Studies of Glycemic Index of Rice and Flour Rice of Indigenous Southern Thai Rice: Sangyod Phatthalung

Ruttikan Inpun

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Functional Food and Nutrition

Prince of Songkla University

2014

Thesis Title	Studies of Glycemic Index of Rice and Flour Rice of Indigenous Southern Thai Rice: Sangyod Phatthalung
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I hereby certify that this work has not been accepted in substance for any degree,
and is not being currently submitted in candidature for any degree.

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ACKNOWLEDGEMENT

I would like to acknowledge many people who enabled me to complete this thesis. I am grateful for the generous support of my advisors, Prof. Dr. Pavinee Chinachoti, who has always been enthusiastic about my research project, and given me much valuable advice on my thesis. Thanks to Associate Prof. Ruttana Leelawattana, MD., who has guided me towards the end in supportive and patient way. I thank Songklanakarind hospital, Prince of Songkla University, Hatyai, Thailand, who provided invaluable help with statistical analysis and responded patiently to my requests for further analyses. I also extend a special thanks to my Co-advisor assistant Prof. Dr. Santad wicheinchot and my committee assistant. Prof. Dr. Piyarat Sirivongpaisal and Prof. Dr. Onanong Naivikul who has been an unending source of all sorts of advice.

I would like to say thank you to all the study volunteers, and my dedicated research assistants, who came early in the morning for fasting blood sugar. There are many other people without whom I would have never completed my study. Thank you to the laboratory technicians of Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla and laboratory technicians of Nutraceutical and Functional Food Research and Development Center, Prince of Songkla University, Hatyai, Thailand for giving me lots of valuable advice on the study and thesis. Thanks to Higher Education Research Promotion and National Research University Project of Thailand (NRU) for giving a generous funding my research project.

Finally, I would like to say special thank you to my family for their continued support. Most of all, I thank my husband and best friend, Mathee Suwaluk, who has been extremely supportive throughout this journey, and shared my stress and joy. This completion is equally his achievement. He made this all possible for me.

Ruttikan Inpun

ชื่อเรื่องวิทยานิพนธ์ การศึกษาดัชนีไกลซีมิกของข้าวและแป้งข้าวพันธุ์พื้นเมืองของภาคใต้: สังข์หยดพัทลุง

ผู้เขียน รัตติกาล อินปั๋น

สาขาวิชา อาหารเพื่อสุขภาพและโภชนาการ

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บทคัดย่อ

ข้าวสังข์หยดพัทลุงเป็นพันธุ์พื้นเมืองของภาคใต้ของประเทศไทยและใช้ในการศึกษานี้ ประกอบด้วยข้าวทั้งเมล็ดและแป้งข้าวของ ข้าวขัดขาว ข้าวกล้อง และข้าวกล้องงอก โดยเปรียบเทียบสมบัติการย่อยและดัชนีไกลซีมิก จากการศึกษาสมบัติทางเคมีพบว่า มีปริมาณโปรตีนร้อยละ 7.81, 8.22 และ 8.30 ปริมาณไขมัน 0.51, 3.18 และ 2.80 และปริมาณเถ้า 0.75, 1.36 และ 1.03 ตามลำดับ ข้าวกล้องมีปริมาณโพแตสเซียมและฟอสฟอรัสสูงกว่าข้าวกล้องงอกและข้าวขัดขาว แต่ข้าวกล้องงอกมีปริมาณเหล็ก สังกะสี โซเดียม แคลเซียม และแมกนีเซียม สูงกว่าข้าวกล้องและข้าวขัดขาว ปริมาณใยอาหารรวมของข้าวขัดขาว ข้าวกล้อง และข้าวกล้อองอก มีปริมาณรอยละ 1.40, 3.12 และ 2.91 ตามลำดับ โดยข้าวกล้องและข้าวกล้องงอกมีใยอาหารรวมสูงกว่าข้าวขัดขาวอย่างมีนัยสำคัญ ($P<0.05$) พบว่าข้าวกล้องเป็นอาหารที่มีใยอาหารรวมสูง (มากกว่าร้อยละ 3 ของตัวอย่างแห้ง) การเกิดเจลาติไนซ์ของแป้งข้าวทั้งสามตัวอย่างพบว่ามี T_0 และ T_p ใกล้เคียงกัน โดยที่ T_p ของข้าวกล้องงอกมีค่าต่ำสุด (87 องศาเซลเซียส) ซึ่งสูงกว่าข้าวขัดขาว (91 องศาเซลเซียส) และข้าวกล้อง (96 องศาเซลเซียส) การเปลี่ยนแปลงเอนทัลปีของขัดขาวมีค่าต่ำสุด เอนทัลปีของข้าวแต่ละชนิดไม่แตกต่างกันอย่างมีนัยสำคัญ ($P<0.05$) ซึ่งมีผลในการลด T_e อย่างเห็นได้ชัด ประกอบกับการลดลงของเอนทัลปีหรือปริมาณเกลียวของแป้ง มีผลทำให้ข้าวกล้องงอกหลังผ่านการหุงมีเนื้อสัมผัสนุ่มขึ้น ปริมาณเถ้าสูงของข้าวกล้องพบว่าทำให้ T_e เพิ่มขึ้นอย่างมีนัยสำคัญ ($P<0.05$) จากข้อมูลองค์ประกอบและอุณหภูมิการเกิดเจลาติไนซ์ของแป้งอาจส่งผลต่อลักษณะเนื้อสัมผัสของข้าวสังข์หยดพัทลุง

การศึกษาการย่อยของข้าวทั้งเมล็ดและแป้งข้าวสังข์หยดพัทลุง พบว่าข้าวทั้งเมล็ดถูกย่อยในอัตราที่ช้ากว่าแป้งข้าวทำให้ดัชนีไกลซีมิกแป้งสูงกว่าข้าวทั้งเมล็ด ($P<0.05$) ซึ่งแป้งข้าวมีค่าดัชนีไกลซีมิกสูงและข้าวทั้งเมล็ดมีค่าดัชนีไกลซีมิกปานกลาง ผลการตรวจสอบชนิดและปริมาณของสารประกอบฟีนอลลิกพบว่า สารประกอบฟีนอลลิกหลักในข้าวสังข์หยดพัทลุง คือ กรดโปรโตคาเตชูอิก ปริมาณ 1.1 และ 1.5 mg/100 g ตัวอย่างแห้งของข้าวกล้องที่ผ่านการให้ความร้อนและไม่

ผ่านการให้ความร้อน ตามลำดับ ปริมาณของกรดโปรโตคาเตชูอิกของข้าวขัดขาวที่ผ่านการให้ความร้อนและไม่ผ่านการให้ความร้อน คือ 0.7 และ 0.85 มิลลิกรัมต่อ 100 กรัมตัวอย่างแห้ง ตามลำดับ และยังพบว่า ข้าวกล้องงอกมีปริมาณสารประกอบฟีนอลลิกลดลงเนื่องจากการสูญเสียในระหว่างกระบวนการเตรียมและการงอก สมบัติทางกายภาพ-เคมีของการก่อเกลียวระหว่างอะไมโลสกับอะไมโลเพกติน และอะไมโลสกับไขมันมีอยู่ในตัวอย่างข้าวทั้ง 3 ชนิด มีความแตกต่างกัน แต่ไม่มีความสัมพันธ์กับกิจกรรมของอัลฟา-อะไมเลส ดังนั้นการบริโภคข้าวกล้องสังข์หยดช่วยลดการย่อยและดูดซึมน้ำตาลส่งผลให้อัตราการดูดซึมแป้งลดลงและยังทำให้ดัชนีไกลซีมิกต่ำ ซึ่งสมบัตินี้มีความสำคัญในการควบคุมน้ำหนักและเหมาะสมในการประยุกต์ใช้กับผู้ป่วยเบาหวาน

การศึกษาดัชนีไกลซีมิกทางคลินิกของข้าวสังข์หยดพัทลุง 3 ชนิด ได้แก่ ข้าวขัดขาว ข้าวกล้อง แป้งข้าวกล้อง และอาหารมาตรฐาน คือ น้ำตาลกลูโคส ขนมปังขาว พบว่า ในกลุ่มข้าว แป้งข้าวกล้องมีการเปลี่ยนแปลงของระดับน้ำตาลในเลือดสูงสุด (high GI) ในนาทีที่ 30 หลังจากการบริโภค นอกจากนี้แป้งข้าวมีผลต่อการลดลงของระดับน้ำตาลในเลือดเร็วกว่าขนมปังขาว ข้าวกล้องและข้าวขัดขาว (low-medium GI) และนาทีที่ 120 และ 180 นาทีหลังบริโภคพบว่าแป้งข้าวมีระดับน้ำตาลในเลือดต่ำกว่าระดับน้ำตาลในเลือดตกลงในนาทีเริ่มต้น ตัวอย่างข้าวทั้ง 3 ชนิด ให้พื้นที่ใต้กราฟน้อยกว่าอาหารมาตรฐาน (กลูโคสและขนมปัง) อย่างมีนัยสำคัญ ($P<0.05$) พื้นที่ใต้กราฟที่ 2 และ 3 ชั่วโมงหลังจากบริโภคตัวอย่างข้าวขัดขาวและข้าวกล้องมีการดูดซึมกลูโคสไม่แตกต่างกัน แต่แตกต่างกับแป้งข้าวอย่างมีนัยสำคัญ ($P<0.10$) จากการศึกษาดัชนีไกลซีมิกของข้าวสังข์หยดพัทลุงพบว่ามีค่าอยู่ในช่วง 38-64 โดยข้าวกล้องและข้าวขัดขาวมีค่า GI ไม่แตกต่างกันอย่างมีนัยสำคัญ ($P<0.10$) แต่มีความแตกต่างของการตอบสนองต่ออินซูลิน ซึ่งพบว่าการบริโภคแป้งข้าวกล้องตอบสนองต่ออินซูลินมากกว่าข้าวกล้องและข้าวขัดขาวอย่างมีนัยสำคัญ ($P<0.10$) ดังนั้นการบริโภคข้าวสังข์หยดพัทลุงเป็นประโยชน์กับผู้ที่มีความบกพร่องในการหลั่งอินซูลิน

Thesis Title Studies of Glycemic Index of Rice and Flour Rice of Indigenous Southern Thai Rice: Sangyod Phatthalung

Author Miss Ruttikan Inpun

Major Program Functional Food and Nutrition

Academic Year 2013

ABSTRACT

Polished (white), unpolished (brown) and germinated (brown, unpolished) Sangyod Phattalung rice were compared in starch hydrolysis and glycemic indeces (HI and GI). Comparing nutrient and composition, the three grains were similar in protein (7.81, 8.22 and 8.30 %, respectively) but varied in lipid contents (0.51, 3.18 and 2.80 %) and ash contents (0.75, 1.36 and 1.03 %), respectively. Brown rice contains higher potassium and phosphorus than germinated and white rice, but germinated rice contains higher amounts of iron, zinc, sodium, calcium and magnesium than brown rice and white rice ($P<0.05$). Gelatinization of rice flours was found to show similar T_0 and T_p for all three samples. Final gelatinization temperature for germinated rice was lowest at 87 ^{o}C followed by that of white rice at 91 ^{o}C and brown rice at 96 ^{o}C. No significant difference in transition enthalpy was observed although white rice had a tendency to have less enthalpic values due to heat destruction during the polishing process. The process of wetting and digestion in germination of rice resulted in starch hydrolysis of the amorphous chain that resulted in lowering in T_e markedly without lowering the enthalpy or helical content this resulted in texture softening of the cooked rice grains. The higher ash content in brown rice was found to raise T_e of the sample significantly contributing to relatively tougher or harder texture.

Starch digestibility of Sangyod Phattalung brown rice grain was found to be were hydrolyzed at rates slower than its flour counterpart. Estimated GI for flour was considered high GI and whole grains low GI (significantly different $P<0.05$). The main phenolic compound was found to be protocatechuic acid (PCA) at levels 1.1 and 1.5 mg/100 g dry sample for cooked and uncooked brown (unpolished) rice, respectively, and 0.7 and 0.85 mg/100 g (dry sample) for cooked and uncooked unpolished, germinated rice, respectively. PCA (and together with other phenolics) was found to inhibit α-amylase activities. Physico-chemical change in amylopectin

and amylose helical formation and amylose-lipid complex (AMC) were present in the three rice samples differently and showed a presence of damaged starch but did not seem to correlate with α-amylase activities, i.e., the GI (*in vitro*) values of white rice was found to be highest but its damaged rice proportion the lowest. Hence GI or amylase activity in this case did not increase with the degree of rice damage.

In 10- human volunteers, it was indicated that whole grain Sangyod Phattalung rice were lower in glycemic and insulin response. The serum glucose level 30 minutes after ingestion of the two controls (glucose and white bread) declined rate of serum glucose was more rapidly than brown rice and white rice giving the rapid drop of the glucose level at 120 minutes to lower than base line but could sustain levels through 180 minutes flour and whole grain. All rice meals gave much lower area under curve (AUC) than of glucose and were similar to of bread. AUC insulin from rice meals were lower than of bread and glucose significantly ($P<0.05$). Among rice meals, white rice as well as brown rice provided further glucose absorption into blood stream leading to larger area of serum glucose at 3 hour than at 2 hour similarly to white bread. Sangyod rice grains showed a low GI of 40 % whereas the rice flour showed a medium GI of 60%. Blood insulin level (and insulin index or II) for volunteers fed with rice flour was significantly higher than those fed with rice grains. Sangyod rice is of great benefits to people with limited pancreatic beta-cell reserve.

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I. Inpun, R., Wichienchot, S. and Chinachoti, P. 2013. Chemical, physico-chemical and nutritive values of grain and flour of Sangyod Phatthalung rice. Proceeding of Congress on Science and Technology of Thailand, 39: 964-969.

II. Inpun, R., Wichienchot, S. and Chinachoti, P. 2014. Digestibility and α-amylase inhibition of Sangyod Phatthalung rice (*Oryza sativa* L.). J. Funct. Foods. Submitted, (in process).

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ABSTRACTS

39th Congress on Science and Technology of Thailand (STT 39)

Innovative Science for a Better Life

October 21 - 23, 2013
Venue: Bangkok International Trade & Exhibition Centre (BITEC), Bangkok, Thailand

Organized by:
The Science Society of Thailand under the Patronage of His Majesty the King
in Association with Faculty of Science,
King Mongkut's University of Technology Thonburi

I_I0007: CHEMICAL, PHYSICO-CHEMICAL AND NUTRITIVE VALUES OF GRAIN AND FLOUR OF SANGYOD PHATTHALUNG RICE

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Abstract: Sangyod Phattalung rice is an indigenous Southern Thai rice cultivar that is purple-black in color. In this study, polished (white) Phattalung rice, unpolished (brown) rice and germinated (brown, unpolished) rice were obtained. The protein contents were 7.81, 8.22 and 8.30% (total basis), respectively. The lipid contents were 0.51, 3.18 and 2.80% (total basis), and ash contents of 0.75, 1.36 and 1.03% (total basis), respectively. Brown rice contains potassium and phosphorus higher than germinated and white rice, but germinated rice contains higher amounts of iron, zinc, sodium, calcium and magnesium than brown rice and white rice. Gelatinization of rice flours was found to show similar T_0 and T_p for all three samples. Final gelatinization temperature for germinated rice was lowest at 87 °C followed by that of white rice at 91 °C and brown rice at 96 °C. No significant difference in transition enthalpy was observed although white rice had a tendency to have less enthalpic values due to heat destruction during the polishing process. The process of wetting and digestion in germination of rice resulted in starch hydrolysis of the amorphous chain that resulted in lowering in T_e markedly without lowering the enthalpy or helical content this resulted in texture softening of the cooked rice grains. The higher ash content in brown rice was found to raise T_e of the sample significantly. In this work, it could be concluded that data from composition and gelatinization temperature are useful information for describing white rice, brown rice and germinated rice and in understanding texture attributes of Sangyod Phatthalung rice. (full paper available on CD)

I_I0008: QUALITY OF FRESH AND RETORTED NARROW-STRIP RICE NOODLE CONTAINING CROSS-LINKED STARCH AND HYDROCOLLOIDS

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Abstract: The main objective of this study was to investigate the effect of cross-linked tapioca starch, hydroxypropyl methycellulose (HPMC) and xanthan gum on physical and sensory properties of fresh and sterilized narrow-strip rice noodle. Three levels of cross-linked starch (5, 10 and 15%) and fixed level of HPMC and xanthan gum (<0.5%) were used to create 6 noodle formulas. Texture and color of the noodles were determined by texture analyzer and colorimeter, respectively. Results showed that, for each type of hydrocolloid used, hardness of the fresh and retorted noodles tended to decrease when the level of cross-linked starch increased. For the same level of cross-linked starch used, hardness of the retorted samples containing xanthan gum was significantly higher than that of the samples containing HPMC ($p<0.05$). Cross-linked starch and hydrocolloids had little effect on the L, a, b color values and whiteness index of fresh and retorted noodles. Only the retorted samples containing 10% and 15% of cross-linked starch (4 formulas) were selected for the sensory evaluation. Results from acceptance test using 7-point hedonic scale showed that hedonic scores of color and cohesiveness of every formula were not significantly difference ($p\geq0.05$). The mean scores of hardness, elasticity and overall acceptability of the control were significantly lower than those of the other formulations ($p<0.05$). Although the overall acceptability scores of the formulas containing cross-linked starch and hydrocolloids were not significantly different ($p\geq0.05$), hedonic scores of elasticity of the samples containing 15% cross-linked starch were significantly higher ($p<0.05$). Therefore, those formulas could be recommended for retorted narrow-strip noodle. (full paper available on CD)

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Adding ORCID to Co-Authored submission

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Journal: Journal of Functional Foods
Title: Digestibility and <alpha>-amylase inhibition of Sangyod Phatthalung rice (Oryza sativa L.)
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Journal of Functional Foods

OBJECTIVES

1. To study chemical composition, physicco-chemical properties and nutritive value of grain and flour of Sangyod Phatthalung rice.
2. To study *in vitro* α-amylase digestion and glycemic index values in grain and flour rice of white rice, brown rice and germinated Sangyod rice
3. To study *in vivo* glycemic and insulin index values in healthy volunteers comparing different Sangyod rice samples.

SCOPE OF STUDIES

Study of nutritional, physical, starch digestion properties and in vitro glycemic index (*in vitro* GI study) will be compared of white, brown and germinated brown of Sangyod Phatthalung rice. The best appropriate starch digestion and *in vitro* GI will be chosen for *in vivo* studies to determine the effects on plasma glucose and plasma insulin response in healthy volunteers.

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Carbohydrate is main component of rice providing energy for the body, but over consumption can lead to obesity and diabetes. Diabetics suffer partly from unhealthy levels of carbohydrate consumption particularly, simple and metabolizable carbohydrates (such as sugar and starch). Today, consumers have become health conscious and desire to control carbohydrate utilization in the body, for example, by chosing slowly digesting carbohydrate, keeping the blood sugar at a moderate level preventing diabetes and metabolic syndrome. In addition**,** slow digesting carbohydrates are attractive for beauty conscious and fashionable consumer. In a world of rapidly changing food habits and stressful life style it is increasingly recognized that a healthy digestive system is essential for overall quality of life. This recognition has led to development of foods that are designed to contribute to a healthy digestive system and eventually to the maintenance of general wellbeing (Shi and Gao, 2011). Rice is a staple food of Asia where 90 % of the world's crop is produced and consumed (Juliano, 1985). That is the major source of energy for most world population. Sangyod Phattalung rice is rice of indigenous southern Thai rice cultivars. Milling of rice in to flour can have positive and negative effect for digestion of starch in the human digestive system. Physical structural change caused by milling, for example, and the composition of rice may affect digestion properties. Unpolished rice has been known to provide healthy effect due to its richness in iron, phenolic compound, fiber and antioxidant. Unpolished rice also contains high level of protein and lipid. Digestion of unpolished rice is believed to result in a reduced rate of glucose absorption and lower glycemic index (GI). Sangyod Phattalung is typically unpolished with outer layer of its grain containing pigment (photochemical) nutritional component. Therefore, this study was to compare *in vitro* and *in vivo* glycemic index of three samples, including white rice, brown rice and germinated rice by rapid *in vitro* α-amylase digestibility assay and *in vivo* glycemic and insulin response. The GI of rice grain and flour was used as indication of the characteristics of the Sangyod grain in its potential rice or carbohydrate

choices for patients with diabetes which was the information used in decision making for consumers.

Literature review

1. Rice

Rice is the major staple foods for a half of the world's population. There are about 90% of its production and consumption in Asia. White rice is polished rice it has removed husk, bran, and germ. Polishing method can improve the texture and appearance of the rice and extend long shelf life of polished rice. The milling and polishing processes both remove important nutrients of bran and germs. Brown rice or unpolished rice it is a kind of whole grain, which is more healthy alternative than polished rice because they are more nutritious than polished rice. Brown rice (unpolished) is more expensive than polished white rice due to difficulty of storage technology and transport. Moreover, brown rice and polished rice have similar of energy and carbohydrates content. Brown rice has been advocated as healthier alternatives. The bran in brown rice contains significant dietary fiber and the germ contains many vitamins and minerals. Comparison between unpolished (brown) and polished (white) rice in terms of protein, mineral and vitamin, carbohydrate and fat content suggests that neither is a complete nutrition source. There is a significant difference in fiber content and nutrients in polished and unpolished. Germinated rice is made by soaking brown rice in water until its root grows to 0.1 mm (Moongngarm, 2010). During germination hydrolytic enzymes activate digestion of starch, fibers and proteins softening the texture of cooked rice (Chung *et al.,* 2012). Germination processes help increase mono-, di-saccharides, amino acids, γ-oryzanol, γ-aminobutyric acid, dietary fiber, ferulic acid, magnesium, potassium, zinc and tocotrienols (Chung *et al*., 2012). Therefore, germinated rice is considered healthy.

2. Sangyod Phattalung rice

Sangyod Phatthalung rice is a traditional rice variety grown in the area of Phatthalung province for hundreds of years. It typically is small and long-slender grain, dark red pericarp, soft and aromatic of cooked rice. Pigmented rice had the highest antioxidant activities (Nam *et al.,* 2006). Red rice had higher total phenolic, flavonoid than not pigmented rice their higher pro-anthocyanidins and anthocyanins contents, respectively (Min *et al.,* 2012). Nam *et al*. (2005) studied pigmented rice extracts found they can inhibit phorbol ester-induced tumor promotion in marmoset lymphoblastoid cells B95-8 in vitro.

3. Glycemic index of rice

GI is a measurement of glucose response after a carbohydrate consumption compared with a carbohydrate reference food typically glucose or white bread of equal amounts. GI is used as criteria for carbohydrate consumption of consumers. A low GI diet results in a slow of blood glucose response than medium GI diet and high GI diet.

Carbohydrates are main component (75-80 percent) of rice (Chan *et al.,* 2001). GI of rice is between 54-121depending on the growing location and cultivars (Miller *et al*., 1992). Therefore, rice and rice products when consumed in high amount result in a high GI, which is not appropriated to diabetics. Rice with high amylose content is generally lower GI than rice with low and medium amylose content and waxy rice. High amylose starch resulted in better health (Sajila *et al.,* 2006). Brown rice contains complex carbohydrates that are digested and absorbed slowly in small intestine, leading to elevated blood glucose response. The U.S. Food and Drug Administration approved brown rice used as whole grain cereal that is low in fat, saturated fat and cholesterol. It is claimed to help prevent heart disease and cancer (U.S. Food and Drug Administration. 2013). Williams (2004) reported that digestion resistance of brown rice could be dependent on the amount and type of fiber and many factors such as the rice species, ratio of amylose and amylopectin. Rice with higher amylose content showed reduced level of sugar in blood (Hu *et al.,* 2004). Essential lipids can help reduce digestion and absorption (Krog, 1971). The combination of amylose and long-chain saturated monoglyceride leads to harder digestion than short-chained fatty acids and long chained unsaturated fatty acids (Guraya *et al*., 1997). Factors such as the amounts and types of phenolic compounds in rice as well as preparation and processing of rice can affect digestion and consequently blood sugar levels. Bjorck, (1996) reported that preparation and cooking temperature can impact the rate of rice digestion. Rashmi and Urooj (2003) reported that heat (steam) shifted amylose structure from open coils to helical structure it is form of crystalline amylose structure. When a lipid is bound amylose helix the hydrophilic and interact with helical structure potentially reducing the rate of digestion.

When brown rice grains are treated with moisture to 35 to 36 percent, the seeds begin germination process (Komatsuzaki *et al.,* 2007). During growth, biochemical process seedings begins when water migrates into the rice grains to stimulate enzymatic activity in the rice grains. Enzyme such as protease enzyme digests proteins into amino acids and peptides. Amylase

enzyme digests the starch or polysaccharides into oligo- di- and monosaccharaides that can be used as instant energy. It also has a collection of compound such as γ-aminobutyric acid (GABA), tocopherol, tocotrienol, γ-orazynol etc.

Wanwilai *et al*. (2553) studied content of enzymes α-amylase of Hom Mali brown rice and Munphoo brown rice during germination increasing time for soaking increases amylase enzyme content as well. Musa *et al*. (2011) studied the physical properties of starches from rice species native of Malaysia. The amounts of amylose in white rice, brown rice and germinated brown rice have been reported as 25.77, 23.83 and 21.78 respectively. Amylose content of germinated brown rice distinctively differs from indicating white rice and brown rice significantly. Hagiwara *et al*. (2004) studied the effect of germinated brown rice on blood glucose response and PAI-1 level in normal and diabetic rats found that can improve positive blood glucose response, serum PAI-1 and lipid peroxide concentration in diabetic rats in comparison with normal rats in same fed.

