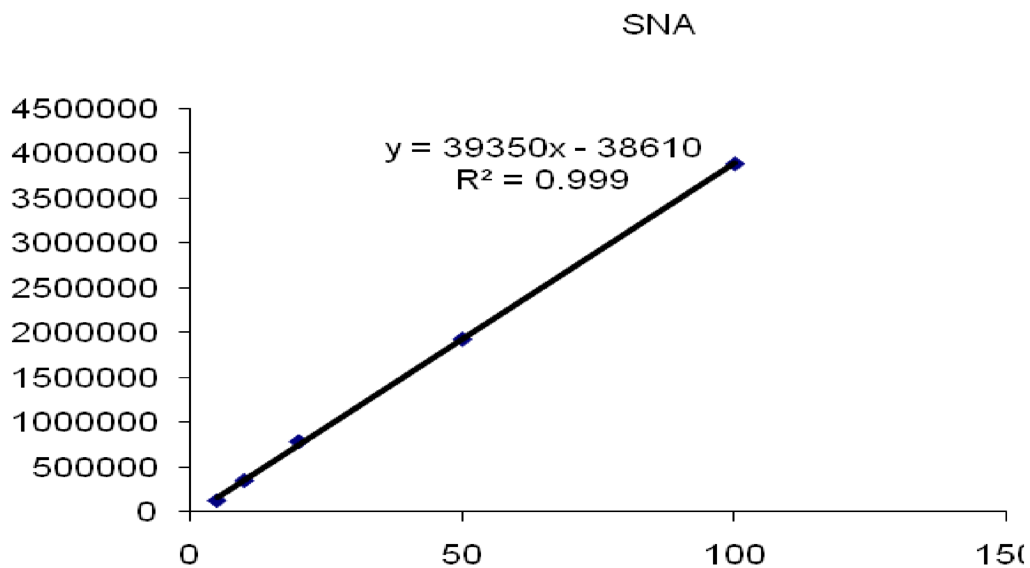


Figure B-10 Calibration curve of standard sinapic acid



Appendix C

GC Chromatograms of fatty acids

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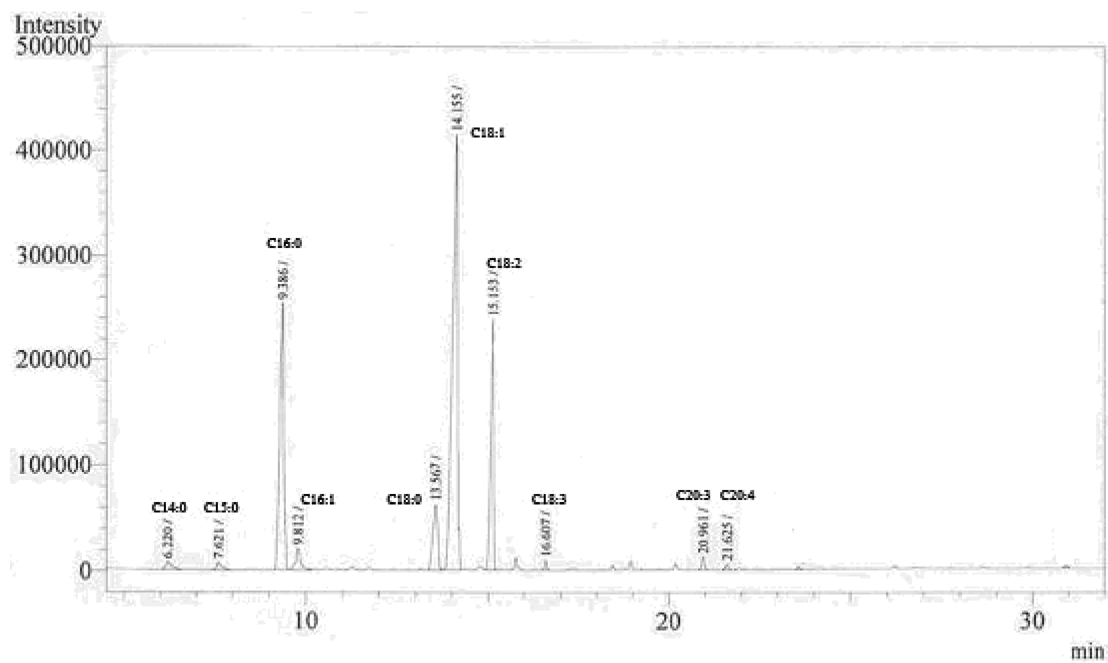
Figure C-1 GC Chromatograms of fatty acids