4. Phenolic compound properties on the inhibiting of the α-amylase enzyme.

4.1 Phenolic compound of rice

Phenolic compounds are a secondary metabolism of plant that had beneficial effects in human (Soobrattee *et al*., 2005). There are rather a small but important food components from the functional health aspect which is characterized by their antioxidative activity (Adum and Lui, 2002; Kim *et al.,* 2005). Generally in rice, ferulic acid and ρ-coumaric acid are the major of phenolics compounds that are present in a form of free -soluble and bound –insoluble forms (Tain *et al*., 2004).

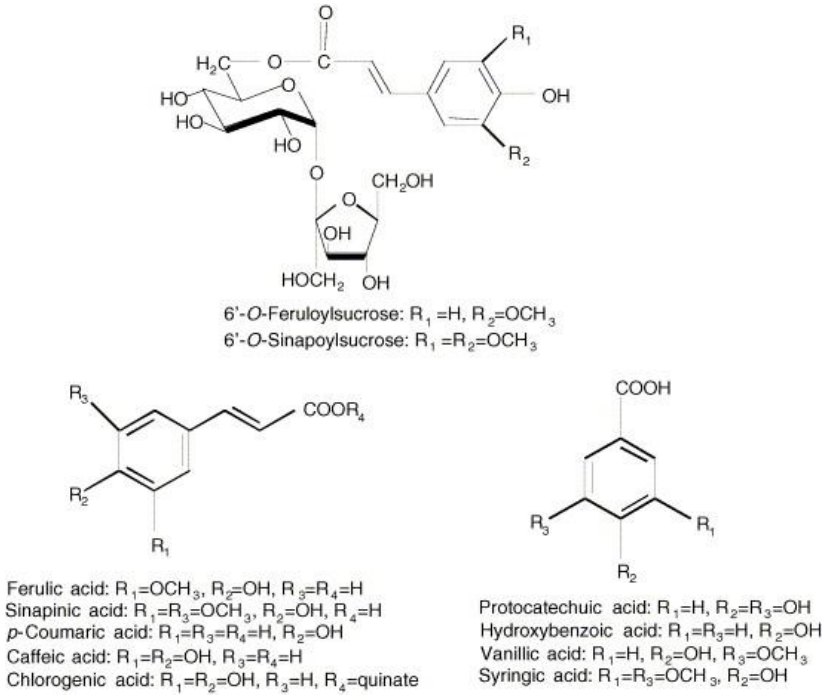

Figure 1 Chemical structure of phenolic compounds of rice

Source: Tain *et al*. (2005)

4.2 Phenolic compound properties on inhibit the alpha amylase enzyme

Manaharan *et al*. (2011) reported that extracts of phenolics compound of plant can give more anti glycemic response than acarbose (commercial carbohydrate inhibitor). The inhibitory activity of ferulic acid on aldose reductase can be related to the prevention of diabetic complications (Lin and Lai, 2011). Moreover, phenolics compounds can inhibitors α-amylase and α-glucosidase, the both enzymes involved in the control of blood glucose level. Donkor *et al*. (2012) report that bioactive compound of geminated grain deducted risk of diabetic agent and colon cancer.

5. Chemical-physical properties of starch

Differential Scanning Calorimetry (DSC) are used to study the thermal properties by measuring the temperature and heat flow during heating (thermal transition) of a material as compared to the reference at a given temperature or time. Gelatinization of starch is a break down the order structure of starch. In the gelatinization process, starch and water are heated to pass characteristic melting or gelatinization temperature. During gelatinization, water is first absorbed

in the amorphous regions of amylopectin chains, in semi-crystalline lamellae which leads to a swelling of the starch granule and increase in viscosity. During heating, the thermal energy is used to break the hydrogen bonds that are in the crystalline regions of amylopectin molecules in the starch granules (Jenkins and Donald, 1998). Water enters amorphous regions of amylopectin which are swollen prior to a disruption of double helical structures as heat energy causing crystalline structure to melt and break free (Zobel, 1984). Thermogram of DSC can be calculated qualitatively from resulting thermal changes caused by a shift in heat capacity during phase change. Extensive heating pass gelatinization temperature leads to leaching of amylose from inside to outside of the granules and a destruction of the granulars structure. DSC study of gelatinization of starches are common (e.g., Normard and Marshall, 1989; Russell and Juliano, 1983). Gelatinization temperatures of starches from different varieties of cereals depend on the presence of proteins, fats, salts and sugars (Marshall *et al*., 1990; Jang *et al*., 2001; Maaurf *et al*., 2001).

6. Digestibility of starch

6.1. Amylose content

Rice starch is composed of two polysaccharides: amylose (15-20% by weight) and amylopectin (80-85% by weight), (Miller *et al*., 1992, Benmoussa *et al.,* 2007). High-amylose rice is likely slowly digested due to shape, chain, portion size of the starch. Amylose is a linear chain with α-(1, 4) linkage, whereas, amylopectin a branched molecule with α-(1, 4) and α-(1, 6) linkages (Sajilata *et al*., 2006). Amylose chain becomes more helical (crystallized) in water than amylopectin chain (Panlasigui *et al*., 1991, Kavita and Prema, 1997). Amylopectin contain more amorphous chain than amylose and hence more susceptible to digestive enzymes (Yang *et al*., 2006; Singh *et al*., 2010).

6.2. Fat content

Lipid content of unpolished rice is higher than polished. Starch in cooked rice, contains amylose that is leached and form helical strands with lipids. The amylose and lipid complex disintegrates at higher temperature. Lipid complex have been suggested to decrease the digestibility of starch and glycemic response (Krog, 1971). Crowe *et al*. (2000) reported fatty acids in lipid complex with starch that inhibited the hydrolysis of α-amylose. Moreover,

consuming rice with milk has been reported to result in lower glycemic response than rice alone (Wolever, 2009)

6.3. Dietary fiber

Dietary fibers usually are non-caloric carbohydrate polymers of cell walls of plant materials, which cannot be digested or absorbed in stomach and small intestine. It is commonly classified info two groups, insoluble (cellulose, hemicellulose and lignin) and soluble dietary fiber (resistant starch, pectin, arabinoxylans and fructans) (Johansson *et al*., 2004). Braaten *et al*. (1991) reported viscosity from dietary fiber can reduce the blood glucose response. Brennan and Cleary (2005) reported β-glucans ability to reduce postprandial blood glucose and insulin response in healthy volunteers.

6.4. Retrogradation

Cooked rice that stored at refrigerated and ambient temperature may retrograde. In this process, amylopectin recrystallizes by forming network among the linear fractions in a helical starch structure. During retrogradation, starch molecules begin to crystallize forming a fringe micelle hardening of the starch gel and weeping/releasing water (Ring *et al*., 1988). The reheating and cooling increase crystalized starch and may lead to slow digestion and absorption of starch. Retrogradation of starch may impact blood glucose response (Nayak *et al*., 2014). Baik *et al*. (1997) reported that recrystallization of waxy rice (non amylose) higher than non-waxy rice which includes amylose and amylopectin. Nayak *et al*. (2014) reported that higher degree of starch gelatinization affects to in vitro starch digestibility and blood glucose response.

6.5. Damaged starch

The integrity of starch granules can be affected by a milling process. Damaged starch granules are the breakage native starch (amylopectin) in flour (Shrestha *et al*., 2010). Damaged starch affects pasting properties, viscosity, solubility of starch (Barrera *et al*., 2011) and the gel formed (Tester, 1997). Leaching of damaged starch was found correlated with swelling properties of starch (Tester and Morrison, 1990). Tester, (1997) reported the amount of damaged starch to be usually greater than the amount of soluble carbohydrate present demonstrating that there was a greater degree of structural integrity in damaged starch granules than it would appear based on susceptibility to erosion by α-amylase digestion.

CHEPTER II

MATERIALS, EQUIPMENTS AND METHODS

1. Materials

1.1 Sangyod Phatthalung rice obtain from Phatthalung Province, Southern Thailand

1.2 Chemical reagents

1.2.1 2-hydroxy-3,5-dinitrobenzoic acid (DNS) (Merck, Germany)

1.2.2 3-(N-morpholino) propanesulfonic acid buffer; MOPS (Sigma, USA)

1.2.3 Acetic acid (J.T. Baker, USA)

1.2.4 Acetone nitrile CH_3CN (HPLC grade) (Merck, Germany)

1.2.5 Calcium chloride; $CaCL_2$ (Ajax Finecchem, New zealand)

1.2.6 Dimethyl sulphoxide; DMSO (Fluka, Germany)

1.2.7 Ethyl alcohol (J.T. Baker, USA)

1.2.8 Ethylenediaminetetraacetic acid ; EDTA (Merck, Germany)

1.2.9 Folin–Ciocalteu reagent (FCR) (Fluka, Germany)

1.2.10 Glucose (HK) Assay Kit (Sigma Chemical Co. Ltd., USA)

1.2.11 Hydro choric acid; HCl (J.T. Baker, USA)

1.2.12 Magnesium chloride; $MgCL_2$ (Ajax Finecchem, New zealand)

1.2.13 Potassium chloride; KCl (Ajax Finecchem, New zealand)

1.2.14 Sodium hydrogen carbonate; $NaHCO_3$ (Ajax Finecchem, New zealand)

1.2.15 Sodium acetate (Ajax Finecchem, New zealand)

1.2.16 Sodium hydroxide; NaOH (Ajax Finecchem, New zealand)

1.2.17 Trifluoroacetic acid (TFA); CF_3CO_2H (Sigma Chemical Co. Ltd., USA)

1.2.18 Water; H_2O (HPLC grade) (Merck, Germany)

1.3 Enzymes

1.3.1 α-amylase (Sigma Chemical Co. Ltd., USA)

1.3.2 Amyloglucosidase from *Aspergillus niger* (Sigma Chemical Co. Ltd.,USA)

1.3.3 Pancreatin from porcine pancreatin (Sigma Chemical Co. Ltd., USA)

1.3.4 Pepsin from gastric porcine mucosa (Sigma Chemical Co. Ltd., USA)

2. Equipment

2.1 Blender (Phillips, China)

2.2 Centrifuged (Mikro 22r, Hettichzentrifugen, UK)

2.3 Desicator

2.4 Glucometor (Accu-Chek Performa, Roche Diagnostics Corporation, Indianapolis, IN, USA)

2.5 Hot air oven (Memmert, D-91126, USA)

2.6 High-performance liquid chromatography; HPLC (Agilent 1200, Waldbronn, Germany)

2.7 Microplate reader (Biotek power wave X, USA)

2.8 pH meter (SCHOTT, USA)

2.9 Sieve 200 mesh

2.10 Timer

2.11 UV-Vis spectrophotometer (Biochrom, Lida S12, USA)

2.12 Water bath (Memmert, D-91126, USA)

2.13 Viscosity graph (Brabender analyzer, Gepruft, Germany)

2.14 Differential Scanning Calorimeter (DSC) (PerkinElmer- DSC7, Waltham, MA, USA)

2.15 Sonicated (Ultrasonic cleaner CD-4820 170w, Zhengzhou Xihua Medical Devices Co. Ltd., China)

2.16 Inductively Coupled Serum Atomic Emission Spectrometer (ICP-AES)

2.17 Hunter-Lab (Colour Flex, Hunter-Lab, USA)

2.18 Milling machine (Daming, made in China)

2.19 Electric rice cooker (International, China)

2.20 Beckman (Coulter, Fullerton, Calif, USA)

2.21 Modular E170 (Roche Diagnostics, USA)

3. Materials and Methods

3.1 Materials

Sangyod rice (*Oryza sativa* L.) from Phatthalung Province, Thailand was dehulled to make unpolished rice (brown and germinated) and polished (white) rice. Each was packed in plastic bags and transported to Prince of Songkla University. White rice and brown rice were washed twice; some washed brown rice was incubated for germination. The samples (500g) were dried by hot air oven at 55^{o}C for 10 h. Half of every sample was milled into flour. The milled samples were packed under vacuum in nylon bags and kept at 4^{o}C throughout the experiment.

3.1.1 Germination process

Germinated rice was prepared according to Tain *et al*. (2004)'s methods. Prepared germinated rice was steeped in distilled water at 27-30^{o}C in the dark for 24 hour. The steep water was changed every 4 h and drained at the end of soaking. The germinated seeds were dried at 55^{o}C

3.1.2 Flour milling

Rice was milled by flour milling machine 25,000 rpm (Daming, made in China). The rice flour was sifted through a 200 mesh sieve.

3.2 Methods

3.2.1 Chemical composition, nutrition, physical and thermal properties

All the samples were analyzed for proximate composition (moisture, lipid, protein and ash) by A.O.A.C. (2000) methods and analyse of minerals, including iron, zinc, sodium, calcium, magnesium, potassium and phosphorus and heavy metal including cadmium and lead by Inductively Coupled Serum Atomic Emission Spectrometer (ICP-AES). Total dietary fiber was analyzed by AACC (2010) 985.29 method. Color of grains and flour were determined by Hunter-Lab (Colour Flex, Hunter-Lab, USA). Prior to color measurements, the instrument was calibrated with light tap and white calibration tile. The colorimeter was set to an illuminant condition D65 and a 10° standard observer. Each sample was put in a cuvette and replaced in to the specular port site, the color parameters (a*, b* and L*) were then read and calculated c*= colour intensity, chroma (c*= $[a^{*}+b^{*}]^{1/2}$), h^{o} = hue angle ($h^{o} = \tan^{-1}[b^{*}/a^{*}]$) . Starch gelatinization properties were analyzed in triplicate using a Differential Scanning Calorimeter (DSC). Rice flour of about 3 milligram was placed in a 40 microliter aluminum pan and water was added to give a

sample-to-water weight ratio of 1: 3. The pan was sealed, and the sample was allowed to equilibrate overnight at 4 ˚C. The DSC temperature scan includes holding at 10˚C for 1 minutesute followed by heating from 10 to 95 ˚C at a rate of 10˚C/minute. Water activity (a_w) was analysed using Novasina Water activity meter.

3.2.2 Digestibility, hydrolysis index and glycemic index

3.2.2.1 *In vitro* rice and flour rice digestion

In vitro digestibility assay of the samples was based on glucometry which a modified method of Mahasukhonthachat *et al*. (2010); Srikaeo and Sopade, (2010) and Sopade and Gidley (2009). A half-gram sample was mixed with 5 mL deionize water and heated in a water bath at 85^{o}C for 30 min. Each sample was treated with 1.0 mL artificial saliva containing porcine α-amylase (Sigma A-3176, 250 U per mL in sodium acetate buffer) and 5 mL pepsin (Sigma P-6887, 1% of protein in sample in deionized water and 0.02 M, HCl pH 2.0 ratio 1:1 (v/v)) was added after incubation at 37˚C for 30 min in a reciprocating water bath. The digesta was neutralized with 0.2 NaOH and pH was adjusted to 6.0 with sodium acetate buffer prior to an addition of 5 mL pancreatin (Sigma P-1750 from porcine pancreas, 2 mg per mL of acetate buffer), 5 mL porcine α-amylase (Sigma A-3176, 250 U per mL in sodium acetate buffer) and 5 mL amyloglucosidase (Sigma A-7420 from *Aspergillusniger*, 28 U per mL in acetate buffer). The mixture was incubated at 37˚C for 4 hours the glucose concentration in the digesta was measured with a glucometor (Accu-Chek Performa, Roche Diagnostics Corporation, Indianapolis, IN, USA). Glucose concentration at specific periods (0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) were measured the amount of digested starch per 100 g dry starch (DS) was calculated using equation 1.

$$DS = \frac{0.9 \times 180 \times GG \times V}{W \times S[100 - M]} \quad \text{…….. [1]}$$

Where DS= digested starch (g per 100 g dry sample)

GG = glucometer reading (mM/L)

V = volume of digesta (mL)

180 = molecular weight of glucose

W = weight of sample (g)

S= starch content of sample (g per 100 g dry sample)

0.9= stoichiometric content for starch from glucose content

M= moisture content of sample (%moisture dry Sample)

Remaining undigested starch of digested samples was analyzed (method by Megazyme International Ireland Ltd., Ireland). Fifty mg sample was mixed with 400 microliter of 80% ethanol, heated in boiling water bath. Two mL dimethyl sulphoxide (DMSO) was added and then the sample was digested with thermo-stable α-amylase (Sigma A-3176) ratio 1:30 (v/v) with 3-(N-morpholino) propanesulfonic acid buffer (MOPS, Sigma M-9381) before sodium acetate buffer and amyloglucosidase (Sigma A-7420) were added and incubated at 37°C for 4 hour. Glucose content was determined using an enzymatic glucose reagent (Sigma, GAHK-20). Absorbance was measured in Biotek Power Wave X Microplate reader (Biotek, Winooski, VT, USA) at 340 nm. Each sample was analyzed in at least duplicate.

3.2.2.2 Total starch analysis

Total starch content of the samples was analyzed using a method derived from Megazyme (Megazyme International Ireland Co. Ltd., Ireland; Srikaeo and Sopade, 2010). About 50 mg of sample was wetted with 400 microliter 80% ethanol, heated (boiling water bath) in 2 mL dimethyl sulphoxide (DMSO) and digested with thermos table α-amylase (Sigma A-3176) (1:30) (v/v) in MOPS (Sigma M-9381) buffer before sodium acetate buffer and amyloglucosidase (Sigma A-7420) were added and incubated as described. Before, the glucose content was determined using an enzymatic glucose reagent (Glucose (HK) assay kit, GAHK20), and the absorbance of the coloration was measured by Microplate reader (Biotek power wave X, USA) at 340 nm. Each sample was analyzed in duplicate.

3.2.2.3 Hydrolysis index (HI) and glycemic index (GI)

The hydrolysis index (HI) of each sample was calculated by dividing the area under its digestogram by the area under the digestogram of a white bread. GI of the samples was also calculated (Goni *et al.,* 1996).

$$GI = 39.71 + (0.549HI) \quad \text{……..... [2]}$$

Values are given as mean ± SD from triplicate.

3.2.3 Total content, identification phenolics and inhibition α-amylase

3.2.3.1 Extraction

Phenolic extraction was done using the method reported by Min *et al*. (2012). Rice flour (5 g) was mixed with 25 mL deionize water and heated in a water bath 85^{o}C for 30 minutes. Twenty five mL ethanol was then added, sonicated (Ultrasonic cleaner CD-4820 170w, Zhengzhou Xihua Medical Devices Co., Ltd., China) for 10 minutes, and left in darkness for 90 minutes at room temperature. The mixture was centrifuged (Mikro 22r, Hettichzentrifugen, UK) at 5,000rpm for 10 minutes. The collected supernatant was filtered through filter paper (Whatman No.1). The filtered liquid was then mixed with 25 mL deionize water and then 20 mL hexane. After 15 minutes hand shaking, the liquid was rested to allow lipid separation; the aqueous portion was then further analyzed.

3.2.3.2 Analysis of total phenolic content

Total phenolic content was determined according to procedure described by Singleton *et al*. (1999). To one hundred microliter extract, 0.2 mL 1N Folin-Ciocalteu's reagent was added and mixed for 5 minutes. The mixture was neutralized with 0.3 mL of NaOH (0.5 M) and placed in 96-well plate allowing to mix for 2 hours. Absorbance at 760 nm was done using Microplate reader. Phenolics content in each extract was calculated using a calibration curve of ferulic acid standard (5–200 mgmL^{-1}). The results were expressed in mg of ferulic acid equivalent per 100 g of whole grain on dry weight basis (mg FA equiv.100g^{-1}). Analyses were performed at least in triplicates.

3.2.3.3 Identification of phenolic compounds

Phenolic compounds in extracts were characterized by reversed-phase High-performance liquid chromatography (HPLC) as described by Tain *et al*., 2004 and Tain *et al.,* (2005) with modification. All extracted samples were filtered through a 0.20-µm pore size syringe-driven filter before injection. A 10-µL aliquot of each sample solution was separated using a Agilent 1100 HPLC system (Agilent 1200, Waldbronn, Germany) equipped with a diode array detector on a 250 mm x 4.6 mm i.d., 5 µm, Eclipse XDB-C18, analytical column (Agilent, Santa Clara, CA, USA). The mobile phase was acetonitrile (A) and purified water with 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.8 mL/min. Gradient elution was done as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent A; from 5 to 15 min, 9% solvent A; from 15 to 22 min, linear gradient from 9 to 11% solvent A; from 22 to 35 min, linear gradient from 11 to 18% solvent A; from 35 to 42 min 18% solvent A. The column temperature was set at 40 °C. PCA, chlorogenic acid and ρ-coumaric acid were detected at 290 nm and ferulic acid at 325 nm. Phenolic compounds in the samples were identified by comparing the retention time and UV spectra with external standard compounds using an external standard method.

3.2.3.4 α-amylase inhibition assay of phenolic extracts

α-amylase inhibition assay was performed following Worthington, (1993); Manaharan *et al*. (2011); Oboh *et al*. (2012). One mL phenolic extract and 0.05 mL 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine α-amylase (0.5 mg/ml) were incubated at 25 ^{o}C for 10 minutes. Fifty microliters of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was mixed and the reaction mixture was incubated at room temperature for 10 min before stopping with 0.2 mL of dinitrosalicylic acid color reagent. After incubated in boiling water bath (5 min) and cooled to room temperature, the reaction mixture was diluted (1 mL of distilled water) and an absorbance at 520 nm was measured. The α-amylase inhibition activity was expressed as percentage inhibition which was calculated as follows.

$$\% = \frac{\text{absorbant of blank} - \text{absorbant of sample}}{\text{absorbant of blank}} \times 100 \qquad \text{…….…. [3]}$$

3.2.4 Starch gelatinization properties

Total starch gelatinization and ungelatinized starch residue were analyzed in triplicate using a Differential Scanning Calorimeter (DSC) (PerkinElmer- DSC7, Waltham, MA, USA). Residue starch gelatinization, rice flour one gram were added DI water ratio 1:10 (w/v) in tube 20 mL heat 85^{o}C for 30 minute in water bath cool in liquid nitrogen and store in -16^{o}C, rice flour (≈1 mg, dry weight basis) was placed in a hermetically-sealed stainless steel pan with o-ring (Perkin Elmer). The sample was heated from -10 to 130^{o}C at rate 10^{o}C/minute in DSC calibrated with Indium. Onset temperature (t_o), peak temperature (t_p), conclusion temperature (t_c) and enthalpy of gelatinization (ΔH) were determined from endothermic peak using Pyris™ Player software (Perkin Elmer).

3.2.6 Solubility and swelling power properties

The methods of solubility and swelling power properties of Hasjim *et al*., (2009) were applied to determine the solubility rice flour with some modifications. Distilled water (30 mL) was transferred into a beaker and the 1 g sample was added. After 85^{o}C for 60 minutes incubating with intermittently stirring, the suspension was centrifuged at 2000 rpm/min for 15 min. Supernatant was transferred to moisture can and dried in an oven at 105^{o}C overnight. Starch solubility is expressed as:

$$\text{Solubility (\%)} = \frac{\text{Weight of solids in supernatant (g)} \times 100}{\text{Weight of dry sample (g)}} \quad \ldots\ldots\ldots\ldots [4]$$

The precipitated weight was measured and the sample swelling power was calculated as:

$$\text{Swelling power} = \frac{\text{Weight of sample swelled sample (g)}}{\text{Total dry weight of original sample (g)} \times (100 - \text{\% solubility})} \quad \ldots\ldots\ldots\ldots [5]$$

3.2.7 Viscosity properties

The pasting characteristics of rice flour were determined by a Viscograph Brabender analyzer (Gepruft, Germany) using a 6% rice flour. The parameter were heating to

50°C before starting the heating cycle, heating up to 95°C at 1.5°C/ minutes, holding at 95°C for 20 minutes, cooling to 50°C at 1.5 °C/minutes, and holding at 50°C for 30 minutes.

3.3 Clinical glycemic and insulin index studies (GI and II)

3.3.1 Test meals

Sangyod rice (*Oryza sativa* L.) was obtained from Phatthalung Province, Thailand. The dehulling process was done to make unpolished rice and polished rice. The grains were packed in nylon bags and transported to Prince of Songkla University and store at ambient temperature before further processed. Five kilograms white rice and ten kilograms brown rice were washed and dried by hot air oven at 55°C for 10 hour (8-10% moisture content) and five kilograms of brown rice was milled into flour by flour milling machine at speed 25,000 rpm (Daming, China). The rice flour was sifted through a 200 mesh sieve and packed in nylon bags and kept at 4°C throughout the experiment. White rice, brown rice and flour brown rice of Sangyod Phatthalung were *in vivo* studied. In order to obtain cooked rice with relatively equal soft texture, white rice with water ratio 1:1.5 (w/v) and brown rice and brown rice flour with water ratio 1:2 (w/v) were cooked using an electric rice cooker (International, China). A cabbage soup, containing very little cabbage, salt and scallions, were cooked using an electric rice cooker for 20 minutes. For the controls, white bread (Farmhouse Co Ltd., Thailand) was purchased locally and glucose (50 g equivalent) was used. Bread in portion containing 50 g carbohydrate equivalent was prepared. Fifty gram glucose was dissolved in 150 mL of warmed water (Table 1).

Table 1 Recipes for test meals used in GI study

	Control		Test meal		
	White bread	glucose	White rice	Brown rice	Brown rice flour
materails					
Grain (g)	-	-	100	100	100
Water (g)	-	100	150	200	200
Glucose (g)	-	50	-	-	-
Control					
g CHO/meal	50	50	50	50	50
Water (mL)	150	150	150	150	150
Soup (mL)	-	50	50	50	50

3.2.2 Volunteer and study protocol

In clinical trial, glycemic and insulin index were tested involving 10 healthy volunteers, 5 man and 5 women with mean age, weight, height and body mass index of 26.8±2.26 years, 60.6±6.93 kg, 1.69±0.04 m and 20.8±1.46 kg/m^2, respectively. All volunteers had normal blood pressure, did not drink alcohol, smoke, or take antibiotics. Female volunteers who were in menstrual period were excluded. The volunteers were served with 5 different test meals (glucose, white bread, white rice, brown rice, and brown rice flour) at random order at 8 A.M. after an overnight fasting. The meals were consumed within 5 minutes along with 200 mL water. An intravenous cannula was inserted into a superficial vein in the forearm, and blood samples were collected immediately before serving the test meals and 30, 60, 90, 120 and 180 minutes after. Serum glucose levels were measured using a hexokinase method (Beckman Coulter, Fullerton, Calif, USA) and insulin in serum was analyzed by Electrochemiluminescence immunoassay (ECLIA) were analyzed with a Modular E170 (Roche Diagnostics). Both were analyzed by laboratory of the Chemical Chemistry Department, Faculty of Medicine, Prince of Songkla hospital. Each subject gave a written informed consent of acceptance, which was previously approved by the Ethical Committee for Prince of Songkla University and conducted in accordance with its rules and regulations. During the study, hunger sensory data recorded were 1) the time period (minutes) when the subjects began feeling hungry after fed and 2) the degree of hunger at three hour after test meal. The degree of hunger was estimated by 5-point scale marking on a horizontal chart (10 cm long) above which 5 vertical ticks were printed as follows (from left to right): ‘extremely hungry’, ‘hungry’, ‘no particular feeling’, ‘satisfied’ and ‘extremely full’.

GI and II were calculated in accordance with calculation method in GI methodology (Brouns *et al*., 2005; Oku *et al*., 2009). Incremental serum glucose and insulin level AUC (Area Under Curve) versus time were calculated using the trapezoidal rule with fasting values as the baseline, and the ratio of AUCs for 2 h versus 50 g of glucose ingestion was considered to be the GI.

The AUCs of serum glucose and insulin for 2 h (AUC-2 h-glucose and AUC-2 h-insulin) and 3 h (AUC-3 h-glucose and AUC-3 h-insulin) after ingestion were calculated with AUC of 50 g of glucose and white bread was comparative controls. The results were expressed in percent. The duration of 3 hours was selected in order to study the correlation of hunger and AUC

glucose. AUC-2 h-glucose and AUC-2 h-insulin of serum glucose and insulin were compared with paired Student's t-test. One-way analysis of variance and Tukey's post hoc test were used to analyze the difference type of rice and Pearson's correlation was analyzed.

The GI calculated as

$$GI = \frac{\text{Serum glucose response area of sample}}{\text{Serum glucose response area of glucose}} \times 100 \quad \text{..... [7]}$$

$$II = \frac{\text{Serum insulin response area of sample}}{\text{Serum insulin response area of glucose}} \times 100 \quad \text{..... [8]}$$

CHAPTER III

RESULTS

Result article 1

1. Chemical, physico-chemical and nutritive values of grain and flour of Sangyod Phatthalung rice

1.1 Introduction

Sangyod Phatthalung rice is traditional rice variety grown in the area of Phatthalung province for more than a hundred years. Its typical grain appearance is small and long-slender grain, dark red pericarp, soft and aromatic of cooked rice. Yodmanee *et al*. (2011) reported polyphenols and anthocyanin contents of Sangyod Phatthalung rice to be relatively high, 82 mg GAE/100g dry basis and 15 mg Cy-3-glc/100g dry basis, respectively.

White rice is milled rice that has had its husk, bran, and germ removed. Brown rice or hulled rice is a kind of unpolished grain which is more nutritious than white rice but goes rancid more quickly because of the germ. Germinated rice is unpolished rice that still has young shoots remained intact, soaked in water until its embryo of seed begins to grow. During germination hydrolytic enzymes activate digestion of starch, fiber and proteins, resulting in improved texture (Tian *et al.,* 2004). The rice varieties with pigmented color (black, purple or red) give slightly pigmented flour upon milling (Finocchiaro *et al.,* 2007). Pigmented rice typically contains high antioxidant activities (Nam *et al.,* 2006; Srisawat *et al.,* 2010).

1.2 Material and Method

Sangyod rice (*Oryza sativa* L.) from Phatthalung Province, Thailand was dehulled to make unpolished rice (brown and germinated) and polished rice. Each was packed in plastic bags and transported to Prince of Songkla University. White rice and brown rice were washed twice some washed brown rice was inculcate for germination. The samples were dried by hot air oven at 55^{o}c for 10 hour. Half of every sample was milled into flour. The milled samples were packed under vacuum in nylon bags and kept at 4±2^{o}C throughout the experiment.

1.2.1 Germination process

Germinated rice was prepared according to Tain *et al*. (2004)'s methods. Prepared germinated rice was steeped in distilled water at 27-30^{o}C in the dark for 24 h. The steep water was changed every 4 h and drained at the end of soaking. The germinated seeds were dried at 55^{o}C

1.2.2 Flour milling

Rice was milled by flour milling machine 25,000r/min (Daming, made in China). The flour rice was sifted through 200 mesh sieve.

1.2.3 Method

All the samples were analyzed proximate (moisture, lipid, protein and ash) by A.O.A.C. (2000) method and analysis of minerals, including iron, zinc, sodium, calcium, magnesium, potassium and phosphorus and heavy metal including cadmium and lead by Inductively Coupled Serum Atomic Emission Spectrometer (ICP-AES). Total dietary fiber was analyzed by AACC, (2010) method. Color of grain and flour were determined by Hunter-Lab (Colour Flex, Hunter-Lab, USA). Prior to color measurements, the instrument was calibrated with light tap and white calibration tile. The colorimeter was set to an illuminant condition D65 and a 10° standard observer. Each sample was put in a cuvette and replaced in to the specular port site, the color parameters (a*, b* and L*) were then read and calculated c*= colour intensity, chroma ($c^* = [a^* + b^*]^{1/2}$), h^o = hue angle ($h^o = \tan^{-1}[b^*/a^*]$).

Starch gelatinization properties were analyzed in triplicate using a Differential Scanning Calorimeter (DSC), (Hasjim *et al*., 2013). Rice flour of about 3 milligram was placed in a 40 microliter aluminum pan; water was added to give a sample: water weight (1: 3). The pan was sealed, and the sample was allowed to equilibrate overnight at 4 ˚C. The DSC temperature scan includes holding at 10˚C for 1 minute followed by heating from 10 to 95 ˚C at a rate of 10˚C/minute. Water activity (a_w) was analysed using Novasina Water activity meter.

1.3 Results and Discussion

Figure 2 shows Sangyod Phatthalung rice samples including white (polished) rice, brown (unpolished) rice and germinated rice. Redness in the grains of Sangyod Phatthalung rice is embedded in the structure of the bran. When the rice is polished, the color is gone leaving a

white grain appearance. During germination, the color intensity decreased because anthocyanin dissolving in water resulting in a change in color particularly b* value (Chung *et al.,* 2012). Anthocyanin is glycosides compounds or acyl glycoside of phenolic compounds in the outer membrane (pericarp) and inner membrane or aleurone layer (Hu *et al.,* 2003). The colors appear, intensity of the color are depending species, cultivation, place of planting, age, duration of germinated etc. (Adom and Liu, 2002).

Figure 2 Sangyod Phatthalung rice sample in studies

Where a = White rice flour, b = Brown rice flour, c = Germinated rice flour,
d = White rice, e = Brown rice, f = Germinated rice

Table 2 shows the color parameters (L*, a*, b*, c* and h^{o}) of grain and flour of Sangyod Phatthalung rice. L* values, which expresses the brightness, were in the range of 35.1-82.0. The values of a*, b*, c* and h^{o} were in the range of 4.2-15.2, 9.9-20.8, 11.8-25.8 and 53.9-69.5 respectively. Polished grain and flour brightness was higher than unpolished. Red of brown rice grain and flour more than germinated and white grain and flour and yellow of unpolished higher than polished grain but yellow of germinated flour were lower than white and brown flour. The intensities of brown grain and flour varied more than white and germinated grain and flour. Sangyod Phatthalung brown rice is dark-red-yellow rice. Polishing removes the bran and

therefore removes the pigment reducing the color intensity and increased the lightness whereas germination lowers the intensity of the colour of Sangyod Phatthalung rice only slightly.

Table 2 CIE color scale from rice and flour of Sangyod Phatthalung rice

	Grain				
	L*	a*	b*	c*	h^o
White rice	60.7±2.4[a]	6.7±0.8[c]	16.1±0.8	17.5±0.6[c]	67.5±3.1[a]
Brown rice	35.1±0.9	15.2±0.7[a]	20.8±1.0[a]	25.8±1.0[a]	53.9±1.8
Germinated rice	36.8±1.4	13.5±0.9[b]	20.2±0.6[a]	24.3±0.6[b]	56.2±2.0
	Flour				
	L*	a*	b*	c*	h^o
White rice	82.0±0.3[a]	4.2±0.1[c]	11.3±0.1[a]	12.1±0.1	69.5±0.4[a]
Brown rice	68.3±0.2[c]	7.0±0.1[a]	11.1±0.1[a]	13.1±0.2[a]	57.7±0.2[b]
Germinated rice	70.8±0.4[b]	6.5±0.2[b]	9.9±0.2	11.8±0.3	56.6±0.2[c]

Value are given as mean ± SD from six replicate

[a,b,c] Superscripts in the same column indicate significant different ($P<0.05$) by Tukey's test

Where The maximum for L* is 100, which represents a perfect reflecting diffuser.

The minimum for L* is zero, which represents black.

a* = Positive is red, Negative is green

b* = Positive is yellow, Negative is blue

c^* = colour intensity, chroma

H^o = hue angle

Chemical composition of white rice, brown rice and germinated rice of Sangyod Phatthalung rice (% dry basis) shows in Table 3. The protein contents were 7.81, 8.22 and 8.30 (% dry basis), respectively (significantly different $P<0.05$). The increase of protein in germination rice was occurring from decarboxylation of L-glutamate by glutamate carboxylase (Komatsuzaki *et al.,* 2007). Comparing among white, brown and germinated rice, lipid contents were 0.51, 3.18 and 2.80 (% dry basis), ash contents were 0.75, 1.36 and 1.03 (% dry basis), and dietary fiber content were 1.40, 3.12 and 2.91 (% dry basis), respectively suggested Sangyod brown rice is high total dietary fiber (more 3 g/100g sample). These were because of the removal during

polishing resulting in a lower value for white (polished rice). In addition, some loss in germinated rice may be due to process of germination that is consistent with the soybean (Park *et al.,* 2002) and rice germination (Moongngarm and Saetung, 2010). This composition of lipids is a healthier composition in brown rice and germinated brown rice because rice bran oil is polyunsaturated and monounsaturated fatty acids. Micronutrients of difference type of Sangyod Phatthalung rice are shown in Table 2. Brown rice contains higher potassium and phosphorus than germinated and white rice (significantly different, $P<0.05$). Germinated rice contains higher amount of iron, zinc, sodium, calcium and magnesium than brown rice and white rice (significantly different ($P<0.05$). Dry-milled flour contained slightly higher amount of lead than others (2.7 ppm vs approx. 1.9 ppm).

Table 3 Chemical composition of white rice, brown rice and germinated rice of Sangyod Phatthalung rice (% dry basis)

	Polished rice	Unpolished rice	
	white rice	brown rice	germinated rice
Moisture	10.60±0.34^{b}	11.56±0.38^{a}	11.25±0.07^{a}
Protein	7.81±0.02^{c}	8.22±0.05^{b}	8.30±0.01^{a}
Lipid	0.51±0.03^{c}	3.18±0.03^{a}	2.80±0.13^{b}
Ash	0.75±0.00^{c}	1.36±0.01^{a}	1.03±0.03^{b}
Total dietary fiber	1.40±0.13^{b}	3.12±0.14^{a}	2.91±0.11^{a}
Micro nutrient (ppm)			
Fe (iron)	12.28±0.06^{b}	12.09±0.05^{c}	19.69±0.05^{a}
Zn (zinc)	20.93±0.29^{c}	22.63±0.05^{b}	31.53±0.11^{a}
Na (sodium)	114.69±1.39^{c}	162.99±2.25^{b}	239.10±0.78^{a}
Ca (calcium)	484.32±1.80^{b}	482.64±4.40^{b}	775.26±4.23^{a}
Mg (magnesium)	513.30±2.77^{c}	985.83±8.69^{b}	1090.97±8.63^{a}
K (potassium)	1192.43±22.06^{b}	2118.14±17.02^{a}	1226.17±18.67^{b}
P (phosphorus)	1012.09±10.55^{c}	2104.58±26.35^{a}	2014.95±30.90^{b}
Heavy metal (ppm)			
Cd (cadmium)	0.095±0.00	0.121±0.052	0.125±0.054
Pb (Lead)	2.735±0.220^{a}	2.019±0.138^{b}	1.807±0.108^{b}

Value are given as mean ± SD from triplicate

Different superscripts in the same row indicate significant different ($P<0.05$) Tukey's test

Table 4 shows water activity of grains were 0.67, 0.50 and 0.54 for white, brown and germinated rice grains, respectively (significantly different, $P<0.05$). a_w of the flours were 0.62, 0.32 and 0.34 for white, brown and germinated rice flour, respectively (significantly different between polished and unpolished rice flour). a_w of the grains and dry-milled white rice flour were 0.60 which was higher than unpolished (brown) and germinated (brown) rice. a_w of flours were lower than that of whole grains.

Table 4 Water activity from rice and flour of Sangyod Phatthalung rice

	Water activity		
	grain	flour	grain-flour *p*-value*
White rice	0.67±0.02[a]	0.62±0.01[a]	<0.05
Brown rice	0.50±0.01[c]	0.32±0.01[b]	<0.05
Germinated rice	0.54±0.01[b]	0.34±0.00[b]	<0.05

Value are given as mean ± SD from triplicate

[a,b,c] Superscripts in the same column indicate significant different ($P<0.05$)

* Grain-flour *p*-value: difference of water activity of sample between grain and flour indicate significant difference ($P<0.05$) by paired sample T-test

Starch gelatinization is the transition of the semi-crystalline structure (Level 3 starch structure) in native starch granules to an amorphous structure (Cooke and Gidley, 1992). Starch gelatinization properties were analyzed both from the rice flour samples using laboratory-scale dry milling and comparison between polished and unpolished rice, which are one factor of starch digestion properties. The gelatinization temperatures of flours and rice studied are presented in Table 4. Peak analysis shows onset (T_0), peak (T_p) and end (T_e) gelatinization temperatures that are characteristics of heat-water treatments that destroy helical structure in starch crystallites. T_0 of Sangyod Phatthalung rice flour was found to be at 74.4-76.5 ^{o}C range where as T_p or peak temperature were in 80.6-88.1^{o}C, not so varied among sample treatments (i.e., polished unpolished or germinated). However, the conclusion temperature T_e varied significantly over 86.6 to 96.0^{o}C range with germinated rice the lowest T_e followed by white (polished) rice and then by brown (unpolished) rice flours. Tukey's analysis of difference preparation of Sangyod

Phatthalung rice shows that T_o and T_p of three rice flour were not significantly different (P<0.05) but the final gelatinization temperature for germinated rice was lowest at 86.62±0.15 ^{o}C followed by that of white rice at 91.08±0.68 ^{o}C and brown rice at 95.96±3.15^{o}C. It is postulated that the dry-heat treatment of polished (white) rice and moist-heat and enzymatic treatments of germinated rice could have contributed to a less stable (lower gelatinization temperature of some elevated temperature fractions).

Table 5 Gelatinization temperatures and transition enthalpy from different flour rice of Sangyod Phatthalung rice

	transition temperatures (^{o}C)			transition enthalpy
	T_o	**T_p**	**T_e**	**(J/g)**
White rice	76.52±0.04	88.10±6.11	91.08±0.68^{b}	2.90±0.08
Brown rice	74.42±3.13	86.73±4.15	95.96±3.15^{a}	3.94±0.92
Germinated rice	75.35±0.28	80.63±0.17	86.62±0.15^{c}	3.57±0.03

Value are given as mean ± SD from triplicate

Different superscripts in the same column indicate significant different (P<0.05) Tukey's test

Where T_o = onset temperature, T_p = peak temperature, T_e = endset temperature

Transition enthalpy of Sangyod Phatthalung rice flour was within 2.90-3.94 J/g (Table 5), not significantly different among samples (P<0.05). However, there was a trend where white rice showed lower enthalpy indicating some possible destruction of helical structure of the starch structure from the milling and polishing processes. Presence of small components such as ionic and non-ionic solutes might influence by retarding gelatinization, i.e., raising the gelatinization temperature of the starch (Chinachoti *et al*. 1991; Rahman 1995). However, based on micronutrient and ash contents, this was not always the case. It was found that the onset gelatinization temperature of brown rice, white rice and germinated rice were only slightly (but not significantly) different base on T_o and T_p comparison. However, T_e was found to be lower in the case of germinated rice (86.62 ^{o}C) meaning the thermal transition was narrower (sharper) than the other two rice treatments. The process of wetting and digestion in germination period could

result in starch hydrolysis in the amorphous domains that are networking in the fraction with higher gelatinization or melting temperature. Note that the thermal enthalpy of germinated rice was not significantly different from brown or white rice indicating similar double helix content but different in the degree of order of crystalline alignment. The process of lowering T_e means the starch are gelatinized more completely at lower temperature and possibly softening the texture. Comparing the compositional difference between white and brown rice (Table 3), small solutes could have had some influence on the gelatinization enthalpy. For example, the presence of higher ash, lipid and protein in brown rice, each could have directly or indirectly impacted the swelling and gelatinization process. But lipid could have additional interaction with the linear portion of starch molecules that could lead to additional lipid-starch interaction enthalpic peak as earlier found (Tian *et al.,* 2010). But in this work this was not observed. It would be possible that most of the lipids are interacted in lipid-protein complex or network outside of the rice grain and hence not able to interact with the starch. The higher ash content in brown rice was found to raise T_e of the sample significantly indicating potential impact of Na, K, Mg and P in the sample. Tester observed brown rice to be slightly leathery or harder in texture than the compounding white rice which could partly from the higher T_e. The pericarp, layer of lipid-protein could also affect the texture (Mestres *et al.,* 2011). In this work it could be concluded that useful data from composition and gelatinization temperature are useful information for describing white rice, brown rice and germinated rice and useful in understanding texture attributes of Sangyod Phatthalung rice. The process of wetting and digestion in germination of rice result in starch hydrolysis of the amorphous chains that could have lowered T_e markedly without lowering the enthalpy or helical contents. This led to texture softening of the cooked rice grains. Some study reported rice gelatinization behavior showing a strong influence of perceived texture of cooked rice; the higher the gelatinization temperature of the grain the harder the texture of the rice (Mestres *et al.,* 2011). In this work, moist-heat treatments in germination and other processes and component distribution may also be important.

1.4 Conclusions

Polished rice showed lower enthalpy indicating some destruction of helical structure of the starch structure from the milling and polishing processes. Note that the thermal enthalpy of germinated rice was not significantly different from brown or white rice indicating similar double helix content. The process of lowering T_e means the starch is gelatinized more completely at lower temperature and possibly softening the texture. But lipids could have additional interaction with the linear portion of starch molecules that could lead to additional lipid-starch interaction enthalpic peak as earlier found. The higher ash content in brown rice was found to raise T_e of the sample significantly indicating potential impact of Na, K, Mg and P in the sample. Chemical compositions and gelatinization temperature are useful information for describing white rice, brown rice and germinated rice and useful in understanding texture attributes of Sangyod Phatthalung rice.

Result article 2

2. Digestibility and α-amylase inhibition of Sangyod Phatthalung rice (*Oryza sativa* L.)

2.1 Introduction

Incidences of diabetes and other metabolic syndrome conditions globally are on the rise. According to a rapid changing and stressful life style and growing elderly population in a world, it is recognized that a healthy digestive system is essentially linked to health status including disease prevention and quality of life among elderly.

Carbohydrate consumption of rice as staple foods is primarily to provide energy. Recent change in life style has caused an imbalance in energy intake resulting in overconsumption of starch and sugar, leading to metabolic syndrome, such as obesity and diabetes. Diabetic patients suffer mainly from elevated post-prandial glycemic response which leads to several clinical complications over the rest of their lives. More health conscious consumers have been increasingly interested in gaining better glycemic control by eating less simple carbohydrates and choosing foods with lower glycemic index (GI). Rice is grown in abundance in Asia (90% of the world's crop, Juliano, 1985) and consumed as the major source of energy for most world population. When unpolished, rice grains contain healthy components including proteins, fats and phenolic compounds and anti-oxidants (such as γ-oryzanol, tocopherol, tocotrienol, and vitamin B) but their shelf-life is much shorter mostly from oxidative and microbial deterioration. When germinated, γ-aminobutyric acid and some antioxidant compositions are produced in a high amount (Tain *et al.,* 2004). Sangyod Phatthalung rice is an indigenous Southern of Thailand rice cultivar with red-brown color. Anthocyanin is glycosides form of phenolics compounds in pericarp and aleurone layer of rice grains (Hu *et al.,* 2003) and color variability is according to species, cultivation, geographic region, maturation (Adom and Liu, 2002).

It has been reported that redish brown color (such as in red bean) may also means an existence of protocatechuic acid (PCA, Archivio *et al.,* 2014) previously shown to inhibit the growth of cancer cells (Hudson *et al.,*2000; Chen *et al.,* 2006) and to regulate heart disease patient by inhibiting factors that cause inflammation (Wang *et al.,* 2007). Consuming unpolished color rice is considered healthy due to its richness in iron, phenolic compound, fiber and antioxidants.

In this work, further investigation was on the impact of indigenous anthocyanin and other antioxidants on glycemic index of rice.

GI of rice grains and flours can be used for choosing for diabetic patient consumption (Sajilata *et al.,* 2006). There are several physical factors that may influence starch digestion. Milling starch, for example, may influence the rate of digestion and glycemic response (Panlasigui *et al.,* 1991; Noda *et al.,* 2008). It has been reported that some but not all phenolic compounds could be a potent inhibitor of α-amylase activity (Yang *et al.,* 2012; Wang *et al.,* 2012). Soybean phenolic-rich extracts have been linked to inhibition of metabolic syndrome-related enzymes, including α-amylase and α-glucosidase for the case of type 2 diabetes (Ademiluyi and Oboh, 2013). PCA is among important phenolic compounds that strongly influence metabolic syndrome including antioxidant activity, anti-inflammatory activity, antihyperglycemic activity, modulation of apoptosis and modulating gut micro flora (Archivio *et al.,* 2014).

The objective for this study is to investigate HI and GI for unpolished, polished and unpolished germinated Sangyod Phatthalung rice comparing the effects of rice phenolics and starch physico-chemical properties.

2.2 Materials and Methods

2.2.1 Materials

Rice grains and flours were prepared from Sangyod Phatthalung rice (*Oryza sativa* L.) with three different grain treatments, i.e., polished (white) rice, unpolished (brown) rice and germinated unpolished (brown) rice. Standard white bread is used as the control digestion study. Dehulled Sangyod rice from Phatthalung Province (Thailand) was collected from calibration year 2012. Unpolished rice (brown) and polished rice (white) were prepared on site. These grains were packed in plastic bags, transported to Prince of Songkla University and stored at ambient temperature (28±2^{o}C) before further processed. Two kilograms of white rice and two kilograms brown rice were washed and dried (hot air oven, 55^{o}C, 10 hours) to 9% moisture content. Germinated brown rice was prepared by steeping in deionize water and incubated in darkness at ambient temperature for 24 hours (Tain *et al.,* 2004). The steep water was changed every 4 hours and drained for 20 minutes and dried (hot air oven, 55^{o}C 10 hours). To prepare rice flours for a comparison study, rice grains (1 Kg) were milled into flours by flour milling machine

(Daming, China) at speed of 25,000 rpm. The rice flour obtained was sifted through 200 mesh sieve and packed under vacuum in nylon bags and kept at 4°C throughout the experiment. The standard for digestion and glycemic study was white bread (obtained from a local market) which was dried by hot air oven at 60°C for 5 hours, milled and sifted through 200 mesh sieve and kept at 4°C throughout the experiment.

2.2.2 Chemical composition analysis

All the samples were analyzed for moisture, lipid, protein and ash contents by A.O.A.C. (2000) methods. The analyses for minerals (iron, zinc, sodium, calcium, magnesium, potassium and phosphorus) and heavy metal (cadmium and lead) were performed by Inductively Coupled Serum Atomic Emission Spectrometer (ICP, Perkin Elmer, USA).

2.2.3. Digestibility, hydrolysis index and glycemic index

2.2.3.1 *In vitro* rice and flour rice digestion

In vitro digestibility assay of the samples was based on glucometry which a modified method of Mahasukhonthachat *et al*. (2010); Srikaeo and Sopade, (2010) and Sopade and Gidley (2009). A half-gram sample was mixed with 5 mL deionize water and heated in a water bath at 85°C for 30 min. Each sample was treated with 1.0 mL artificial saliva containing porcine α-amylase (Sigma A-3176, 250 U per mL in sodium acetate buffer) and 5 mL pepsin (Sigma P-6887, 1% of protein in sample in deionized water and 0.02 M, HCl pH 2.0 ratio 1:1 (v/v)) was added after incubation at 37˚C for 30 min in a reciprocating water bath. The digesta was neutralized with 0.2 NaOH and pH was adjusted to 6.0 with sodium acetate buffer prior to an addition of 5 mL pancreatin (Sigma P-1750 from porcine pancreas, 2 mg per mL of acetate buffer), 5 mL porcine α-amylase (Sigma A-3176, 250 U per mL in sodium acetate buffer) and 5 mL amyloglucosidase (Sigma A-7420 from *Aspergillusniger*, 28 U per mL in acetate buffer). The mixture was incubated at 37°C for 4 hours the glucose concentration in the digesta was measured with a glucometor (Accu-Chek Performa, Roche Diagnostics Corporation, Indianapolis, IN, USA). Glucose concentration at specific periods (0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) were measured the amount of digested starch per 100 g dry starch (DS) was calculated using equation 1.

$$DS = \frac{0.9 \times 180 \times GG \times V}{W \times S[100 - M]}. \qquad \ldots\ldots [1]$$

Where DS = digested starch (g per 100 g dry sample)

GG = glucometer reading (mM/L)

V = volume of digesta (mL)

180 = molecular weight of glucose

W = weight of sample (g)

S = starch content of sample (g per 100 g dry sample)

0.9 = stoichiometric content for starch from glucose content

M = moisture content of sample (%moisture dry Sample)

Remaining undigested starch of digested samples was analyzed (method by Megazyme International Ireland Ltd., Ireland). Fifty mg sample was mixed with 400 microliter of 80% ethanol, heated in boiling water bath. Two mL dimethyl sulphoxide (DMSO) was added and then the sample was digested with thermo-stable α-amylase (Sigma A-3176) ratio 1:30 (v/v) with 3-(N-morpholino) propanesulfonic acid buffer (MOPS, Sigma M-9381) before sodium acetate buffer and amyloglucosidase (Sigma A-7420) were added and incubated at 37°C for 4 hour. Glucose content was determined using an enzymatic glucose reagent (Sigma, GAHK-20). Absorbance was measured in BiotekPower Wave X Microplate reader (Biotek, Winooski, VT, USA) at 340 nm. Each sample was analyzed in at least duplicate.