Appendix D

HPLC Chromatograms of γ-oryzanol

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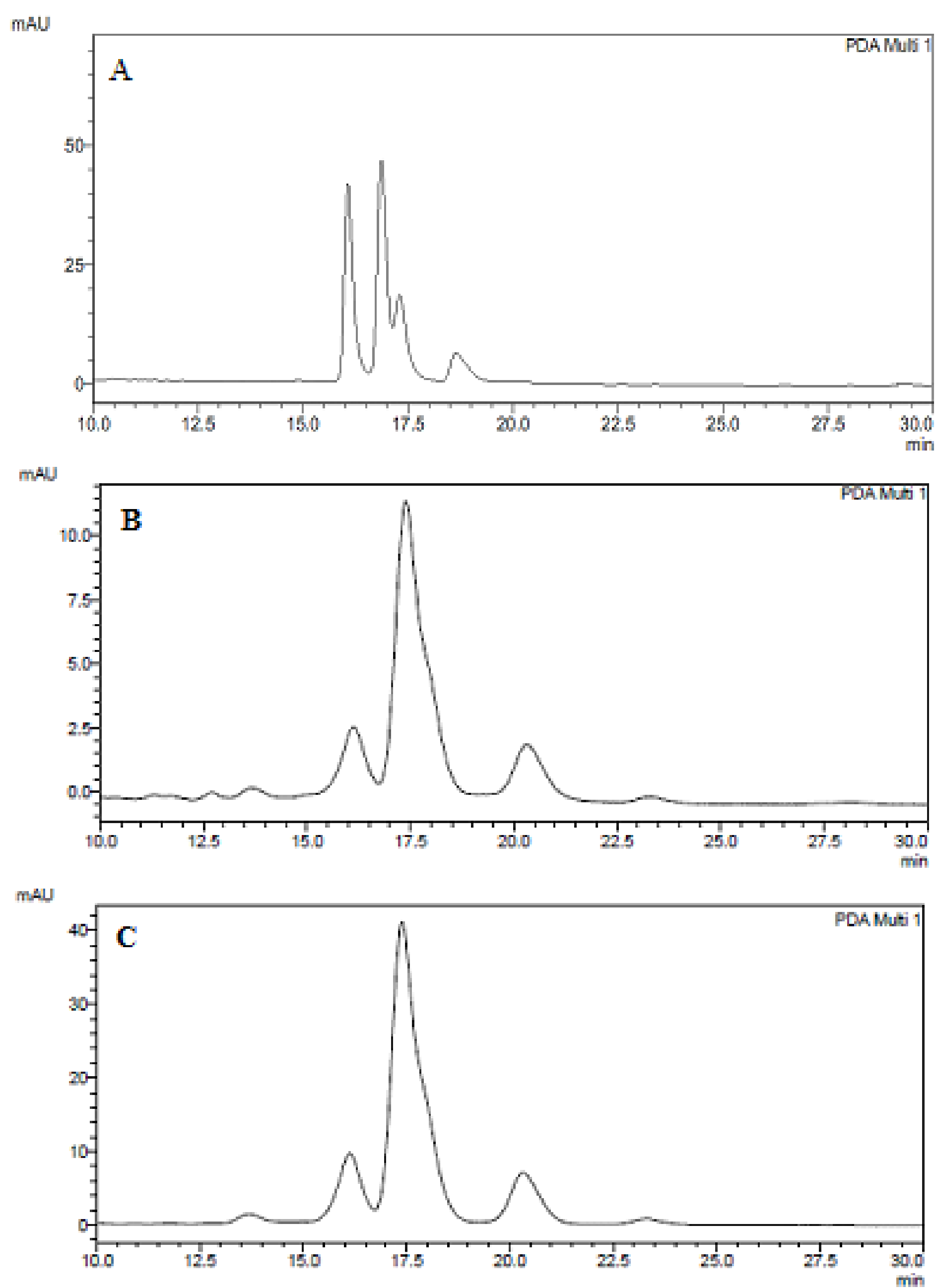
Figure D-1 Typical chromatograms of: (A) standard γ-oryzanol, (B) raw glutinous rice and (C) glutinous rice soaked at the soaking temperature of 60°C and NaCl content of 1.5%.



Appendix E

Lists of publications and oral presentations

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LISTS OF PUBLICATIONS AND ORAL PRESENTATIONS

Publication

Thammapat, P., Meeso, N., Siriamornpun, S. Effects of NaCl and soaking temperature on the phenolic compounds, α-tocopherol, γ-oryzanol and fatty acids of glutinous rice. Food Chemistry 2015; 175 : 218-224.

Oral presentations

18th World Congress on Clinical Nutrition (WCCN) 2014, December 1-3, 2014.

The topic presented was the effect of soaking conditions on resistant starch of glutinous rice : Optimization of soaking conditions.

RGJ seminar series Science and Technology for sustainable development January 31, 2013.

The topic presented was the effect of soaking conditions on DPPH radical scavenging activity of rice : Optimization of soaking conditions.

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Biography

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