2.2.3.2 Total starch analysis

Total starch content of the samples was analyzed using a method derived from Megazyme (Megazyme International Ireland; Srikaeo and Sopade, 2010). About 50 mg of sample was wetted with 400 microliter 80% ethanol, heated (boiling water bath) in 2 mL dimethyl sulphoxide (DMSO) and digested with thermos table α-amylase (Sigma A-3176) (1:30) (v/v) in MOPS (Sigma M-9381) buffer before sodium acetate buffer and amyloglucosidase (Sigma A-7420) were added and incubated as described. Before, the glucose content was determined using an enzymatic glucose reagent (Glucose (HK) assay kit, GAHK20), and the absorbance of the coloration was measured by Microplate reader (Biotek power wave X, USA) at 340 nm. Each sample was analyzed in duplicate.

2.2.3.2 Hydrolysis index (HI) and glycemic index (GI)

The hydrolysis index (HI) of each sample was calculated by dividing the area under its digestogram by the area under the digestogram of a white bread (Goni *et al.,* 1996). GI of the samples was also calculated (equation 2; Sopada and Gidley, 2009). Valus are given as mean ± SD from triplicate.

$$GI = 39.71 + (0.549HI) \quad \text{…….. [2]}$$

2.2.4. Extraction and analyses of phenolics

2.2.4.1 Extraction

Phenolic extraction was done using the method reported by Min *et al*. (2012). Five gram rice flour was mixed with 25 mL deionize water and heated in a water bath 85^{o}C for 30 minutes. Twenty five mL ethanol was then added, sonicated (Ultrasonic cleaner CD-4820 170w, Zhengzhou Xihua Medical Devices Co., Ltd., China) for 10 minutes, and left in darkness for 90 minutes at room temperature. The mixture was centrifuged (Mikro 22r, Hettichzentrifugen, UK) at 5,000rpm for 10 minutes. The collected supernatant was filtered through filter paper (Whatman No.1). The filtered liquid was then mixed with 25 mL deionize water and then 20 mL hexane. After 15 minutes hand shaking, the liquid was rested to allow lipid separation; the aqueous portion was then further analyzed.

2.2.4.2 Analysis of total phenolic content

Total phenolic content was determined according to procedure described by Singleton *et al*. (1999). To one hundred microliter extract, 0.2 mL 1N Folin-Ciocalteu's reagent was added and mixed for 5 minutes. The mixture was neutralized with 0.3 mL of NaOH (0.5 M) and placed in 96-well plate allowing to mix for 2 hours. Absorbance at 760 nm was done using micro plate reader. Phenolics content in each extract was calculated using a calibration curve of ferulic acid standard (5-200 mgmL^{-1}). The results were expressed in mg of ferulic acid equivalent per 100 g of whole grain on dry weight basis (mg FA equiv.100g^{-1}). Analyses were performed at least in triplicates.

2.2.4.3 Identification of phenolic compounds

Phenolic compounds in extracts were characterized by reversed-phase High-performance liquid chromatography (HPLC) as described by Tain *et al*., 2004 and Tain *et al.,*

(2005) with modification. All extracted samples were filtered through a 0.20-µm pore size syringe-driven filter before injection. A 10-µL aliquot of each sample solution was separated using a Agilent 1100 HPLC system (Agilent 1200, Waldbronn, Germany) equipped with a diode array detector on a 250 mm x 4.6 mm i.d., 5 µm, Eclipse XDB-C18, analytical column (Agilent, Santa Clara, CA, USA). The mobile phase was acetonitrile (A) and purified water with 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.8 mL/min. Gradient elution was done as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent A; from 5 to 15 min, 9% solvent A; from 15 to 22 min, linear gradient from 9 to 11% solvent A; from 22 to 35 min, linear gradient from 11 to 18% solvent A; from 35 to 42 min 18% solvent A. The column temperature was set at 40 °C. PCA, chlorogenic acid and ρ-coumaric acid were detected at 290 nm and ferulic acid at 325 nm. Phenolic compounds in the samples were identified by comparing the retention time and UV with external standard compounds using an external standard method.

2.2.4.4 α-amylase inhibition assay of phenolic extracts

α-amylase inhibition assay was performed following Worthington, (1993); Manaharan *et al*. (2011); Oboh *et al*. (2012). One mL phenolic extract and 0.05 mL 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine α-amylase (0.5 mg/ml) were incubated at 25 ^{o}C for 10 minutes. Fifty microliters of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was mixed and the reaction mixture was incubated at room temperature for 10 min before stopping with 0.2 mL of dinitrosalicylic acid color reagent. After incubated in boiling water bath (5 min) and cooled to room temperature, the reaction mixture was diluted (1 mL of distilled water) and an absorbance at 520 nm was measured. The α-amylase inhibition activity was expressed as percentage inhibition which was calculated as follows.

$$\% = \frac{\text{absorbant of blank} - \text{absorbant of sample}}{\text{absorbant of blank}} \times 100 \qquad \ldots\ldots\ldots [3]$$

2.2.5 Starch gelatinization properties

Total starch gelatinization and ungelatinized starch residue were analyzed in triplicate using a Differential Scanning Calorimeter (DSC) (Perkin Elmer- DSC7, Waltham, MA, USA). Residue starch gelatinization, rice flour one gram were added DI water ratio 1:10 (w/v) in

tube 20 mL heat 85°C for 30 minute in water bath cool in liquid nitrogen and store in -16°C, rice flour (≈1 mg, dry weight basis) was placed in a hermetically-sealed stainless steel pan with O-ring (Perkin Elmer). The sample was heated from -10 to 130°C at rate 10°C/minute in DSC calibrated with Indium. Onset temperature (t_o), peak temperature (t_p), conclusion temperature (t_c) and enthalpy of gelatinization (ΔH) were determined from endothermic peak using Pyris™ Player software (Perkin Elmer).

2.2.6 Solubility and swelling power properties

The methods of solubility and swelling power properties of Hasjim *et al*., (2009) were applied to determine the solubility rice flour with some modifications. Distilled water (30 mL) was transferred into a beaker and the 1 g sample was added. After 85°C for 60 minutes inclubating with intermittently stirring, the suspension was centrifuged at 2000 rpm/min for 15 min. Supernatant was transferred to moisture can and dried in an oven at 105°C overnight. Starch solubility is expressed as:

$$\text{Solubility (\%)} = \frac{\text{Weight of solids in supernatant (g)} \times 100}{\text{Weight of dry sample (g)}} \quad \ldots\ldots\ldots\ldots [4]$$

The precipitated weight was measured and the sample swelling power was calculated as:

$$\text{Swelling power} = \frac{\text{Weight of sample swelled sample (g)}}{\text{Total dry weight of original sample (g)} \times (100 - \text{\% solubility})} \quad \ldots\ldots\ldots ..[5]$$

2.2.7 Viscosity properties

The pasting characteristics of rice flour were determined by a Viscograph Brabender analyzer (Gepruft, Germany) using a 6% rice flour. The parameter were heating to 50°C before starting the heating cycle, heating up to 95°C at 1.5°C/ minutes, holding at 95°C for 20 minutes, cooling to 50°C at 1.5 °C/minutes, and holding at 50°C for 30 minutes.

2.2.7 Statistics

All experiments were performed at least in duplicate. Analysis at every time point from each experiment was carried out in triplicates. SPSS statistics package (version 15.0, Chicago, IL, USA) was used ($P<0.05$).

2.3. Results and Discussion

2.3.1. Chemical composition in Sangyod Phatthalung rice

Sangyod Phatthalung rice samples including white (polished) rice, brown (unpolished) rice and germinated brown rice varied in outside color (redish brown) primarily due to anthocyanin embedded in the structure of the bran. After polishing, the red color is removed leaving a white grain appearance. In case of germinated rice the color intensity decreased during germination due to anthocyanin degradation and loss in the soaking water (Chung *et al.,* 2012).

Chemical compositions of all rice studied are in Table 6. Protein content was 7.8-8.3% with brown rice which slightly higher protein content. Lipid contents for both brown rice and germinated brown rice were higher (3.18 and 2.80 % dry basis) than white, polished rice (0.51 % dry basis). Similarly, higher ash contents were found in brown rice samples than white rice. These resulted from pericarp removal from the polishing step; some loss in germinated rice might also be through the germination process which has been earlier reported (Moongmgarm and Seatung, 2010). Micronutrients (Table 6) indicated some more similarity in Fe and Zn contents among samples but more significant differences in Na, Ca, Mg, K and P. Both brown and germinated brown rice contains higher phosphorus than white rice ($P<0.05$) but only brown rice was higher than the other two on potassium. Germinated rice contains higher amount of Fe, Zn, Na, Ca and Mg than brown (unpolished) rice and white (polished) rice ($P<0.05$). This information is useful for controlling some specific diets. Total starch of white rice was obviously greater than those for brown rice samples by approximately 10%. Standard (white bread) have total starch around 62.6% (data not shown).

Table 6 Chemical composition of unpolished (brown) rice and unpolished (brown, germinated) rice of Sangyod Phatthalung rice (% dry basis)

	Polished rice	Unpolished rice	
	polished rice	(brown) rice	(germinated) rice
Protein	7.81±0.02[c]	8.22±0.05[b]	8.30±0.01[a]
Lipid	0.51±0.03[c]	3.18±0.03[a]	2.80±0.13[b]
Ash	0.75±0.00[c]	1.36±0.01[a]	1.03±0.03[b]
Total starch	83.86±2.60[a]	73.05±4.76[b]	75.38±5.79[b]
Micro nutrient (ppm)			
Fe (iron)	12.28±0.06[b]	12.09±0.05[c]	19.69±0.05[a]
Zn (zinc)	20.93±0.29[c]	22.63±0.05[b]	31.53±0.11[a]
Na (sodium)	114.69±1.39[c]	162.99±2.25[b]	239.10±0.78[a]
Ca (calcium)	484.32±1.80[b]	482.64±4.40[b]	775.26±4.23[a]
Mg (magnesium)	513.30±2.77[c]	985.83±8.69[b]	1090.97±8.63[a]
K (potassium)	1192.43±22.06[b]	2118.14±17.02[a]	1226.17±18.67[b]
P (phosphorus)	1012.09±10.55[c]	2104.58±26.35[a]	2014.95±30.90[b]
Total phenolic (mg FA equiv/100g dry sample)	37.50±4.81[c]	385.42±4.17[a]	306.25±7.98[b]

Value are given as mean ± SD from triplicate

Different superscripts in the same row indicate significant different ($P<0.05$)

2.3.2. Digestion rate of rice grains and flours

Porcine α-amylase hydrolysis of cooked rice grains and flours are expressed in a typical starch digestograms, i.e., digested starch (DS) and the increase if glucose versus hydrolysis time (Figure 3) using white bread as the control. Cooked rice flour digestion rates were higher than the corresponding cooked rice grains but no significant difference was found among the three kinds of flour. Amylase hydrolysis of cooked rice grains showed a significant difference. White rice grains were digested more rapidly than germinated brown rice grains. In this work, the

digestion that was more rapid for the respective flour samples was likely to be because the reduction in particle size in flour milling (Ranawana *et al.,* 2010). In addition, it has been reported that ground starch exhibited lower crystallinity and smaller molecular weight (Morrison *et al.,* 1993; Huang *et al.,* 2007). These could have allowed more open structure after gelatinization and easier digestion. Brown rice tends to be more resistant and relatively lower in digestibility (Sajilata *et al.,* 2006). It was found that DS (digested starch) increased in a curvilinear fashion with hydrolysis time reaching an asymptotic level after approx. Two hour of digestion (Figure 3A and 3B). The white bread control was highest in digestion rate approaching almost 80%. Cooked rice flours were less rapidly digested with polished (white) rice flour slightly more rapid than white rice grains (Figure 3A and 3B). White (polished) rice grains were digested only slightly more than brown rice grains indicating that removal of bran by polishing could not accelerate the digestion but the extent of whole grain digestion was about 2/3 of that of flour (i.e., 40% vs 60% DS).

The increase in glucose (Figure 3C and 3D) mirrored the DS curves indicating less in vitro glucose release in grain hydrolysis than flour hydrolysis, respectively. Brown rice appears to sustain glucose release over longer time than white bread and white rice, suggesting its potential satiety sustaining property.

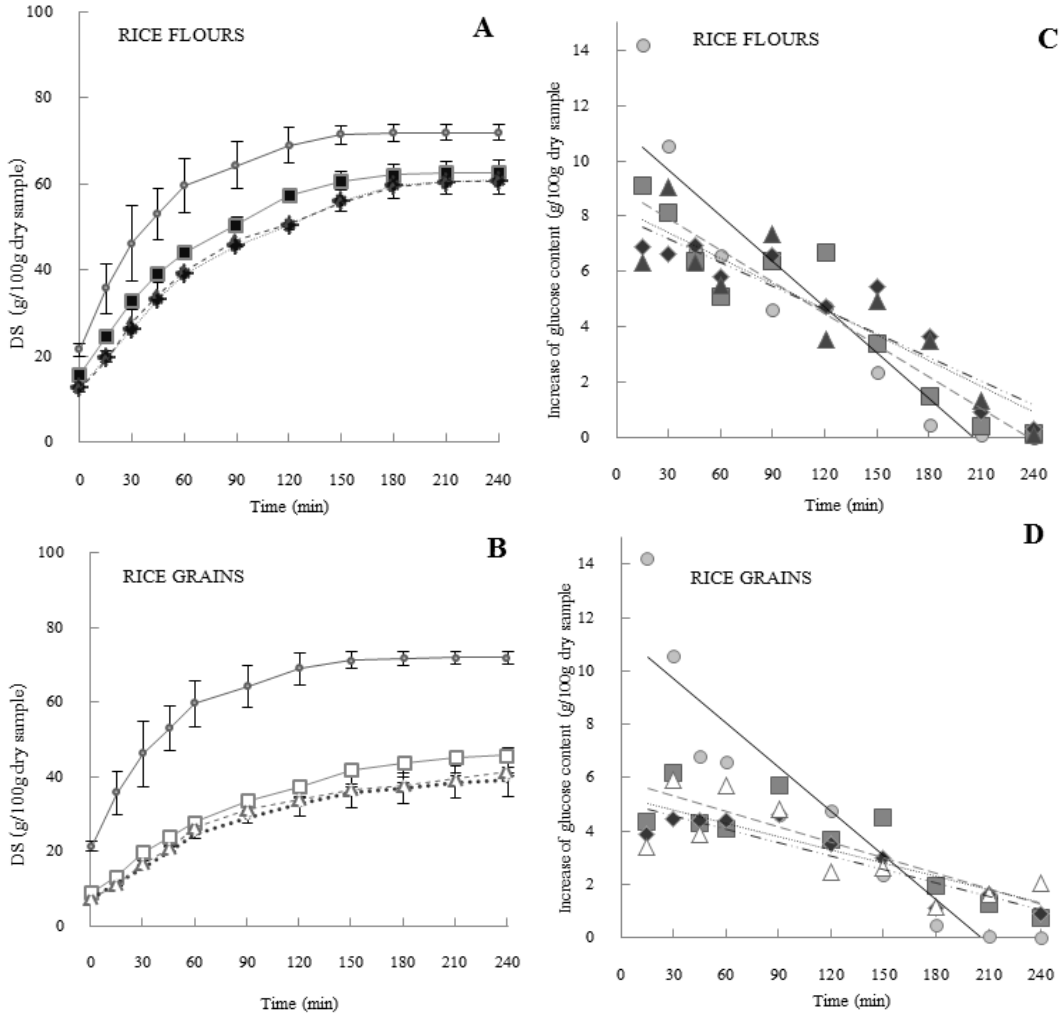

Figure 3 Starch digestograms for cooked rice flours (A) and grains (B); increase of glucose contents during hydrolyses of rice grains and rice flours (C) and grains (D), respectively; standard (white bread (—◆—), white rice flour (- ■ -), brown rice flour (···◆···), brown germinated rice flour (- - ▲ - -), white rice grain (—□—), brown rice grain (···◇···) and brown and germinated rice grain (- -△- -)

2.3.3. *In vitro* Glycemic index of Sangyod Phatthalung

Glycemic index (GI) and hydrolysis index (HI) values of Sangyod Phatthalung rice are shown Table 6. HI values ranged from 40-71 whereas GI's ranged from 62-79 with values relatively higher in white rice (polished) rice grains than brown rice grains and flours. GI of white rice flour and grains are 78.7 and 67.2, respectively. GI's of all flours were consistently

higher than GI's of the three corresponding grains (Table 7). All of the flours can be classified in the high GI group whereas the grains classified in the medium GI group. White rice grains and flour showed higher GI's than brown rice grains and flour and GI's of germinated rice grains and flour, respectively ($P<0.05$). Compared to other Thailand rice, GI' for Sangyod Phatthalung rice grains are lower than GI reported for brown Jasmine rice 70.3 (Jaisut *et al.,* 2008) and rice porridge (Srikaeo and Sopade, 2010). This confirms earlier suggestion that Sangyod Phatthalung rice is a reasonable candidate to moderate glycemic index (Hu *et al.,* 2004).

Table 7 *In vitro* glycemic index in grain and flour of Sangyod Phatthalung rice

	HI	GI	Interpretation
Flours			
White (polished)	70.9±1.45[a]	78.7±0.80[a]	High GI
Brown (unpolished)	68.0±1.72[b]	77.1±0.94[b]	High GI
Brown (unpolished) germinated)	67.9±0.97[b]	77.0±0.53[b]	High GI
Grains			
Polished rice	50.0±2.14[c]	67.2±1.17[c]	Medium GI
Brown (unpolished)	40.7±1.02[d]	62.0±0.57[d]	Medium GI
Germinated (unpolished)	42.5±1.62[d]	63.0±0.89[d]	Medium GI

Different superscripts in the same column indicate significant different ($P<0.05$)

2.3.4. Total phenolic content and α-amylase inhibition

Phenolic extracts from polished and unpolished rice contained various total phenolics with white rice the lowest in phenolic content and brown rice the highest (Table 8). This indicated that much of the phenolics were removed with the pericarp that was removed in the polishing process. Germination also led to some lowering of total phenolics which could be from the loss in processing water, thermal decomposition, possibly, interaction with other components (Walter *et al.,* 2013).

Table 8 Total phenolic compounds and percentage of porcine alpha-amylase inhibition activity of Sangyod Phatthalung rice

	Phenolic content (mg/mL)	Starch content (mg/mL)	Ratio Phenolic: starch	% inhibition
Polished				
White rice	0.015±0.002[c]	10	0.15:1	2.44±0.67[b]
Unpolished				
brown rice	0.154±0.002[a]	10	1.54:1	15.62±0.65[a]
germinated rice	0.123±0.003[b]	10	1.23:1	16.30±0.66[a]

Value are given as mean ± SD from triplicate

Different superscripts of type of rice in the same column indicate significant different (P<0.05)

Phenolic extracts were further studied in order to investigate the influence of phenolics on α-amylase inhibition comparing against three antioxidants (ferulic acid, ρ-coumaric acid catechin and protocatechuic acid). For the experiment with these controls, the inhibition of α-amylase activities was performed phenolic: starch ratio. The rice phenolic extracts were tested for amylase inhibition similarly but because different amounts of phenolics and hence different concentration of phenolics in the extracts differed, the experiments were therefore varied in phenolic: starch ratio (Figure 4). The inhibition varied drastically from the sample with very low phenolics (2.44% inhibition) to samples with higher phenolics (15.6-16.3% inhibition) for extracts from brown rice (Table 8). These data are superimposed against the three controls on Figure 3. It was concluded that phenolic components might have played an important role in α-amylase digestion of starch lowering GI and HI in the cases of brown rice samples. But it could be argued that the starches might have different physico-chemical properties (e.g., number of double helices or crystallinity) and, therefore, they were tested by DSC.

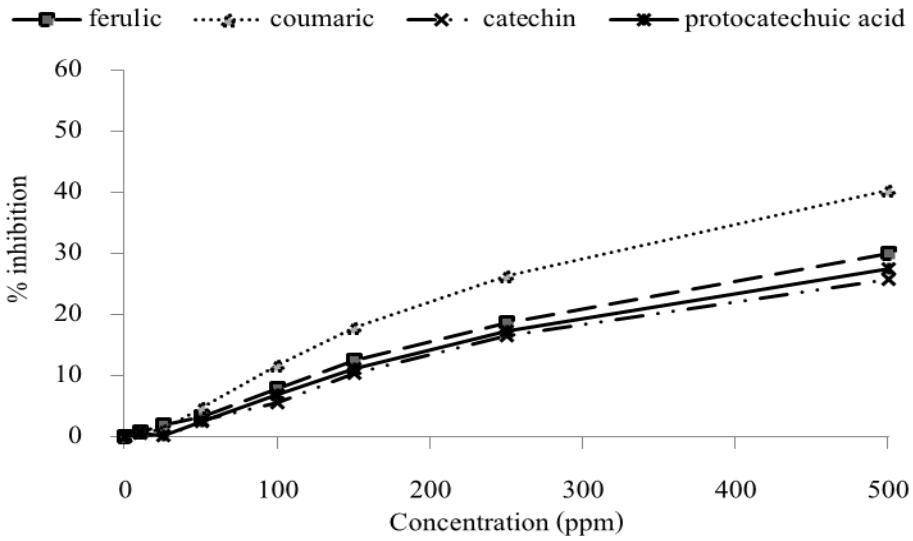

Figure 4 Relationships between phenolic content (standards) and % inhibition porcine α-amylase activity, including ferulic acid, coumaric acid, catechin and protocatechuic acid.

Comparing add phenolics content against α-amylase inhibition data also shown in Figure 4 as horizontal lines, it can be seen that the inhibition of α-amylase did increase at elevated phenolics content. α-amylase inhibition of ρ-coumaric was likely higher other standards (ferulic acid, catechin and PCA). Therefore, it was concluded that Sangyod Phatthalung rice digestion that resulted in a lower GI was due to the presence of phenolics which was the major contributor of α-amylase inhibition.

2.3.5 Type and content of phenolics in rice

HPLC spectra for all cooked and uncooked rice samples and the standard controls are shown in and Figure 5 and 6. The spectral peaks were identified for PCA, ρ-coumaric acid, and ferulic acid and the contents of each are shown in Table 9. It was clear that Sangyod Phatthalung rice contains PCA as the major phenolic component at levels approximately 1.1 and 1.5 mg/100 g dry sample for cooked and uncooked brown rice, respectively and 0.7 (cooked) and 0.85 (uncooked) unpolished germinated rice respectively. The ρ-coumaric and ferulic acids were found to be more similar among samples although some significant difference was observed but the differences were relatively small. The results demonstrated that PCA was mostly lost through polishing whereas ρ-coumaric and ferulic acids were also lost but to a much less extent. Cooking resulted in a slight (5-10%) decrease in phenolics content.

Table 9 Phenolic compounds of Sangyod Phatthalung rice (mg/100g dry sample)

	Polished (white)		Unpolished (brown)		Unpolished (germinated)	
	Cooked	Not cooked	Cooked	Not cooked	Cooked	Not cooked
Protocatechuic acid	0.334±0.015[c]	0.322±0.021[c]	1.105±0.049[a]	1.148±0.042[a]	0.716±0.013[b]	0.851±0.013[b]
ρ-coumaric acid	0.251±0.001[a]	0.317±0.015[a]	0.297±0.025[a]	0.345±0.015[a]	0.224±0.003[b]	0.226±0.007[b]
Ferulic acid	0.204±0.010[b]	0.238±0.015[c]	0.342±0.006[a]	0.357±0.010[b]	0.371±0.046[a]	0.483±0.016[a]
Total phenolics	0.790±0.004[c]	0.858±0.039[c]	1.672±0.054[a]	1.823±0.029[a]	1.477±0.082[b]	1.561±0.037[b]

Superscript notation on the same row indicates significant difference ($P<0.05$)

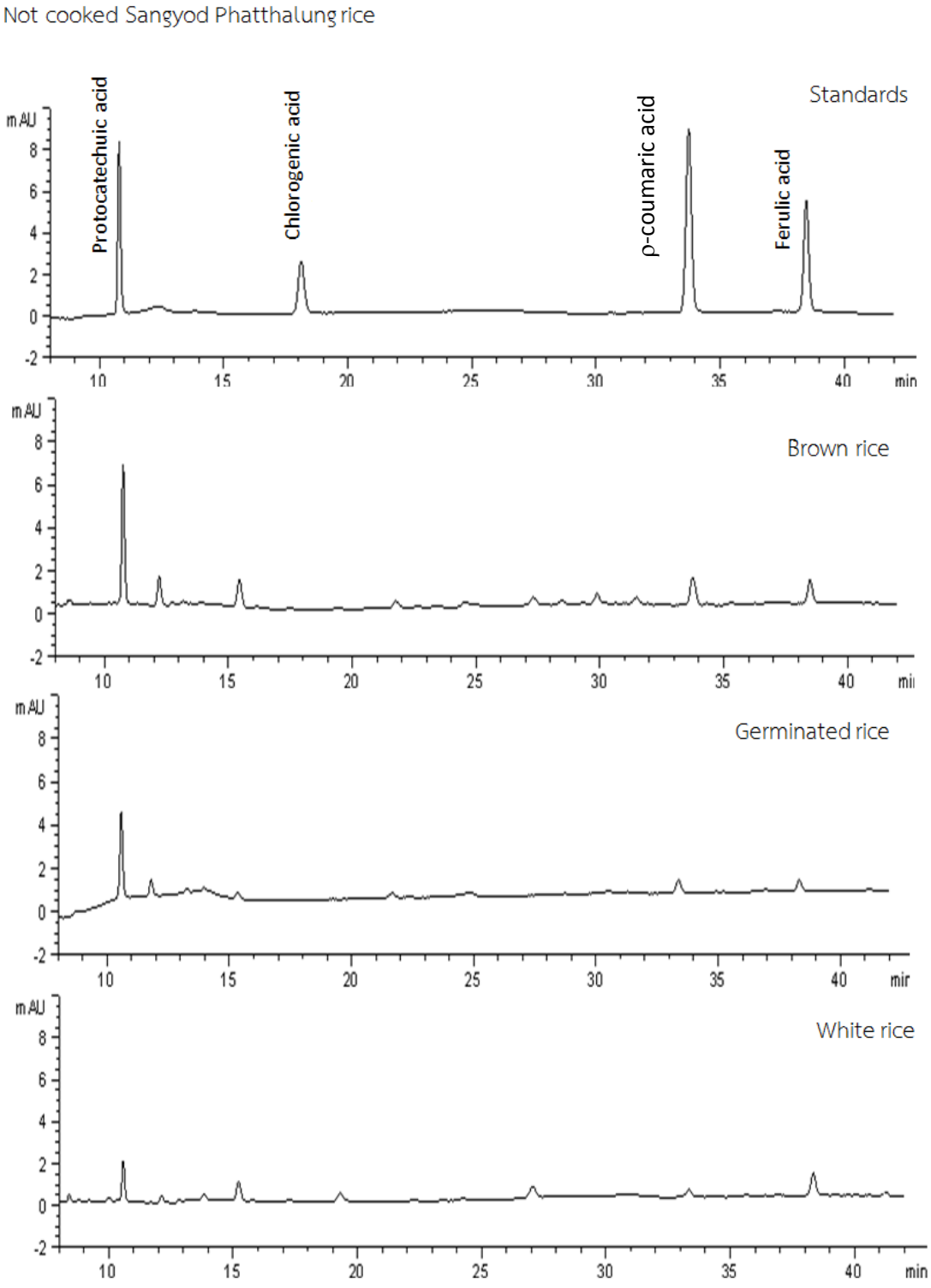

Figure 5 HPLC chromatograms (290 nm.) of phenolic extracts from not cooked Sangyod Phatthalung rice comparing polished (white), unpolished (brown) rice, germinated unpolished (brown) rice.

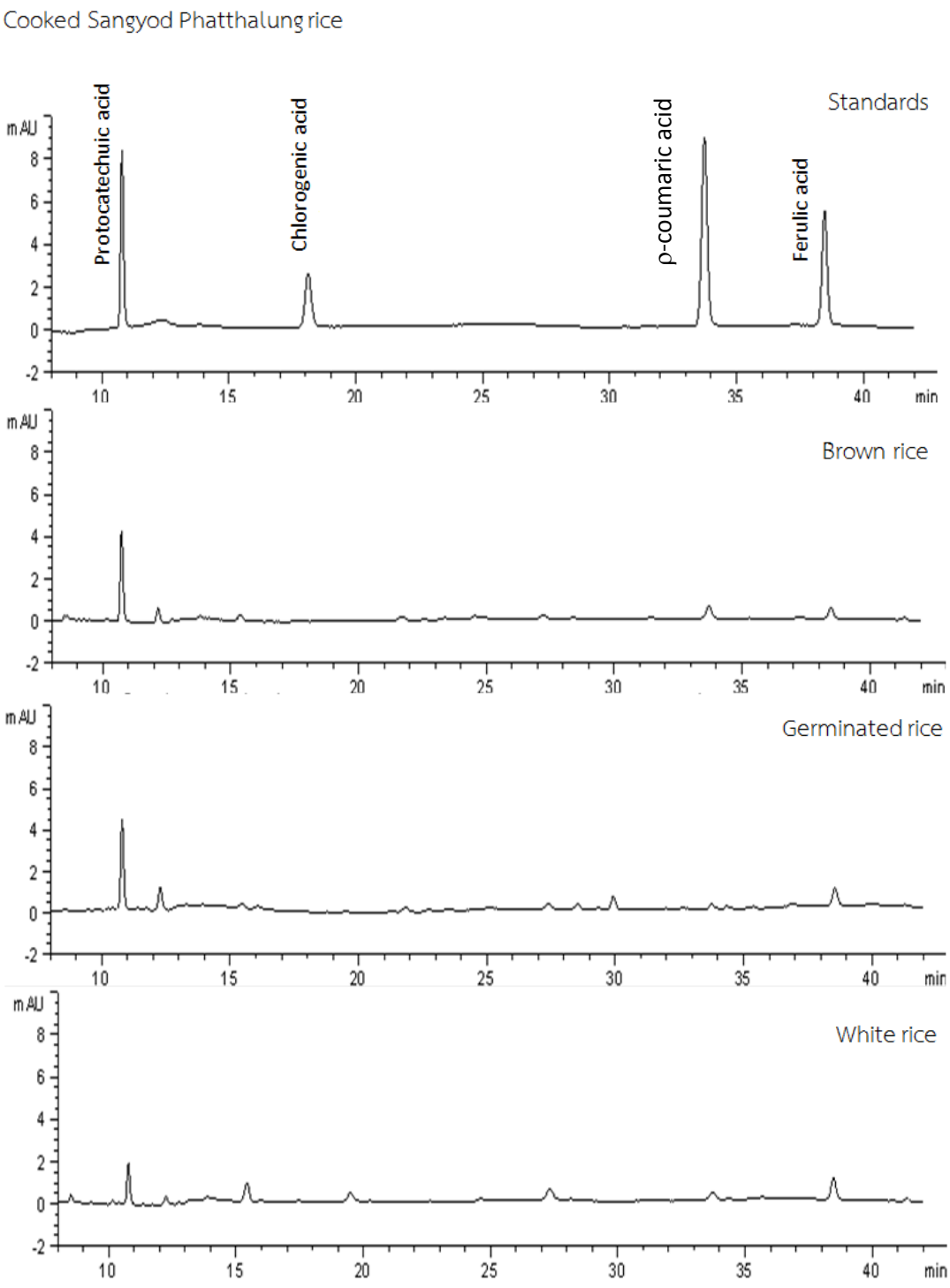

Figure 6 HPLC chromatograms (290 nm.) of phenolic extracts from cooked Sangyod Phatthalung rice comparing polished (white), unpolished (brown) rice, germinated unpolished (brown) rice.

2.3.6 Physico-chemical properties (DSC) of starch

Other factors may also vary and affect amylase digestion of starch among all rice samples studied. Since polishing of rice grains may have removed protein, lipids and modified some physical nature of the starches. In order to investigate the physicochemical effects, rice grains were milled, cooked (as in the digestion experiments) and frozen before tested for DSC endothermic transition analysis. The objective was to determine endothermic melting transition of amylopectin and amylase as well as any other endothermic starch-lipid complex (Figure 7).

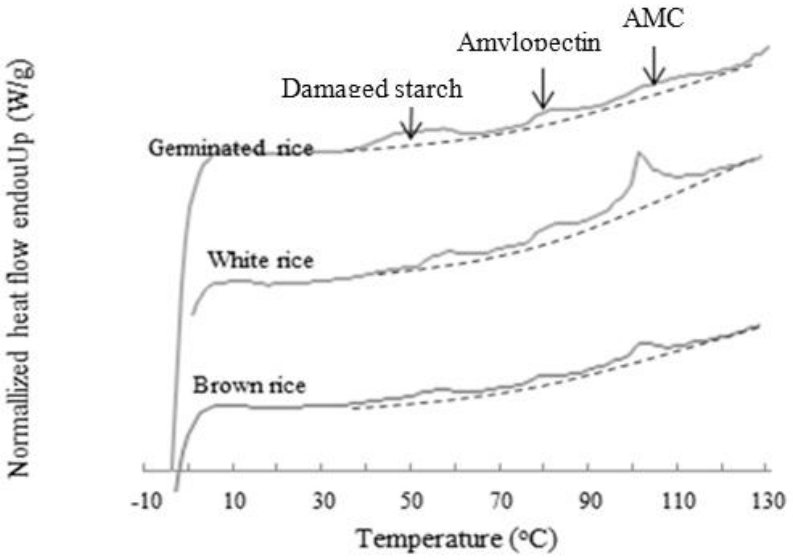

Figure 7 DSC Thermogram of cooked and cooled white (polished), brown (unpolished) and germinated brown (unpolished) Sangyod Phatthalung rice at 90 percentage moisture content.

The gelatinization temperature and transition enthalpy of cooked rice samples are shown in Table 10. All samples showed three endothermic peaks, 40-62 ^{o}C, 75-86^{o}C, and 93-110^{o}C. The low peak was likely damaged starch (40-60^{o}C) and seconded peak was likely due to amylopectin (75-86 ^{o}C) transition. The third peak was assigned as at 93-110^{o}C amylose lipid complex (AMC) and/or amylose melting transition (peaks for brown rice exhibited a broad multiple transition). These complexes have been earlier reported to be in this range (between 95 and 105^{o}C, Galloway *et al.,* 1989; Karkalas and Raphaelides, 1995). It was found that the three rice samples exhibited some small ungelatinized or unmelted starch remained (endothermic peaks

at 80°C, T_p). Comparing the DSC and starch digestion results (that white rice more distribute than brown and germinated), it was found that there was no correlation between amylopectin endothermic starch melting (second) enthalpy (i.e., double helix content) and starch digestibility by α-amylase (Table 8). This suggested that the helical structure of starch was not the primary prefer that prevented the starch digestion in this case.

Table 10 Gelatinization temperatures and transition enthalpy from different flours of Sangyod Phatthalung rice

Peak	Rice	transition temperatures (°C)			ΔH (J/g)
		T_o	T_p	T_e	
First	Polished (white)	51.93±0.18[a]	58.06±0.06[a]	62.42±0.51[a]	0.20±0.05[ab]
(Damage starch)	Unpolished (brown)	51.67±0.03[a]	58.00±0.29[a]	62.24±0.35[a]	0.06±0.01[b]
	Unpolished (germinated)	39.41±0.71[b]	50.17±1.78[b]	62.45±0.49[a]	0.44±0.14[a]
Second	Polished (white)	75.72±0.11[a]	80.39±0.28[a]	86.81±0.23[a]	0.19±0.08[a]
(amylopectin)	Unpolished (brown)	75.87±0.11[a]	80.44±0.61[a]	86.06±0.31[a]	0.04±0.01[a]
	Unpolished (germinated)	75.57±0.17[a]	80.61±0.46[a]	85.64±0.44[a]	0.11±0.05[a]
Third	Polished (white)	98.70±0.04[a]	101.72±0.28[a]	106.99±0.40[b]	0.83±0.15[a]
(AMC)	Unpolished (brown)	93.93±2.94[a]	104.94±1.02[ab]	110.52±1.22[b]	0.15±0.01[b]
	Unpolished (germinated)	93.82±2.73[a]	107.05±1.13[a]	116.70±1.00[a]	0.21±0.10[b]

Value are given as mean ± SD from triplicate

Different superscripts of type of rice in the same column indicate significant different ($P<0.05$)

The lower endothermic peaks over the 39-62 °C range were identified as damaged starch found in all rice samples (white-polished, brown-unpolished and unpolished-germinated (Table 10). This identification was investigated to prove this potential origin. Since these samples were ground by a high speed blender which could have partially damaged the starch granules and destroyed some of the crystalline structure, an experiment was performed in order to determine the swelling properties. Comparing dry ground starch and wet ground starch data (Table 10)

showed % solubility and swelling power dry mill rice flour (DF) and wet mill rice flour (WF) (control) were each mixed with water and heated in a water bath > 85^{o}C. Swelling and solubility of the starch was seen according to gelatinization. In a process of gelatinization, amylopectin structure of the granular starch underwent a major change by expansion and becoming solubilized and amylose chain leaving the granules. Swelling is caused by water uptake of the amorphous chains of the amylopectin in amorphous regions of semi-crystalline lamellae and measured as the degree of water absorption by rice flour was found lower. Starch solubility of all DF was higher than that of corresponding WF rice significantly ($P<0.05$). Moreover, white, brown and germinated rice flours showed 4.3, 5.1 and 6.5 % solubility, respectively. Swelling power of WF of brown rice were slightly swollen than other samples and swelling power of WF and DF were not significant different ($P<0.05$). The result suggested that contained more damage starch than WF because of the damage during the dry milling process.

The viscosity properties of DF and WF of rice flour showed a duplex pattern by a Figure 8. The pasting profile of the three flours were markedly different. Viscosity of DF in all case as lower than WF indicating more damaged starch might be present in DF (Barrera *et al*., 2011). When starch is damaged some structure might be destroyed in terms of granule structure resulting in decreasing viscosity from fragment/remnants of destroyed granule. Moreover, upon cooling the viscosities at set back of WF of all samples were higher in DF for white rice than brown rice suggesting that more long-chained amylose might have been released during gelatinization, resulting different characteristics of the gel formed. Since an increase swelling was observed in dry ground starch, the endothermic peak observed at 39-62 ^{o}C are most likely due to damaged starch as earlier reported by Tester (1997).

Mechanical damage of dry starch granules (i.e., ball milling) could lead to starch granule damage resulting in a materail containing a mixture of intact granules, gel-forming fragments, cracked gel-forming, ordered a material fragments and soluble low-MW amylopectin (Tester, 1997). Hatjim *et al*. (2012) reported that dry milling increased damage of starch which showed solubility increasing with increasing surfaces and/or exposure to loosely packed inner segments of starch granules.

The data on the increasing starch solubility (Table 11) and reducing viscosity in (Figure 8) in damaged starch (DF) as compared with undamaged starch (WF) suggested that there are

fragments of broken starch granules and remnants exposed of surfaces of inner parts of amylopectin that could have been physically modified in a way that resulted in separated, lower melting transition at 39-62^{o}C in these rice samples. The level of impact of damaged rice on solubility was found in following order: germinated rice, brown rice and white rice. The level of impact of damaged rice on viscosity profile was found to be in the following (reverse) order: white rice, brown rice and germinated rice. The levels of swelling power although were found in the following order: germinated, white and brown rice; the difference were only within 1-2 %. All case showed dry milled flours higher in solubility, swelling and viscosity than wet milled flours.

Table 11 Solubility and swelling power of Sangyod Phattalung dry milled and wet milled rice flours

		Dry milled flour (DF)	Wet milled flour (WF)	Difference P-value
Solubility (%)	White rice	4.30±0.60^{b}	3.18±0.56^{d}	0.044
	Brown rice	5.06±0.57^{b}	3.43±0.12^{c}	0.038
	Germinated rice	6.47±0.37^{a}	4.39±0.51^{b}	0.008
Swelling power	White rice	7.60±0.05^{b}	7.48±0.01^{bc}	0.047
	Brown rice	7.54±0.05^{ab}	7.41±0.04^{c}	0.042
	Germinated rice	7.71±0.03^{a}	7.55±0.04^{ab}	0.001

Values are given as mean ± SD from triplicates

Different superscripts in the same column indicate significant difference ($P<0.05$)

However, the endothermic transition showed the T_0 of germinated rice much lower than T_0 of white rice and brown rice, suggesting the relationships with DSC transition was not a direct one. It has been discussed that smaller and highly crystalline starch granule such as rice starch are more susceptible to milling damage (Tester, 1997). The data presented have may be limited from drawing specific, concrete relationship since 1) size also plays a more important role in addition to composition and architectural properties of granule, 2) lipid and protein present in brown rice also impact the melting transition of the starch which in turn also influence the susceptibility to

be damaged (Tester, 1997). In the other words, there are likely more sample-specific parameters that impacted endothermic transition, starch solubility, swelling properties and viscosity in different way that one could not find a direct and simple cause-effect relationship with the degree of starch damage. Since an increase swelling was observed in dry ground starch, the endothermic peaks observed at 39-62 ^{o}C are most likely due to damaged starch as earlier reported by Tester (1997).

The total dietary fiber (Table 4) of brown rice and germinated rice were higher than white rice significantly ($P<0.05$) that resulting with *in vitro* GI. In this study, it is possible that total dietary fiber may be associated with the activities of digestive of α-amylase.

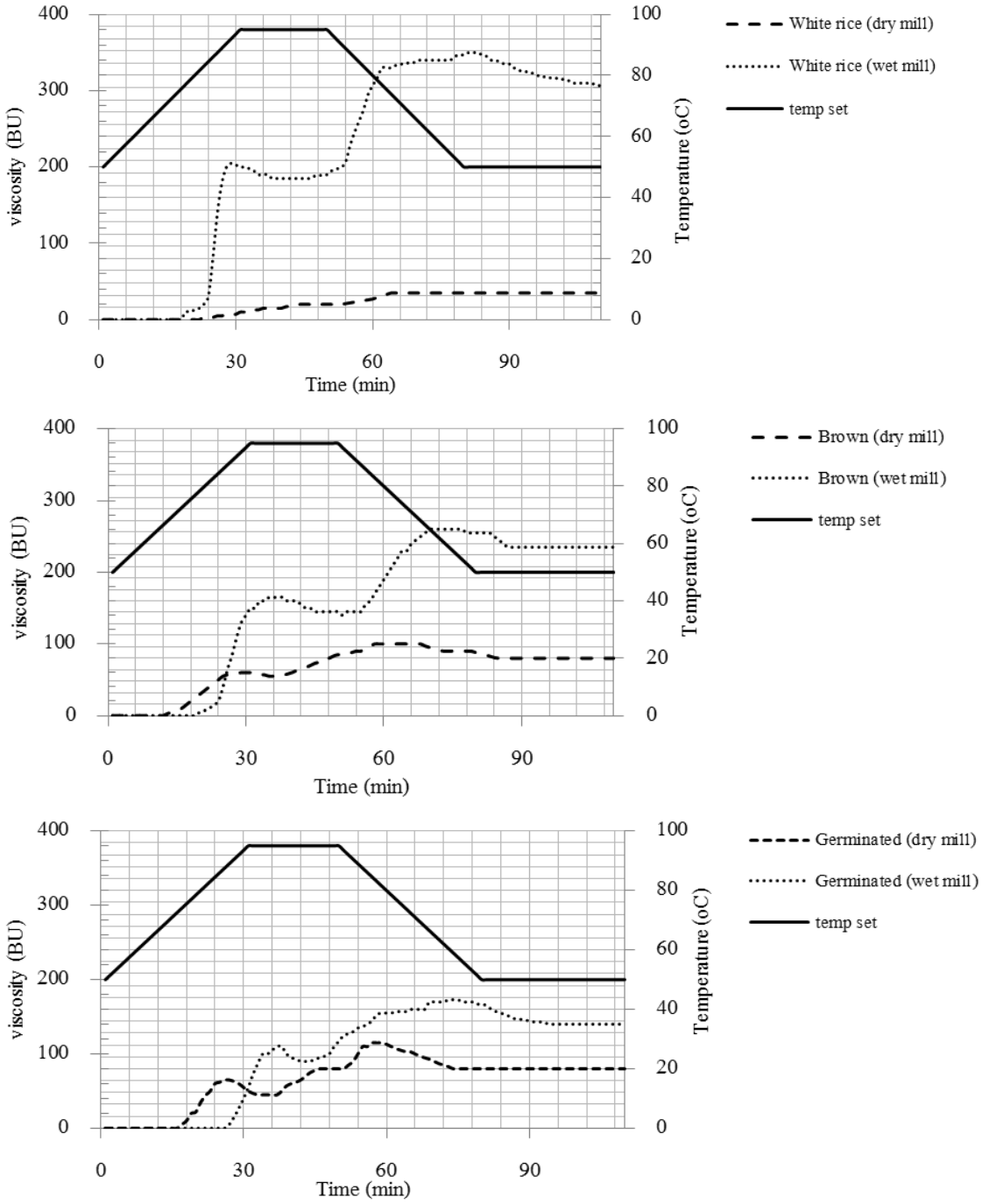

Figure 8 Viscosity profiles of wet and dry milled flours from white, brown and germinated Sangyod Phatthalung rice as determined by a Viscosity Graph Brabender analyzer.

2.4 Conclusion

Unpolished Sangyod Phatthalung rice in form of whole grains is hydrolyzed at rate slower than its flour counterpart. The reddish brown color of the pericarp is shown to be rich of phenolics particularly PCA which (and together with other phenolics) exhibited α-amylase inhibition *in vitro*. Germinated brown rice also had similar effects but slightly less phenolics found due to some loss in the pre-treatment and germination process. Physico-chemical changes in amylopectin and amylose helical formation and amylose-lipid complex were present in the three rice samples differently and showed a presence of damaged starch but did not seem to correlate with α-amylase activities, i.e., the GI values of white rice were found to be highest but its damaged rice proportion the lowest. Hence GI or amylase activity in this case did not increase with the degree of rice damage. Consuming brown Sangyod rice should help reduce starch digestion (in vitro results) and consequently lower HI and GI. This is of great importance to weight control and diabetic control application. However, in vivo study is needed to validate this postulation.

Next studies, we will study glycemic index in human. Samples included white, brown rice grains and brown rice flour. Because starch digestion of germinated rice and brown rice were no significant different but starch digestion of flour and grain were significant different. It is interesting for studies in clinical GI.

Result article 3

3. Clinical studies of Glycemic of Sangyod Phatthalung Indigenous Southern Thai rice

3.1. Introduction

Diabetes and obesity are major health problems and diet is the essential key management, especially, carbohydrate and its glycemic index. The glycemic index (GI) is a classification of food based on glucose response to a food relative to reference food such as glucose or white bread (Trinidad *et al.,* 2010). However, in normal glucose metabolic people, glucose excursion after ingestion of carbohydrate may not be obvious according to the intact of beta cell in secretion of insulin to handle the high glucose level. In this group of people, diet stimulate less insulin respond may cause less beta cell exhaustion, in metabolic studies of Bata-cell function (Florez, 2008; Gerich, 2000). Changes of blood glucose of low GI foods are slower than high GI and more gradual lead to response of insulin secretion (Willett *et al.,* 2002). High blood glucose level causes complications such as nerve damage, kidney disease, eye disease, and cardiovascular disease (Leed, 2002). Moreover, studies show that low GI food were full hunger than high GI. In additional, consuming low GI foods can prevent diabetes mellitus as well as obesity (Miller *et al.,* 2002).

Rice is main food for half of the world population (Butsat and Siriamornpun, 2010). When consuming rice, there are some related preparation processes including removed husk that protect rice grain (brown rice). When brown rice has polished bran, and germ removed that is white rice. The main component of carbohydrates of rice is carbohydrate. Such processes lead to a wide range of GI in rice (54% to 121%) with different properties (Miller *et al.,* 1985; Jenkins *et al.,* 1988). In addition to the different of physical property of rice, there may be some special features like pigment of rice affecting GI. Moreover, pigmented rice typically contains high antioxidant activities (Srisawat *et al.,* 2011). Consumption of pigmented rice improves human health due to the antioxidant activities, which have been reported to contain acetylated procyanidin, anthocyanins, and other phenolic with significant free radical scavenging activity (Hu *et al.,* 2003). The phenolics in controlling glycemic response are positive effects on glucose response have been obtained *in vitro* and *in vivo* studies (Nizamutdinova *et al.,* 2009). Takikawa *et al.* (2010) reported that anthocyanins improve insulin and glucose response in diabetic rats. Sangyod Phatthalung rice is a traditional pigmented rice grown in the area of Phatthalung

province for more than a hundred years. Its typical grain appearance is small and long-slender grain, with a dark red pericarp, giving soft and aromatic cooked rice. Yodmanee *et al*. (2011) reported polyphenols and anthocyanin contents of Sangyod Phatthalung rice to be relatively high. Rice has inconsistent GI report. This inconsistency could be the result of special type of rice (pigmented, shape, fiber and different other composition), the production of grain (polished, brown) or the cooking method. Sangyod has interesting characters; those are red pigment (which may contribute to antioxidant property), good aroma, shape, color and soft texture which make it palatable. As GI is an essential factor as a choice of carbohydrate choosing, Sangyod rice characteristics that might influence GI should be under more in depth investigation. In earlier investigation (Inpun *et al.,* 2013), we have discovered major phenolics compounds in Sangyod that were shown to play an important role in alpha-amylase inhibition (*in vitro*). The objective of this work was then to further explore if this can be observed *in vivo*. This information may be helpful for consumer to choose the proper carbohydrate foods. Our hypothesis is Sangyod rice that its brown rice has more favorable GI than white rice and rice flour.

3.2 Materials and Methods

From chapter II, we choose sample for GI clinical studies including white rice, brown rice and brown rice flour of Sangyod Phatthalung rice. White rice and brown rice were comparing GI (glycemic index) and II (insulin index) between polished rice and unpolished rice. Brown rice and brown rice flour were comparing GI and II between flour and grain.

3.2.1 Test meals

Sangyod rice was obtained from Phatthalung Province, Thailand. The dehulling process was done to make unpolished rice and polished rice. The grains were packed in nilon bags and transported to Prince of Songkla University and store at ambient temperature before further processed. Five kilograms white rice and ten kilograms brown rice were washed and dried by hot air oven at 55^{o}C for 10 hour (8-10% moisture content) and five kilograms of brown rice was milled into flour by flour milling machine at speed 25,000 rpm (Daming, China). The rice flour was sifted through a 200 mesh sieve and packed in nylon bags and kept at 4^{o}C throughout the experiment. White rice, brown rice and flour brown rice of Sangyod Phatthalung were *in vivo* studied. In order to obtain cooked rice with relatively equal soft texture, white rice with water

ratio 1:1.5 (w/v) and brown rice and brown rice flour with water ratio 1:2 (w/v) (Table 12) were cooked using an electric rice cooker (International, China).

Table 12 Recipes for test meals used in GI study

	Control		Test meal		
	White bread	glucose	White rice	Brown rice	Brown rice flour
Material					
Grain (g)	-	-	100	100	100
Water (g)	-	100	150	200	200
Glucose (g)	-	50	-	-	-
Control					
g CHO/meal	50	50	50	50	50
Water (mL)	150	150	150	150	150
Soup (mL)	-	50	50	50	50
Weight of meal (g)	175.1 ± 2.1^{ab}	50.00	170.8 ± 4.0^{b}	179.8 ± 2.0^{a}	146.6 ± 2.6^{c}
Energy (Kcal)	318.7 ± 1.4^{a}	200.00	266.3 ± 3.0^{c}	317.3 ± 2.1^{a}	296.2 ± 1.4^{b}

Value are given as mean ± SD

Different superscripts in the same roll indicate significant different ($P<0.05$)

A cabbage soup, containing very little cabbage, salt and scallions, were cooked using an electric rice cooker for 20 minutes. For the controls, white bread (Farmhouse Co Ltd., Thailand) was purchased locally and glucose (50 g equivalent) was used. Bread in portion containing 50 g carbohydrate equivalent was prepared. Fifty gram glucose was dissolved in 150 mL of warmed water (Figure 9). Clinical GI samples are shown in Figure 10.

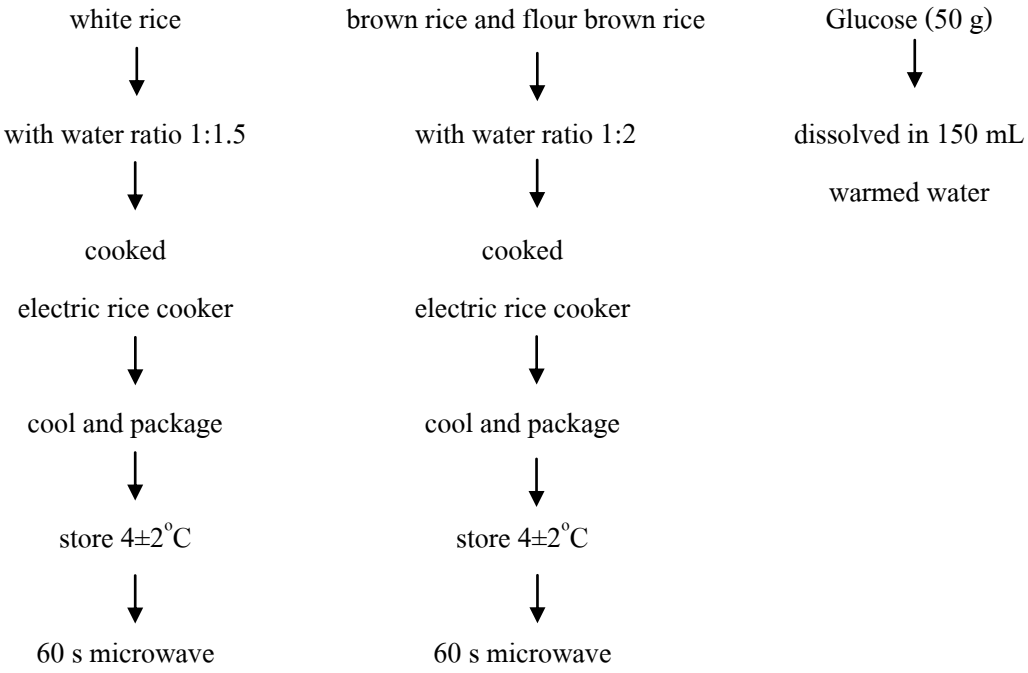

Figure 9 Prepare test meals for GI clinical study

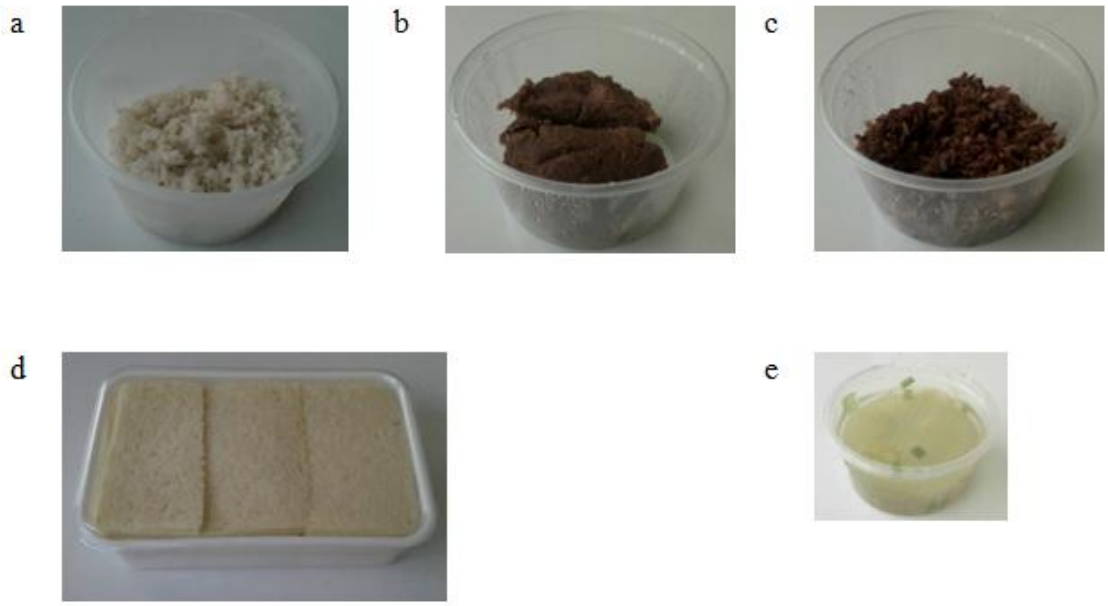

Figure 10 Sangyod Phatthalung rice and cabbage soup soup was standard food of glycemic index study (a = white rice, b = brown rice, c = brown rice flour, d = white bread and e = cabage)

3.2.2 Volunteer and study protocol

In clinical glycemic index study, 10 healthy volunteers were consisted of 5 men and 5 women with mean age, weight, height and body mass index of 26.8±2.26 years, 60.6±6.93 kg, 1.69±0.04 m and 20.8±1.46 kg/m^2, respectively. All volunteers had a normal blood pressure, did not drink alcohol, smoke, or take antibiotics. Female volunteers who were in menstrual period were excluded. The volunteers were served with 5 different test meals (glucose, white bread, white rice, brown rice, and brown rice flour) at random order at 8 A.M. after an overnight fasting. The meals were consumed within 5 minutes along with 200 mL water. An intravenous cannula was inserted into a superficial vein in the forearm, and blood samples were collected immediately before serving the test meals and 30, 60, 90, 120 and 180 minutes after. Serum glucose levels were measured using a hexokinase method (Beckman Coulter, Fullerton, Calif, USA) and insulin in serum was analyzed by Electrochemiluminescence immunoassay (ECLIA) were analyzed with a Modular E170 (Roche Diagnostics). Both were analyzed by laboratory of the Chemical Chemistry Department, Faculty of Medicine, Prince of Songkla hospital. Each subject gave a written informed consent of acceptance, which was previously approved by the Ethics Committee for Prince of Songkla University. During the study, hunger sensory data recorded were 1) the time period (minutes) when the subjects began feeling hungry after fed and 2) the degree of hunger at three hour after test meal. The degree of hunger was estimated by 5-point scale marking on a horizontal chart (10 cm long), (Figure 11).

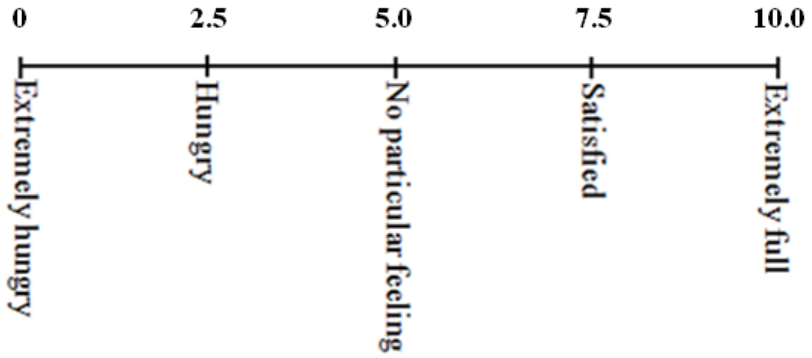

Figure 11 Marking at the horizontal scale of hunger estimate

Glycemic index (GI) and Insulin index (II) were calculated in accordance with in GI methodology (Brouns *et al*., 2005; Oku *et al*., 2009). Incremental serum glucose and insulin level

AUC versus time were calculated using the trapezoidal rule with fasting values as the baseline, and the ratio of AUCs for 2 h versus 50 g of glucose ingestion was considered to be the GI. Calculating AUC is as follows assuming that at time t_0, t_1, …., t_n, the blood glucose concentrations are G_0, G_1, …, G_n respectively.

$$AUC = \sum_{n}^{x=1} Ax$$

Where Ax = AUC for Xth time interval

Xth = interval between time t_{x-1} and t_x

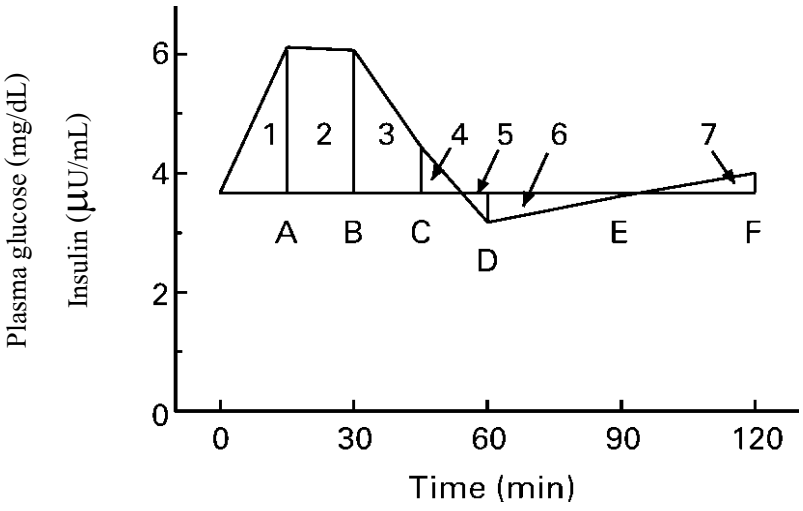

Figure 12 Calculation of incremental area under the curve (AUC)

Source: Brouns *et al*. (2005)

Application of the formula to the above-cited example (incremental areas for segment 1-7 (Figure 12)

1= (A/2) x (t_x-t_{x-1})

2= (A/2 + B/2) x (t_x-t_{x-1})

3= (B/2 +C/2) x (t_x-t_{x-1})

4= (C^2/(C - D)) x (t_x-t_{x-1})/2

5 and 6 = area below baseline not including = 0

7= (F^2/(F - E)) x (t_x-t_{x-1})/2

The AUCs of serum glucose and insulin for 2 h (AUC-2 h-glucose and AUC-2 h-insulin) and 3 h (AUC-3 h-glucose and AUC-3 h-insulin) after ingestion were calculated with AUC of 50

g of glucose and white bread was comparative controls. The results were expressed in percents. The duration of 3 hours was selected in order to study the correlation of hunger and AUC glucose. AUC-2 h-glucose and AUC-2 h-insulin of serum glucose and insulin were compared with paired Student's t-test. One-way analysis of variance Tukey's post hoc test and Pearson's correlation was and used to analyze the effect of difference types of rice.

The GI calculated as

$$GI = \frac{\text{Serum glucose response area of sample}}{\text{Serum glucose response area of glucose}} \times 100 \quad \text{..... [7]}$$

$$II = \frac{\text{Serum insulin response area of sample}}{\text{Serum insulin response area of glucose}} \times 100 \quad \text{..... [8]}$$

3.2.3 Statistical analyses

All statistical analyses were performed using the Statistical Package for Social Sciences version 10.0 (SPSS Inc, Chicago, Ill). The changes in serum glucose and serum insulin after the ingestion of each test meal by time interval were analyzed by repeated-measurement analysis of variance (ANOVA) and Tukey multiple tests. Differences in GI and serum glucose levels among the 5 test meals were also analyzed by pair T-test. Results were reported as means ± SE ($P< 0.05$).

3.3 Result and discussion

3.3.1 The nutrient composition of Sangyod Phatthalung rice products

The nutrient compositions of tested meals are shown in Table 13, including standard foods (white bread and glucose) and rice samples (white rice grains, brown rice grains and brown rice flours). Tested meal composition ranges were as follow 27.81-34.12 % carbohydrate, 51.39-60.38% moisture; 1.59-4.21% ash, 7.97-8.51% protein and 0.77-3.62% fat contained. White rice and white bread contained highest amounts of carbohydrates and proteins. Fats and proteins in foods have been suggested to significantly lower GI (Jenkins *et al.,* 1988).

Table 13 Proximate composition, and caloric energy of rice products as well as glucose and bread control used in this study.

	Glucose	White bread	White rice	Brown rice	Brown rice flour
Chemical composition (%)					
Carbohydrate	100.00	28.56±0.34^{bc}	29.28±0.69^{c}	27.81±0.31^{b}	34.12±0.60^{a}
Moisture	-	55.38±0.11^{b}	60.38±0.60^{a}	58.37±0.22^{a}	51.39±0.44^{c}
Ash	-	4.21±0.28^{a}	1.59±0.01^{c}	2.04±0.06^{b}	2.46±0.29^{b}
Protein	-	14.97±0.08^{a}	7.97±0.01^{d}	8.16±0.01^{c}	8.51±0.02^{b}
Fat	-	0.88±0.02^{b}	0.77±0.10^{b}	3.62±0.06^{a}	3.51±0.10^{a}

Value are given as mean ± SD

Different superscripts in the same row indicate significant different (P<0.05)

Volunteers began to perceive their hunger at 86±12.13 minutes after having glucose (P<0.05), followed by 126.50±11.05 minutes for brown rice flour, 129.20±8.77 minutes for white rice, 149.38±10.58 minutes for white bread and 157.40±8.30 minutes for brown rice. The degrees of hunger after fed incrementally decreased in following order; glucose, white rice, brown rice flour, brown rice and white bread (8.22±0.87, 7.85±0.47, 4.98±0.54, 4.12±0.99 and 2.66±0.54, respectively) (where, 10.0 = extremely full, 7.5 = satisfied, 5.0 = no particular felling, 2.5 = hungry and 0 = extremely hungry).

3.2.2 Biochemical analysis

Serum glucose and serum insulin after consumption of tested meals are shown in Figure 13. All volunteers exhibited similar fasting concentrations of serum glucose. After ingestion of a meal, 30-min incremental serum glucose elevated from the baseline values to 53.04±14.96 mg/dL, 58.44±18.83 mg/dL, 37.67±22.54 mg/dL and 42.42±21.33 mg/dL for white bread, brown rice flours, white rice grains and brown rice grains, respectively. All were significantly different from the baseline (P<0.05). The incremental serum glucose was highest with the glucose solution control which was higher than that of the bread control and rice meals significantly (P<0.05). The incremental glucose level was sustained for the glucose meal at 60

minutes which was at level higher than other meals (67.85 mg/dL, P<0.05). The patterns of blood glucose elevation were similar among white bread, brown rice, white rice and brown rice flour. It was clearly observed that the treatments with lower blood glucose peaks and prolonged decline of serum glucose levels differed from of glucose meal control which showed a rapid increase of blood glucose led to a high peak followed by a rapid decline of glucose after 60 minutes. At 60 minutes after meal, serum glucose excursion was maximum from glucose higher levels than other meals (53.40 mg/dL, P<0.05). At 120 minutes, serum glucose were differed among treatments (P>0.05). Serum glucose from rice meals and glucose dropped to levels close to or lower than the base line but still sustained from white bread (26.50 mg/dL, P<0.05). However, brown rice flour gave lower serum glucose level at -7.50 mg/dL, P<0.05). Interestingly, at 180 minutes, serum glucose from the glucose control dropped maximally and deeply to level (66.00 mg/dL) much lower than the baseline (80.50mg/dL) (Figure 13). One volunteer had a asymptomatic serum glucose level of 58 mg/dL. At 180 minutes, serum glucose of bread and rice meals were significantly higher than of glucose (P<0.05) with no strikingly low level reported. For brown rice flour meal, the decline rate of serum glucose was more rapid than that of white bread despite similar peak serum glucose level at 30 minutes. For brown rice and white rice, a rapid drop of the glucose level was observed at 120 minutes to lower than base line but could be maintained at a sustained level through 180 minutes.

Changes in serum insulin levels are also shown in Figure 13. Serum insulin levels at 0 minute from each meal were not significantly different (P<0.05). The serum insulin levels at 30 minutes were high for glucose (930.0±525.3 mIU/dL) and white bread control (917.6±461.5 μU/mL) which were higher than of white rice (378.2±234.4 mIU/dL), brown rice flour (473.4±270.5 mIU/dL) and brown rice (646.7±323.2 mIU/dL) (p<.05). At 60 minutes, serum insulin level of glucose standard still sustained at a high level of 915.0±470.0 mIU/dL, while insulin levels from other meals showed some decline with patterns parallel to that of the glucose level counterpart but at a different rate. Insulin levels of white bread standard (728.7±317.5 mIU/dL) and brown rice flour (477.3±315.3 mIU/dL) were significantly higher than those of white rice (209.2±150.5 μU/mL) and brown rice (203.3±166.1 μU/mL) (p<.05). At 120 minutes, insulin levels of all meals dropped in according to the dropping of serum glucose of the glucose control, white bread, white rice, brown rice, and brown rice flour (23.94±32.38 mIU/dL,

34.85±27.04 mIU/dL, 7.99±10.35 mIU/dL, 4.62±3.88 mIU/dL and -0.47±3.33 mIU/dL, respectivly). All rice meals resulted in similar insulin levels and were close to the base line values. However, both glucose and bread controls were higher in serum insulin levels which were higher than that of rice meals ($P<0.05$) and higher than the baseline values. Noticeably, the serum insulin level of the glucose meal remained high despite back-to-baseline serum glucose levels. At 180 minutes, all meals gave similar serum insulin levels equilibrated back to its baseline values which were not different between the treatments and the control meals (0.93±9.71 mIU/dL, 10.72±11.77 mIU/dL, -0.43±4.17 mIU/dL, 0.61±2.40 mIU/dL, -2.46±1.68 mIU/dL for baselines of glucose, bread, white rice, brown rice and brown rice flour, respectively).

Area under curve (AUC) 2 hours and 3 hours of glucose and AUC-2, AUC-3 of insulin are shown in Figure 14. At 2 hours, AUC-2 and AUC-3 glucose for the glucose standard were 4423.22±1742.48, 4558.94±1847.01 mg.min.dL, respectively ($P=0.431$) while AUC-2 and AUC-3 glucose for the bread standard were 2588.72±687.09, 3083.84±992.27 mg.min.dL, respectively ($P<0.05$). The latter AUC was lower than that of glucose despite higher calories. Rice grains and flour gave much lower AUC than of glucose and were similar to of the bread.

Interestingly, AUC insulin was highest for bread and high for glucose despite different AUC glucose. AUC insulin for rice meals were lower than that for bread and glucose significantly ($P<0.05$).

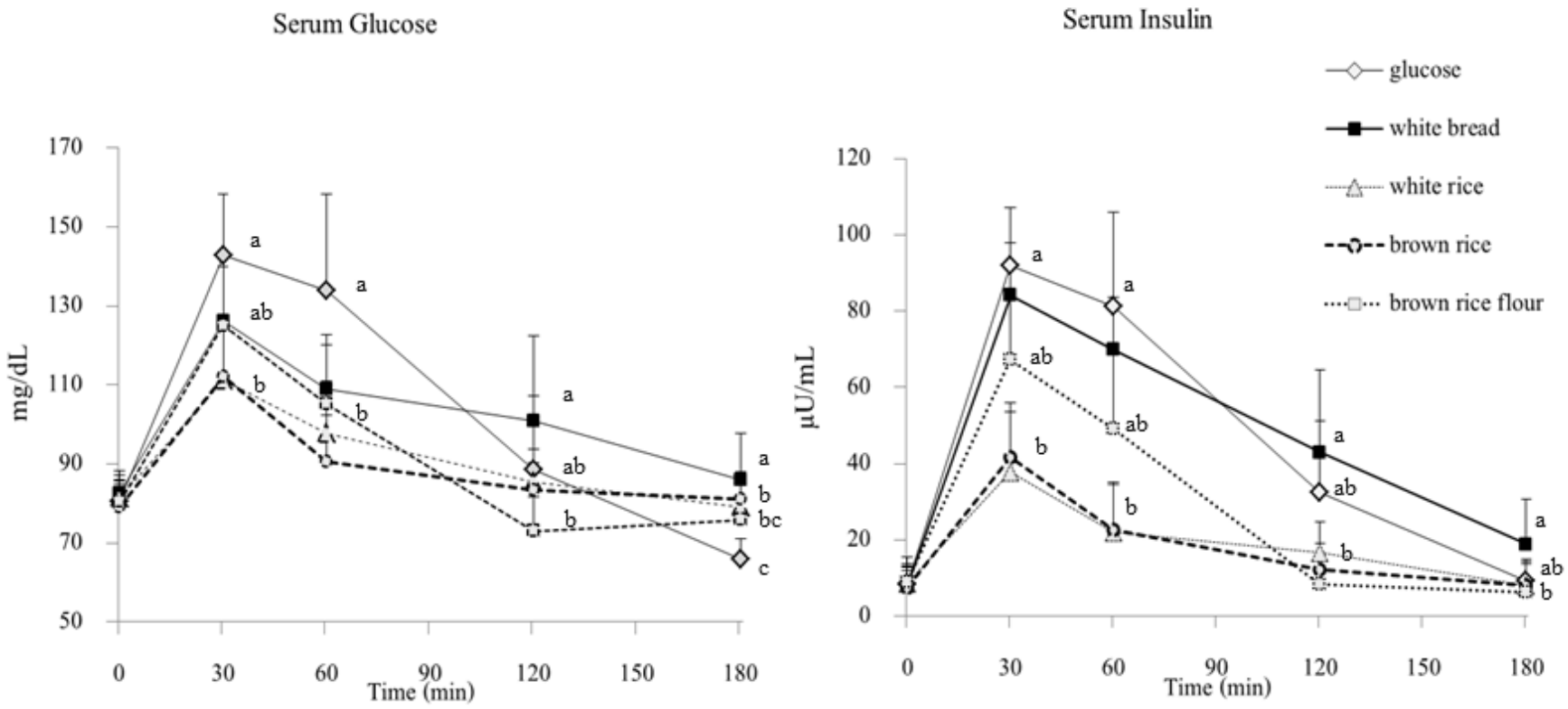

Figure 13 Curves of elevation of serum glucose and serum insulin after the ingestion of test foods white rice, brown rice and brown rice flour) and standard food, glucose and white bread). Values are expressed as mean ± SD. A significant different was found between values with same lowercase letters at each time point, at $P<0.05$ by Tukey's test.

AUC-3 of glucose and insulin were quite similar to AUC-2 with glucose, brown rice flour, whereas of bread, white rice and brown rice differed (Figure 14). Bread gave a larger AUC-3 of glucose than AUC-2. AUC-3 of insulin was higher than AUC-2 indicating further absorption of glucose from intestine and this higher serum glucose stimulates insulin secretion. This prolonged hyperglycemia from bread may explain less hunger level at 3 hours after consuming bread. For the case of rice meals, white rice as well as brown rice provided further glucose absorption into the blood stream leading to a larger area of serum glucose at 3 hour than at 2 hour similarly to that of bread but white rice meal caused unchanged insulin secretion while brown rice meal caused higher AUC insulin at 3 hours (greater than at 2 hours). This finding might be from the variability among volunteers and small number of volunteers used leading to relatively large variation of insulin secretion. However AUC-3 of glucose or insulin had no correlation with hunger. There was no correlation between glucose and insulin level and time-onset of hunger.

Comparing area under curve of serum glucose and serum insulin between food standards and food samples, it was found that there was a significant difference between AUC-2-glucose and AUC-3-glucose of the two standards. Time period of 2 and 3 hours did not affect the absorption of polished rice and brown rice flour ($P>0.05$) but did affect the absorption of brown rice ($P>0.05$). AUC-2, 3-insulin difference between brown rice and brown rice flour cases were significant but AUC-insulin between brown rice and polished rice was not.

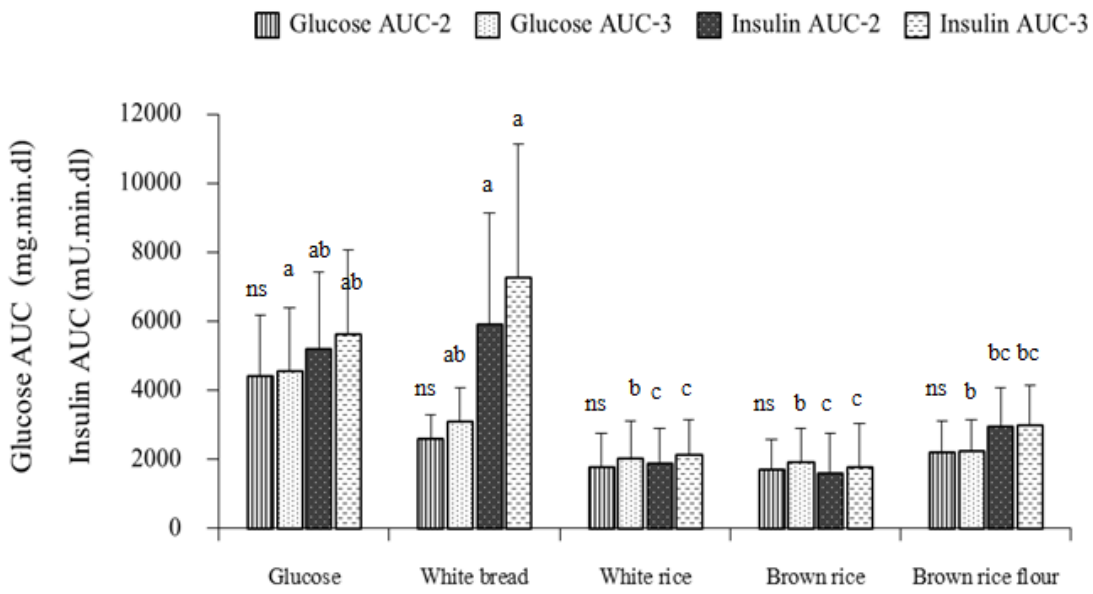

Figure 14 Comparative of area under curve of serum glucose individual of volunteers for standards foods and rice samples for 2 (AUC-2) and 3 (AUC-3) hour and serum insulin for 2 (AUC-2) and 3 (AUC-3) after ingestion. The total AUC and AUC were calculated using the trapezoid rule, ignoring the area below the baseline. A significant difference found between AUC of samples ($P<0.05$).

3.3.3 Glycemic index and insulin index

GI is regularly calculated on the basis of the AUC of incremental serum glucose until 2 hour as shown in Figure 15. Compared to glucose, Sangyod rice meal exhibited GI ranging from 38.0 to 56.5. Interestingly, Sangyod white (polished) rice gave a good (low) GI of 39.4 similarly to of brown (unpolished) rice of 38.8, and much better than brown rice flour. It indicated that fiber and lipids on unpolished grain surfaces–might not influence much on GI for Sangyod rice but the processing method (i.e., milling) might influence more. We can see that brown rice flour gave GI of 56.5 which is not as low as white rice. This finding suggests that Sangyod rice has some properties that make GI low independently of fiber or lipids. That property may be destroyed by the flour milling process. Significant difference between glucose and white bread standards was also found. GI of sample ranged from 39.4 to 65.1 and from 38.8 to 69.4 during the rice flour ranged from 56.5 to 81.7 respectively and GI of all samples was no different significantly.

Insulin index (II) of white rice ranged from 35.7 to 37.2, II for brown rice flour ranged from 30.7 to 33.5, and II for brown rice ranged from 53.9 to 64.0. II's of 2 and 3 hours of white rice, brown rice and brown rice flour (that used white bread as standard) were significant different ($p<0.05$). The difference between the two and three hour insulin index of brown rice flour were significantly different but polished rice and brown rice were not. Food insulin index shows the ability of foods to secretion of insulin within a body. It is an important indicator for insulin that uptake the serum glucose into the cells and tissues and used as marker symptoms in stimulating the secretion of this enzyme in large quantities over a very long time.

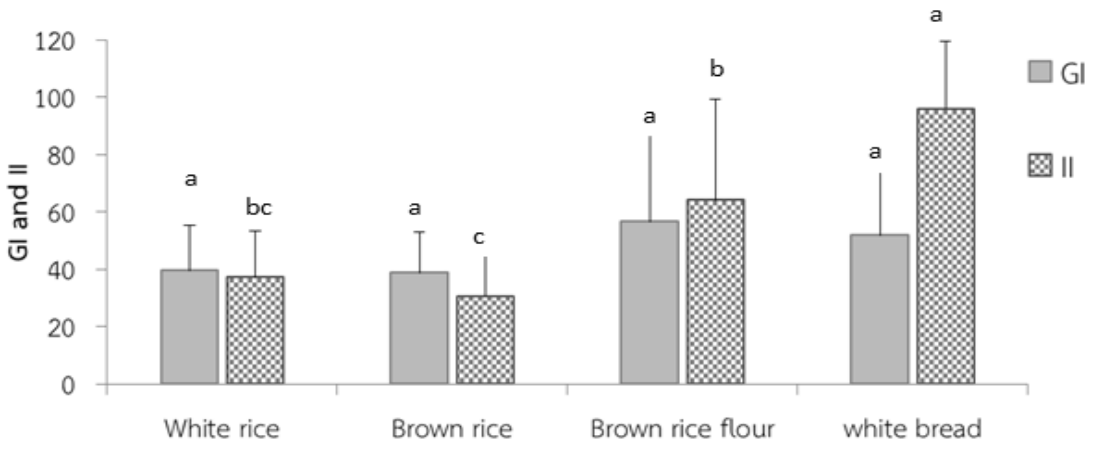

Figure 15 Comparative of glycemic index (GI) and insulin index (II) of individual serum glucose of volunteers for rice sample at 2 hour. A significant different superscripts was found between GI and II value of samples, at $P<0.05$ by Tukey's test.

Despite being consumed by over half of world population, glycemic indexes (GI) of rice were classified as high (Juliano *et al*., 1989), medium (55-70) or low (less 50) which cause difficulty for consumers to choose. These variations could be from the way rice was cooked (parboiling, surface area of starch), the variety of rice (amylopectin, amylose content, protein content), or the preparation of grain (content of fiber, fat and protein). There was no information of GI of our local traditional rice, Sangyod. Our study provided the GI of Sangyod rice in various forms, i.e., white rice, brown rice, brown rice flour and comparing those results against standard meals those were glucose and white bread. Sangyod rice was found low GI's for brown rice

(38.8), and white rice (39.4) and medium GI for rice flour GI of bread (53.6) considered a medium GI. When explored in details, it was found that even GI's were similar among treatment, Sangyod rice caused much less insulin response than bread and glucose and there were some variations in insulin response among different forms of Sangyod rice. Moreover, the glucose patterns among different meals are also different. In this study, Sangyod brown rice had similarly good GI as Sangyod white rice. Thus fiber of brown rice does not affect GI in Sangyod rice, this neutral effect of fiber was also found by Jenkins *et al*. (1988). For rice flour, which was produced from brown rice, contained fiber but caused a higher GI. Thus, fiber in Sangyod rice may play lesser role in GI than particle size. However, the preparation of rice and cooking method may contribute to a larger magnitude on GI. Sangyod rice flour had medium GI, which is not as low as Sangyod rice grain. The difference between flour and grain is the larger surface area of flour which may allow more contact area to digestive enzyme. We could demonstrate the higher peak of glucose at 30 minutes, suggesting of more rapid absorption of carbohydrate from brown rice flour. This rapid rise of plasma glucose was also found in glucose and bread meal. All meals showed the declining of plasma glucose after 30 minute but in different rates. At 60 minute, plasma glucose levels by all meals dropped but at a slow rate, but from 60 minute to 120 minute, rates of decline were much faster in glucose and flour meals while grain meals and bread were parallel and much slower. At 120 minute, rice flour gave a lower plasma glucose than baseline but still in normal range and the level of plasma glucose maintained at similar level through 180 minute. On the other hand, at 180 minute, glucose meal caused significant further dropped in plasma glucose to much lower level and lower than baseline and below normal range (66 mg/dL data not shown). This unusually low plasma glucose level at 180 minute was not observed in other meals which were similar to baseline levels.

The plasma glucose response in normal human is the effects of insulin, however; there are some controllers of insulin as well. Other factors affecting insulin secretions in same individual other than glucose level which is the most important one are, incretin, rate of glucose rising, counter-regulatory hormones and previous glucose levels. Our study showed that all meals with similar GI gave different insulin responses. As expect, most insulin responses were parallel to glucose curves. The glucose meal gave the highest insulin level at 30 minute and rapidly dropped but level at 120 minute was higher than baseline while plasma glucose at that time was

back to baseline. This finding can explain the low plasma glucose level at 180 minute from glucose meal because of the inappropriately high plasma insulin level at 120 minute. This could be the very high insulin levels secondary to rapid rising in plasma glucose level. The half-life of insulin is 3-5 minutes but biological half-life is much longer (Duchworth *et al*., 1998). Bread also caused a high insulin response but to a lesser degree than glucose meal. Rate of insulin decline in bread meal is slower than other meals which were parallel to it slower drop of plasma glucose level. Bread is a combination of carbohydrate, fat and protein. Thus this complex diet leads to prolong glucose absorption unlike glucose meal. For this complex meal, there was no low glucose level found in our volunteer at 180 minute. This rapid and high level of insulin responses were not observed from rice meals even for rice flour which gave similarly high plasma glucose at 30 minute with bread but the insulin level was lower (Figure 13) and AUC of insulin was smaller with similar AUC of glucose. For brown and white Sangyod rice, they contribute to good GI and very low insulin response which were significant lower than glucose and bread. Even among Sangyod rice, rice flour gave no better insulin response than glucose meal while rice grain gave lower AUC of insulin. The effect of Sangyod to insulin could not explain by glucose level alone. However, our study was design to explore GI, thus unable to explain this finding. But this finding is very interesting for application of Sangyod rice for people with limit beta cell function. This meal can minimize beta cell exhaustion and preserve beta cell function.

The difference in insulin response from consuming Sangyod rice is of unknown mechanism. It could be the effect of incretin which suppresses glucacon secretion and allows better insulin action. This unknown effect of Sangyod on insulin action and secretion is independent of fiber component of rice because white rice has effect on plasma glucose and plasma insulin similar to brown rice. Thus high fiber of rice was not effective on plasma glucose.

There are some limitations in this study. This study comprised of only 10 subjects, all of which were healthy, lean and young. Standard error of GI is wide, as GI is a biological biochemical index with previous study showing a mean coefficient of variation of as large as 25% (Wolever *et al*., 2009). Secondly, the food component in 4 meals studied was not similar in the term of calories, protein and fat components. Thirdly, the design was a cross-sectional study whereas the consumption of food is long-term and we do not know if there will be any biological

adjustment or not. In addition, all subjects were of same ethnic, Thais. This study did not design to demonstrate the mechanism of insulin sensitivity effect of Sangyod rice. And finally, this study was a short term study. We cannot extrapolate that the duration this effect will be long-lasting. However, this study is the first study of effect of Sangyod rice in various forms on GI, and II. In addition, this study is done in same group of population, and sequence of food consumed in done in a random order and all test meals were done within a short period, 1 week, thus it is very unlikely that that subject would have any physiologic change.

In summary, Sangyod Phattalung rice, either white or brown, has low GI and favorable II and is considered as a good choice for the consumers. However, milling or decreasing size of rice into small particles (flour) will abolish some beneficial effects on plasma glucose and insulin response.

Despite the beneficial effects of Sangyod Phatthalung rice were shown in this study, it is premature to conclude that this rice should be recommended for diabetic patients. The study of the effect of this rice in diabetic patients in a larger and longer trial as well as in other groups of patients, such as obese, is definitely needed. The mechanism of Sangyod rice on better insulin response warrants further investigation.

3.4. Conclusions

In this study, we showed that Sangyod rice gave GI of 38-64 depending on the process of preparation. In general, Sangyod white (polished) rice gave similarly low GI as Sangyod brown rice. Sangyod rice is classified as low GI food irrespectively of the form, i.e., whole grain or not. Sangyod rice also gives favorable insulin response, far lower than of glucose and bread. This could be of great benefits to people with limited pancreatic β-cell reserve.

CHAPTER VI

CONCLUSION AND SUGGESTION

Conclusions

Polished (white), unpolished (brown) and germinated (brown, unpolished) Sangyod Phattalung rice were compared in starch hydrolysis and glycemic indeces (HI and GI). By comparing nutrients and composition, the three grains were similar in protein (7.81, 8.22 and 8.30 %, respectively) but varied in lipid contents (0.51, 3.18 and 2.80 %) and ash contents (0.75, 1.36 and 1.03 %), respectively. Brown rice contains higher potassium and phosphorus than germinated and white rice, but germinated rice contains higher amounts of iron, zinc, sodium, calcium and magnesium than brown rice and white rice. Gelatinization of rice flours was found to show similar T_0 and T_p for all three samples. Final gelatinization temperature for germinated rice was lowest at 87 ^{o}C followed by that of white rice at 91 ^{o}C and brown rice at 96 ^{o}C. No significant difference in transition enthalpy was observed although white rice had a tendency to have less enthalpic values due to heat destruction during the polishing process. The process of wetting and digestion in germination of rice resulted in starch hydrolysis of the amorphous chain that resulted in lowering in T_e markedly without lowering the enthalpy or helical content this resulted in texture softening of the cooked rice grains. The higher ash content in brown rice was found to raise T_e of the sample significantly contributing to relatively tougher or harder texture. It was concluded that data from composition and gelatinization temperature are useful information for describing Sangyod Phatthalung rice in understanding texture attributes.

Starch digestibility of Sangyod Phattalung rice grain and flour it was found that unpolished grains were hydrolyzed at rate slower than its flour counterpart. Estimated glycemic index (GI) for flour was considered high GI and whole grains low GI (significantly different $P<0.05$). The main phenolic compound was found to be protocatechuic acid (PCA) at levels 1.1 and 1.5 mg/100 g dry sample for cooked and uncooked brown (unpolished) rice, respectively, and 0.7 and 0.85 mg/100 g dry sample for cooked and uncooked unpolished, germinated rice, respectively. PCA (and together with other phenolics) was found to inhibit α-amylase activity resulting in lower HI and GI compared to white rice. Germinated brown rice showed some loss in PCA in the process.

Total dietary fiber brown rice and germinated rice were higher than white rice (significant difference, $P<0.05$) that corresponded with *in vitro* GI but it is not in the GI clinical trial. In this study, total dietary fiber could contribute to that effecting α-amylase activity. *In vivo* GI is not only a function of digestion but also endocrine function absorption of blood glucose. Moreover, lipid and total dietary fiber for example could also affect the blood glucose response.

Starch physico-chemical properties by endothermic transition analysis showed presence of amylopectin and amylose helical formation. Amylose-lipid complex was also present in the three rice samples differently but did not correlate with α-amylase activities. i.e., the GI values of white rice was found to be highest but its damaged rice proportion the lowest. Hence GI or amylase activity in this case did not increase with the degree of rice damage.

In 10-volunteers human trial whole grain Sangyod rice was found to have an ability to lower glycemic and insulin response (compared to glucose and white bread). The serum glucose level 30 minutes after ingestion of the two controls (glucose and white bread) showed declined rates of serum glucose more rapidly (than brown Sangyod rice and white Sangyod rice), the rapid drop of the glucose level at 120 minutes to lower than base line but could sustain levels through 180 minutes. Flour and whole grains rice seeds gave much lower AUC (Area Under Curve) than of glucose and were similar to of bread.

Serum insulin for whole grain and flours of Sangyod rice (white and brown) was lower significantly than glucose and the white bread control ($P<0.05$). Insulin levels were calibrated back to the base line slower in the glucose and the white bread control. The insulin response for the rice flour was significantly higher than those for the whole grain. Some wide range of the standard error of the result partly caused by normal behavior of small human subject number. This limited biological differences among subjects lowering glycemic index comparison power and thus some ability to distinguish glycemic index of our samples.

Suggestions

Sangyod white (polished) rice gave similarly low GI as Sangyod brown rice in vivo (but higher GI in vitro). Sangyod rice is classified as low-medium GI food. This could be of great benefits to people with limited pancreatic beta-cell reserve. The mechanism of the digestion of Sangyod starch should be investigated further. In vitro results indicated that the α-amylase inhibitory roles of phenolic compounds were not supported by in vivo glycemic human study. i.e., volunteers were equally low in glycemic response but the insulin response was significantly greater in rice flours than in whole grains (polished and/or white, unpolished or brown). This certainly warrants further investigation on the impact of α-amylase activity and other physiological factors involved in glycemic response, including individual pancreatic function and corresponding related factors.

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I_I0007: CHEMICAL, PHYSICO-CHEMICAL AND NUTRITIVE VALUES OF GRAIN AND FLOUR OF SANGYOD PHATTHALUNG RICE

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Abstract: Sangyod Phattalung rice is an indigenous Southern Thai rice cultivar that is purple-black in color. In this study, polished (white) Phattalung rice, unpolished (brown) rice and germinated (brown, unpolished) rice were obtained. The protein contents were 7.81, 8.22 and 8.30% (total basis), respectively. The lipid contents were 0.51, 3.18 and 2.80% (total basis), and ash contents of 0.75, 1.36 and 1.03% (total basis), respectively. Brown rice contains potassium and phosphorus higher than germinated and white rice, but germinated rice contains higher amounts of iron, zinc, sodium, calcium and magnesium than brown rice and white rice. Gelatinization of rice flours was found to show similar T_0 and T_p for all three samples. Final gelatinization temperature for germinated rice was lowest at 87 ^oC followed by that of white rice at 91 ^oC and brown rice at 96 ^oC. No significant difference in transition enthalpy was observed although white rice had a tendency to have less enthalpic values due to heat destruction during the polishing process. The process of wetting and digestion in germination of rice resulted in starch hydrolysis of the amorphous chain that resulted in lowering in T_e markedly without lowering the enthalpy or helical content this resulted in texture softening of the cooked rice grains. The higher ash content in brown rice was found to raise T_e of the sample significantly. In this work, it could be concluded that data from composition and gelatinization temperature are useful information for describing white rice, brown rice and germinated rice and in understanding texture attributes of Sangyod Phatthalung rice.

Introduction: Sangyod Phatthalung rice is traditional rice variety grown in the area of Phatthalung province for more than a hundred years. Its typical grain appearance is small and long-slender grain, dark red pericarp, soft and aromatic of cooked rice. Yodmanee *et al.* (2011) reported polyphenols and anthocyanin contents of Sangyod Phatthalung rice to be relatively high, 82 mg GAE/100g db and 15 mg Cy-3-glc/100g db, respectively.

White rice is milled rice that has had its husk, bran, and germ removed. Brown rice or hulled rice is a kind of unpolished grain which is more nutritious than white rice but goes rancid more quickly because of the germ. Germinated rice is unpolished rice that still has young shoots remained intact, soaked in water until its embryo begins to bud. During germination hydrolytic enzymes activate digestion of starch, fiber and proteins, resulting in improved texture.[1] The rice varieties with pigmented color (black, purple or red) give slightly pigmented flour upon milling.[2] Pigmented rice typically contains high antioxidant activities.[3]

Methodology: Sangyod rice (*Oryza sativa* L.) from Phatthalung Province, Thailand was dehulled to make unpolished rice (brown and germinated) and polished rice. Prepared germinated rice was steeped in distilled water at 27-30 ^oC in the dark for 24 h. The steep water was changed every 4 h and drained at the end of soaking. The germinated seeds were dried at 50 ^oC. All the samples were analyzed proximate (moisture, lipid, protein and ash) by AOAC method (2000) and analysis of minerals, including iron, zinc, sodium, calcium, magnesium, potassium and phosphorus by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). Color of grain and flour were determined by Hunter-Lab (Colour Flex, Hunter-Lab, USA). Prior to color measurements, the instrument was calibrated with light tap and white calibration tile. The colorimeter was set to an illuminant condition D65

and a 10 ° standard observer. Each sample was put in a cuvette and replaced in to the specular port site, the color parameters (a*, b* and L*) were then read and calculated c*=colour intensity, chroma ($c^*=[a^*+b^*]^{1/2}$), h^o=hue angle ($h^o=\tan^{-1}[b^*/a^*]$). Starch gelatinization properties were analyzed in triplicate using a Differential Scanning Calorimeter (DSC). Rice flour of about 3 milligram was placed in a 40μL aluminum pan and water was added to give a sample-to-water weight ratio of 1 to 3. The pan was sealed, and the sample was allowed to equilibrate overnight at 4 ^oC. The DSC temperature scan includes holding at 10 ^oC for 1 minute followed by heating from 10 to 95 ^oC at a rate of 5 ^oC/minute. Water activity (a_w) was analysed using Novasina Water activity meter.

Results, Discussion and Conclusion: Figure 1 shows Sangyod Phattalung rice samples including white (polished) rice, brown (unpolished) rice and germinated rice. Redness in the grains of Sangyod Phatthalung rice is embedded in the structure of the bran. When the rice is polished, the color is gone leaving a white grain appearance. During germination, the color intensity decreased because anthocyanin dissolving in water resulting in a change in color particularly b* value.[4] Anthocyanin is glycosides compounds or acyl glycoside of phenolic compounds in the outer membrane (pericarp) and inner membrane or aleurone layer.[5] The colors appear, intensity of the color are depending species, cultivation, place of planting, age, duration of germinated etc.[6]

Figure 1. Sangyod Phattalung rice sample in studies
Where a=White rice flour, b=Brown rice flour, c=Germinated rice flour, d=White rice, e=Brown rice, f=Germinated rice

Table 1 shows the color parameters (L*, a*, b*, c* and h^o) of grain and flour of Sangyod Phattalung rice. L* values, which expresses the brightness, were in the range of 35.1-82.0. The values of a*, b*, c* and h^o were in the range of 4.2-15.2, 9.9-20.8, 11.8-25.8 and 53.9-69.5 respectively. Polished grain and flour brightness was higher than unpolished. Red of brown rice grain and flour more than germinated and white grain and flour and yellow of unpolished higher than polished grain but yellow of germinated flour were lower than white and brown flour. The intensities of brown grain and flour varied more than white and germinated grain and flour. Sangyod Phatthalung brown rice is dark-red-yellow rice. Polishing removes the bran and therefore removes the pigment reducing the color intensity and increased the lightness whereas germination lowers the intensity of the colour of Sangyod Phatthalung rice only slightly.

Table 1. CIE color scale from rice and flour of Sangyod Phatthalung rice

	Grain				
	L*	a*	b*	c*	h^o
White rice	60.7±2.4[a]	6.7±0.8[c]	16.1±0.8	17.5±0.6[c]	67.5±3.1[a]
Brown rice	35.1±0.9	15.2±0.7[a]	20.8±1.0[a]	25.8±1.0[a]	53.9±1.8
Germinated rice	36.8±1.4	13.5±0.9[b]	20.2±0.6[a]	24.3±0.6[b]	56.2±2.0
	Flour				
	L*	a*	b*	c*	h^o
White rice	82.0±0.3[a]	4.2±0.1[c]	11.3±0.1[a]	12.1±0.1	69.5±0.4[a]
Brown rice	68.3±0.2[c]	7.0±0.1[a]	11.1±0.1[a]	13.1±0.2[a]	57.7±0.2[b]
Germinated rice	70.8±0.4[b]	6.5±0.2[b]	9.9±0.2	11.8±0.3	56.6±0.2[c]

Value are given as mean±SD from six replicate, [a,b,c]Superscripts in the same column indicate significant different (P<0.05) by Tukey's test

Where The maximum for L* is 100, which represents a perfect reflecting diffuser.
The minimum for L* is zero, which represents black.
a*=Positive is red, Negative is green
b*=Positive is yellow, Negative is blue
c*=colour intensity, chroma
H^o=hue angle

Chemical composition of white rice, brown rice and germinated rice of Sangyod Phatthalung rice (%dry basis) shows in table 2. Proximate composition of white rice, brown rice and germinated brown rice of Sangyod Phatthalung rice are shown in Table 2. The protein contents were 7.81, 8.22 and 8.30% (dry basis), respectively (significantly different P<0.05). The increased of protein in germination rice occuring from decarboxylation of L-glutamate by glutamate carboxylase.[7] Comparing among white, brown and germinated rice, lipid contents were 0.51, 3.18 and 2.80% (dry basis), and ash contents were 0.75, 1.36 and 1.03% (dry basis), respectively. These were because of the removal during polishing resulting in lower values for white (polished rice). In addition, some loss in germinated rice may be due to process of germination that is consistent with the soybean (Park et al., 2002) and rice germination.[8] This composition of lipids is a healthier composition in brown rice and germinated brown rice because rice bran oil is polyunsaturated and monounsaturated fatty acids. Micronutrients of difference type of Sangyod Phatthalung rice shows in Table 1. Brown rice contains higher potassium and phosphorus than germinated and white rice (significantly different, P<0.05), Germinated rice contains higher amount of iron, zinc, sodium, calcium and magnesium than brown rice and white rice (significantly different, P<0.05). Dry-milled flour contained slightly higher amount of lead than others (2.7 ppm vs approx. 1.9 ppm).

Table 2. Chemical composition of white rice, brown rice and germinated rice of Sangyod Phatthalung rice (%dry basis)

	% dry basis		
	Polished rice	Unpolished rice	
	white rice	brown rice	germinated rice
Macro nutrient			
Moisture	10.60±0.34[b]	11.56±0.38[a]	11.25±0.07[a]
Protein	7.81±0.02[c]	8.22±0.05[b]	8.30±0.01[a]
Lipid	0.51±0.03[c]	3.18±0.03[a]	2.80±0.13[b]
Ash	0.75±0.00[c]	1.36±0.01[a]	1.03±0.03[b]
Micro nutrient (ppm)			
Fe (iron)	12.28±0.06[b]	12.09±0.05[c]	19.69±0.05[a]
Zn (zinc)	20.93±0.29[c]	22.63±0.05[b]	31.53±0.11[a]
Na (sodium)	114.69±1.39[c]	162.99±2.25[b]	239.10±0.78[a]
Ca (calcium)	484.32±1.80[b]	482.64±4.40[b]	775.26±4.23[a]
Mg (magnesium)	513.30±2.77[c]	985.83±8.69[b]	1090.97±8.63[a]
K (potassium)	1192.43±22.06[b]	2118.14±17.02[a]	1226.17±18.67[b]
P (phosphorus)	1012.09±10.55[c]	2104.58±26.35[a]	2014.95±30.90[b]
Heavy metal (ppm)			
Cd (cadmium)	0.095±0.00	0.121±0.052	0.125±0.054
Pb (Lead)	2.735±0.220[a]	2.019±0.138[b]	1.807±0.108[b]

Value are given as mean±SD from triplicate, Different superscripts in the same row indicate significant different (P<0.05) Tukey's test

Table 3 shows water activity of grains were 0.67, 0.50 and 0.54 for white, brown and germinated rice grains, respectively (significantly different, P<0.05). a_w of the flours were 0.62, 0.32 and 0.34 for white, brown and germinated rice flour, respectively (significantly different between polished and unpolished rice flour). a_w of the grains and dry-milled white rice flour were 0.60 which was higher than unpolished (brown) and germinated (brown) rice. a_w of flours were lower than that of whole grains.

Table 3. water activity from rice and flour of Sangyod Phatthalung rice

	Water activity (A_w)		
	grain	flour	grain-flour *p*-value*
White rice	0.67±0.02[a]	0.62±0.01[a]	<0.05
Brown rice	0.50±0.01[c]	0.32±0.01[b]	<0.05
Germinated rice	0.54±0.01[b]	0.34±0.00[b]	<0.05

Value are given as mean±SD from triplicate, [a,b,c]Superscripts in the same column indicate significant different (P<0.05) by Tukey's test

*Grain-flour *p*-value: difference of water activity of sample between grain and flour indicate significant difference (P<0.05) by paired sample T-test

Starch gelatinization is the transition of the semi-crystalline structure (Level 3 starch structure) in native starch granules to an amorphous structure.[9] Starch gelatinization properties were analyzed both from the rice flour samples using laboratory-scale dry milling and comparison between polished and unpolished rice, which are one factor starch digestion properties. The gelatinization temperature of difference flours from rice studied is presented in Table 3. Peak analysis shows onset (T_0), peak (T_p) and end (T_e) gelatinization temperatures that are characteristics of heat-water treatments that destroy helical structure in starch crystallites. T_0 of Sangyod Phatthalung rice flour was found to be at 74.4-76.5 ^oC range where as T_p or peak temperature were in 80.6-88.1 ^oC, not so varied among sample treatments (i.e, polished unpolished or germinated). However, the conclusion temperature T_e varied

significantly over 86.6 to 96.0 ^{o}C range with germinated rice the lowest Te followed by white (polished) rice and then by brown (unpolished) rice flours. Tukey's analysis of difference preparation of Sangyod Phatthalung rice shows that T_0 and T_p of three rice flour of Sangyod Phatthalung rice were not significantly different (p<0.05) but the final gelatinization temperature for germinated rice was lowest at 86.62±0.15 ^{o}C followed by that of white rice at 91.08±0.68 ^{o}C and brown rice at 95.96±3.15 ^{o}C. It is postulated that the dry-heat treatment of polished (white) rice and moist-heat and enzymatic treatments of germinated rice could have contributed to a less stable (lower gelatinization temperature of some elevated temperature fractions). During germination, hydrolytic enzymes activate digest starch, fibers and proteins and making the rice cooked with improved texture.[10,11]

Table 4. Gelatinization temperatures and transition enthalpy from different flour rice of Sangyod Phatthalung rice

	transition temperatures (^{o}C)			transition enthalpy
	T_o	**T_p**	**T_e**	**(J/g)**
White rice	76.52±0.04	88.10±6.11	91.08±0.68^{b}	2.90±0.08
Brown rice	74.42±3.13	86.73±4.15	95.96±3.15^{a}	3.94±0.92
Germinated rice	75.35±0.28	80.63±0.17	86.62±0.15^{c}	3.57±0.03

Value are given as mean±SD from triplicate, Different superscripts in the same column indicate significant different (P<0.05) Tukey's test

Where T_o=onset temperature, T_p=peak temperature,T_e=endset temperature

Transition enthalpy of Sangyod Phatthalung rice flour were 2.90-3.94 j/g**,** not significantly different (p<0.05). However, there was a trend found. White rice showed lower enthalpy trend indicating some destruction of helical structure of the starch structure from the milling and polishing processes. Presence of small components such as ionic and non-ionic solutes may influence by retarding gelatinization, i.e., raising the gelatinization temperature of the starch.[12,13] However, based on micronutrient and ash content, this was not always the case. It was found that the onset gelatinization temperature of brown rice, white rice and germinated rice were only slightly (but not significantly) different base on T_o and T_p comparison. However, T_e was found to be lower in the case of germinated rice (86.62 ^{o}C) meaning the thermal transition was narrower (sharper) than the other two rice treatments. The process of wetting and digestion in germination period could result in starch hydrolysis in the amorphous domains that are networking in the fraction with higher gelatinization or melting temperature. Note that the thermal enthalpy of germinated rice was not significantly different from brown or white rice indicating similar double helix content. The process of lowering T_e means the starch are gelatinized more completely of lower temperature and possibly softening the texture. Comparing the compositional difference between white and brown rice (table 2), small solutes could have had some influence on the gelatinization enthalpy. For example, the presence of higher ash, lipid and protein in brown rice, each could have directly or indirectly impacted the swelling and gelatinization process. But lipid could have additional interaction with the linear portion of starch molecules that could lead to additional lipid-starch interaction enthalpic peak as earlier found.[11] But in this work this was not observed. It would be possible that most of the lipids are interacted in lipid-protein complex or network outside of the rice grain and hence not able to interact with the starch. The higher ash content in brown rice was found to raise T_e of the sample significantly indicating potential impact of Na, K, Mg and P in the sample. Tester observed brown rice to be slightly leathery or harder in texture than the compounding white rice which could partly from the higher T_e. The pericarp, layer of lipid-protein could also affect the texture. In this work it could be concluded that useful data from composition and gelatinization temperature are useful information for describing white rice, brown rice and germinated rice and useful in understanding texture attributes of Sangyod Phatthalung rice. The process of wetting and digestion in germination of rice result in starch

hydrolysis of the amorphous chains that could have lowered T_e markedly without lowering the enthalpy or helical contains. This led to texture softening of the cooked rice grains. Some study reported rice gelatinization behavior showed strong influence of perceived texture of cooked rice; the higher the gelatinization temperature of the grain the harder the texture of the rice.[14] In this work, moist-heat treatments in germination and other processes and component distribution may also be important.

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Acknowledgements: Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand.

Keywords: Sangyod Phattalung rice, gelatinization, composition, germinated rice, brown rice

Appendix 2

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Digestibility and α-amylase inhibition of Sangyod Phatthalung rice (*Oryza sativa* L.)

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Abstract

Starch digestibility of Sangyod Phattalung rice (red rice) was studied varying polishing and germination, i.e., unpolished rice, polished rice, and brown and germinated rice grain and flour. Unpolished grains were hydrolyzed at rate slower than its flour counterparts. Estimated GI for flour was higher than for whole grains ($P<0.05$). GI values of rice flours were high GI whereas grain was medium GI. HPLC results showed that the main phenolic compound is protocatechuic acid (PCA). PCA (and together with other phenolics) exhibited α-amylase inhibition. Germinated brown rice contained less phenolics due to some losses in the pre-treatment and germination processes. Physico-chemical amylopectin and amylose helical formation and amylose-lipid complex were present in three rice samples differently but did not correlate with α-amylase activities. Consuming brown Sangyod rice should help reduce starch digestion and consequently lower HI and GI. This is of great importance to weight control and diabetes control application.

Keywords: unpolished rice, glycemic index, digestibility, α-amylase inhibition, phenolics compound.

Introduction

Incidences of diabetes and other metabolic syndrome conditions, globally are in the rise. According to a rapid changing and stressful life style and growing elderly population in a world, it is recognized that a healthy digestive system is essentially linked to health status including disease prevention and quality of life among elderly.

Carbohydrate consumption of rice as staple foods is primarily to provide energy arch. Recent change in life style has caused an imbalance in energy intake resulting in overconsumption of starch and sugar, leading to metabolic syndrome, such as obesity and diabetes. Diabetic patients suffer mainly from elevated post-prandial glycemic response which leads to several clinical complication over the rest of their lives. More health conscious consumers have been increasingly interested in gaining better glycemic control by eating less simple carbohydrates and choosing foods with lower glycemic index (GI). Rice is grown in abundance in Asia (90% of the world's crop, Juliano, 1985) and consumed as the major source of energy for most world population. When unpolished, rice grains contain healthy components including proteins, fats and phenolic compounds and anti-oxidants (such as γ-oryzanol, tocopherol, tocotrienol, vitamin B) but their shelf-life is much shorter mostly from oxidative and microbial (mold) deterioration. When germinated, gamma-aminobutyric acid (GABA), an anti-oxidant, is produced in a high amount (Tian, Nakamura & Kayahara, H. 2004). Sangyod Phattalung rice is an indigenous Southern of Thailand rice cultivar with reddish-brown color when unpolished primarily from anthocyanin 85% of which in the form of cyaniding-3-glucosideand peonidin-3-glucosid (Hu, Zawistowski, Ling & Kitts (2003). Anthocyanin is glycosides compounds or acyl

glycoside of phenolic compounds in the rice grain outer membrane (pericarp) and inner membrane or aleurone layer (Hu, Zawistowski, Ling & Kitts (2003) and color variability is according to species, cultivation, geographic region, maturation (Adom & Liu, 2002).

It has been reported that redish brown color (such as in red bean) may also means an existence of protocatechuic acid (PCA, Archivio, Scazzocchio, Giovannini, & Masella, 2014) previously shown to inhibit the growth of cancer cells (Chen, Kuo, Chiang, Chiou, Hsieh & Chu, 2006) and to regulate heart disease patient by inhibiting factors that cause inflammation (Wang, Huang, Shao, Qian, & Xu, 2007). Consuming unpolished color rice is considered healthy due to its richness in iron, phenolic compound, fiber and antioxidants. In this work, further investigation was on the impact of indigenous anthocynanins and other antioxidants on glycemic index of rice.

Digestion of starch can be described by hydrolysis index (HI) which is digestibility of the starch in foods in relation to the digestibility of starch in a reference material, i.e., white bread (Sandhu & Lim, 2008). Glycemic index (GI) is calculated from HI as the incremental postprandial blood glucose area after injection of the test product as a percentage of the corresponding area after injection of an equicarbohydrate portion of the reference product (Jenkins et al., 1981). GI of rice grains and flours can be used for choosing for diabetic patient consumption. (Sajilat, Singhal & Kulkarni, 2006). There are several physical factors that may influence starch digestion. Milling starch, for example, may influence the rate of digestion, digestibility and glycemic response (Panlasigui, Thompson, Juliano, Perez, Yiu, & Greenberg, 1991). It has been reported that some but not all phenolic compounds could be a potent inhibitor of α--amylase activity (Yang, Huang, Jin, Sun, Song &

Chen, 2012; Wang Huang, Shao, Qian & Xu, 2012). Soybean phenolic-rich extracts have been linked to inhibition of metabolic syndrome-related enzymes, including α-amylase and α-glucosidase for the case of type-2 diabetes (Ademiluyi & Oboh, 2013). PCA is among important phenolic compounds that strongly influence metabolic syndrome including antioxidant activity, anti-inflammatory activity, antihyperglycemic activity, modulation of apoptosis and modulating gut microflora (Archivio, Scazzocchio, Giovannini & Masella, 2014).

The objective for this study is to investigate HI and GI for unpolished, polished and unpolished germinated Sangyod Phatthalung rice comparing the effects of rice phenolics and starch physico-chemical properties.

Materials and Methods

1. Materials

Rice grains and flours were prepared from Sangyod Phatthalung rice (*Oryza sativa* L.) with three different grain treatments, i.e., polished (white) rice, unpolished (brown) rice and germinated unpolished (brown) rice. Standard white bread is used as the control digestion study.

Dehulled Sangyod rice from Phatthalung Province (Thailand) was collected from calibration year 2012. Unpolished rice (brown) and polished rice (white) were prepared on site. These grains were packed in plastic bags, transported to Prince of Songkla University and stored at ambient temperature (28±2^{o}C) before further processed. Two kilograms of white rice and two kilograms brown rice were washed and dried (hot air oven, 55^{o}C, 10 hours) to 9% moisture content. Germinated brown rice was prepared by steeping in deionize water and incubated in darkness at ambient temperature for 24 hours (Tain, Nakamura & Kayahara, 2004). The steep water was

changed every 4 hours and drained for 20 minutes and dried (hot air oven, 55^{o}C 10 hours).

To prepare rice flours for a comparison study, rice grains (1 Kg) were milled into flours by flour milling machine (Daming, China) at speed of 25,000 rpm. The rice flour obtained was sifted through 200 mesh sieve and packed under vacuum in nylon bags and kept at 4^{o}C throughout the experiment.

The standard for digestion and glycemic study was white bread (obtained from a local market) which was dried by hot air oven at 60^{o}C for 5 hours, milled and sifted through 200 mesh sieve and kept at 4^{o}C throughout the experiment.

2. Chemical composition analysis

All the samples were analyzed for moisture, lipid, protein and ash contents by AOAC methods (2000). The analyses for minerals (iron, zinc, sodium, calcium, magnesium, potassium, phosphorus, cadmium and lead) were performed by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP, Perkin Elmer, USA).

3. Digestibility, hydrolysis index and glycemic index

3.1 *In vitro* rice and flour rice digestion

In vitro digestibility assay of the samples was based on glucometry which a modified method of Mahasukhonthachat, Sopade & Gidley (2010) and Sopade and Gidley (2009). A half-gram sample was mixed with 5 mL deionize water and heated in a water bath at 85^{o}C for 30 min. Each sample was treated with 1.0 mL artificial saliva containing porcine α-amylase (Sigma A-3176, 250 U per mL in sodium acetate buffer) and 5 mL pepsin (Sigma P-6887, 1% of protein in sample in deionized water and 0.02 M, HCl pH 2.0 ratio 1:1 (v/v)) was added after incubation at 37◦C for 30 min

in a reciprocating water bath. The digesta was neutralized with 0.2 NaOH and pH was adjusted to 6.0 with sodium acetate buffer prior to an addition of 5 mL pancreatin (Sigma P-1750 from porcine pancreas, 2 mg per mL of acetate buffer) and amyloglucosidase (Sigma A-7420 from *Aspergillusniger*, 28 U per mL in acetate buffer). The mixture was incubated at 37°C for 4 hours the glucose concentration in the digesta was measured with a glucometor (Accu-Chek Performa, Roche Diagnostics Corporation, Indianapolis, IN, USA). Glucose concentration at specific periods (0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) were measured the amount of digested starch per 100 g dry starch (DS) was calculated using equation 1.

$$\mathrm{DS} = \frac{0.9 \times 180 \times \mathrm{GG} \times \mathrm{V}}{\mathrm{W} \times \mathrm{S}[100 - M]} \ldots. \quad [1]$$

Where DS = digested starch (g per 100 g dry sample)
GG = glucometer reading (mM/L)
V = volume of digesta (mL)
180 = molecular weight of glucose
W = weight of sample (g)
S = starch content of sample (g per 100 g dry sample)
0.9 = stoichiometric content for starch from glucose content
M = moisture content of sample (%moisture dry Sample)

Remaining undigested starch of digested samples was analyzed (method by Megazyme International Ireland Ltd., Ireland). Fifty mg sample was mixed with 400 µL of 80% ethanol, heated in boiling water bath. Two mL dimethyl sulphoxide (DMSO) was added and then the sample was digested with thermo-stable α-amylase (Sigma A-3176) ratio 1:30(v/v) with 3-(N-morpholino) propanesulfonic acid buffer (MOPS, Sigma M-9381) before sodium acetate buffer and amyloglucosidase (sigma A-7420) were added and incubated at 37°C for 4 hour. Glucose content was

determined using an enzymatic glucose reagent (Sigma, GAHK-20). Absorbance was measured in BiotekPower Wave X Microplate reader (Biotek, Winooski, T, USA) at 340 nm. Each sample was analyzed in at least duplicate.

3.2 Hydrolysis index (HI) and glycemic index (GI)

The hydrolysis index (HI) of each sample was calculated by dividing the area under its digestogram by the area under the digestogram of a white bread (Goni, AIonso & Calixto 1997).

$$GI = 39.71 + (0.549HI) \quad \text{... [2]}$$

Valus are given as mean ± SD from triplicate.

4. Extraction and analyses of phenolics

4.1 Extraction

Phenolic extraction was done using the applied method reported by Min, Gub, McClung, Bergman & Chen (2012). Five gram rice flour was mixed with 25 mL deionize water and heated in a water bath 85^{o}C for 30 minutes. Twenty five mL ethanol was then added, sonicated (Ultrasonic cleaner CD-4820 170w, Zhengzhou Xihua Medical Devices Co., Ltd., Chaina) for 10 minutes, and left in darkness for 90 minutes at room temperature. The mixture was centrifuged (Mikro 22r, Hettichzentrifugen, UK) at 5,000rpm for 10 minutes. The collected supernatant was filtered through filter paper (Whatman No.1). The filtered liquid was then mixed with 25 mL deionize water and then 20 mL hexane. After 15 mins hand shaking, the liquid was rested to allow lipid separation; the aqueous portion was then further analyzed.

4.2 Analysis of total phenolic content

Total phenolic content was determined according to procedure described by Singleton Orthofer & Lamuela (1999). To one hundred microliter extract, 0.2 mL 1N Folin-Ciocalteu's reagent was added and mixed for 5 minutes. The mixture was neutralized with 0.3 mL of NaOH (0.5 M) and placed in 96-well plate allowing to mix for 2 hours. Absorbance at 760 nm was done using Microplate reader. Phenolics content in each extract was calculated using a calibration curve of ferulic acid standard (5–200 $mgmL^{-1}$). The results were expressed in mg of ferulic acid equivalent per g of whole grain on dry weight basis (mg FA equiv.$100g^{-1}$). Analyses were performed at least in triplicates.

4.3 Identification of phenolic compounds

Phenolic compounds in extracts were characterized by reversed-phase High-performance liquid chromatography (HPLC) as described by Tain, Nakamura & Kayahara (2004) with modification. All extracted samples were filtered through a 0.20-µm pore size syringe-driven filter before injection. A 10-µL aliquot of each sample solution was separated using a Agilent 1100 HPLC system (Agilent 1200, Waldbronn, Germany) equipped with a diode array detector on a 250 mm x 4.6 mm i.d., 5 µm, Eclipse XDB-C18, analytical column (Agilent, Santa Clara, CA, USA). The mobile phase was acetonitrile (A) and purified water with 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.8 mL/min. Gradient elution was done as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent A; from 5 to 15 min, 9% solvent A; from 15 to 22 min, linear gradient from 9 to 11% solvent A; from 22 to 35 min, linear gradient from 11 to 18% solvent A; from 35 to 42 min 18% solvent A. The column temperature was set at 40 °C.

Hydroxybenzoic acid compounds (PCA, chlorogenic acid and *p*-coumalic acid) were detected at 290 nm and hydroxycinnamic (ferulic) acid at 325 nm. Phenolic compounds in the samples were identified by comparing the retention time and UV spectra with external standard compounds using an external standard method.

5. α-amylase inhibition assay of phenolic extracts

α-amylase inhibition assay was performed following Manaharan, Teng, Appleton, Ming, Masilamani & Palanisamy (2011). One mL phenolic extract and 0.05 mL 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine α-amylase (0.5 mg/ml) were incubated at 25 ^{o}C for 10 minutes. Fifty microliters of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was mixed and the reaction mixture was incubated at room temperature for 10 min before stopping with 0.2 mL of dinitrosalicylic acid color reagent. After incubated in boiling water bath (5 min) and cooled to room temperature, the reaction mixture was diluted (1 mL of distilled water) and an absorbance at 520 nm was measured. The α-amylase inhibition activity was expressed as percentage inhibition which was calculated as follows.

$$\% = \frac{\text{absorbant of blank} - \text{absorbant of sample}}{\text{absorbant of blank}} \times 100 \ldots [3]$$

6. Starch gelatinization properties

Total starch gelatinization and ungelatinized starch residue were analyzed in triplicate using a Differential Scanning Calorimeter (DSC) (PerkinElmer-DSC7, Waltham, MA, USA)**.** Residue starch gelatinization, rice flour one gram were added DI water ratio 1:10 (w/v) in tube 20 mL heat 85^{o}C for 30 minute in water bath cool in liquid nitrogen and store in -16^{o}C, rice flour (≈1 mg, dry weight basis) was placed in a hermetically-sealed stainless steel pan with o-ring (Perkin Elmer). The sample was

allowed to equilibrate one hour at 4^{o}C. Total starch gelatinization, one milligram rice flour was placed in a stainless steel pan was added with deionized water at ratio 1:10 (w/v). The pan was sealed with rubber O-ring stainless covers and the sample was allowed to equilibrate about 1 hour on 4◦C. The sample was heated from -10 to 130^{o}C at rate 10^{o}C/minute in DSC calibrated with Indium. Onset temperature (t_o), peak temperature (t_p), conclusion temperature (t_c) and enthalpy of gelatinization (ΔH) were determined from endothermic peak using Pyris^{TM} Player software (Perkin Elmer).

7. Statistics

All experiments were performed at least in duplicate. Analysis at every time point from each experiment was carried out in triplicates. SPSS statistics package (version 15.0, Chicago, IL, USA) was used ($P< 0.05$).

Results and Discussion

1. Chemical composition in Sangyod Phattalung rice

Sangyod Phattalung rice samples including white (polished) rice, brown (unpolished) rice and germinated brown rice (Fig. 1) varied in outside color (redish brown) primarily due to anthocyanins embedded in the structure of the bran. After polishing, the red color is removed leaving a white grain appearance. In case of germinated rice the color intensity decreased during germination due to anthocyanin degradation and loss in the soaking water (Chang, Cho, Park, Kweon & Lim, 2012).

Chemical compositions of all rice studied are in Table 1. Protein content was 7.8-8.3% with brown rice which slightly higher protein content. Lipid contents for both brown rice and germinated brown rice were higher (3.18 and 2.80 % dry basis) than white, polished rice (0.51 % dry basis). Similarly, higher ash contents were

found in brown rice samples than white rice. These resulted from pericarp removal from the polishing step; some loss in germinated rice might also be through the germination process which has been earlier reported. Micronutrients (Table 2) indicated some more similarity in Fe and Zn contents among samples but more significant differences in Na, Ca, Mg, K and P. Both brown and germinated brown rice contains higher phosphorus than white rice ($P<0.05$) but only brown rice was higher than the other two on potassium. Germinated rice contains higher amount of Fe, Zn, Na, Ca and Mg than brown (unpolished) rice and white (polished) rice ($P<0.05$). This information is useful for controlling some specific diets. Total starch of white rice was obviously greater than those for brown rice samples by approximately 10%. Standard (white bread) have total starch around 62.6% (data not shown).

2. Digestion rate of rice grains and flours

Porcine α-amylase hydrolysis of cooked rice grains and flours are expressed in a typical starch digestograms, i.e., digested starch (DS) and the increase if glucose versus hydrolysis time (Fig. 2) using white bread as the Control. Cooked rice flour digestion rates were higher than the corresponding cooked rice grains but no significant difference was found among the three kinds of flour. Amylase hydrolysis of cooked rice grains showed a significant difference. White rice grains were digested more rapidly than germinated brown rice grains. In this work, the digestion that was more rapid for the respective flour samples was likely to be because the reduction in particle size in flour milling. In addition, it has been reported that ground starch exhibited lower crystallinity and smaller molecular weight (Morrison et al., 1993; Huang, Schols, Soest, Jin, Sulmann & Voragen, 2007). These could have allowed

more open structure after gelatinization and easier digestion. Brown rice tends to be more resistant and relatively lower in digestibility (Sajilata, Singhal & Kulkarni 2006). It was found that DS (digested starch) increased in a curvilinear fashion with hydrolysis time reaching an asymptotic level after approx. Two hour of digestion (Fig. 2A and 2B). The white bread control was highest in digestion rate approaching almost 80%. Cooked rice flours were less rapidly digested with polished (white) rice flour slightly more rapid than white rice grains (Fig 2A and 2B). White (polished) rice grains were digested only slightly more than brown rice grains indicating that removal of bran by polishing could not accelerate the digestion but the extent of whole grain digestion was about 2/3 of that of flour (i.e., 40% vs 60% DS).

The increase in glucose (Fig 2C and 2D) mirrored the DS curves indicating less in vitro glucose release in grain hydrolysis than flour hydrolysis, respectively. Brown rice appears to sustain glucose release over longer time than white bread and white rice, suggesting its potential satiety sustaining property.

3. *In vitro* Glycemic index of Sangyod Phatthalung

Glycemic index (GI) and hydrolysis index (HI) values of Sangyod Phatthalung rice are shown Table 2. HI values ranged from 40-71 whereas GI‘s ranged from 62-79 with values relatively higher in white rice (polished) rice grains than brown rice grains and flours. GI of white rice flour and grains are 78.7 and 67.2, respectively. GI’s of all flours were consistently higher than GI’s of the three corresponding grains (Table 2). All of the flours can be classified in the high GI group whereas the grains classified in the medium GI group. White rice grains and flour showed higher GI’s than brown rice grains and flour and GI’s of germinated rice grains and flour, respectively ($P<0.05$). Compared to other Thailand rice, GI’ for Sangyod Phattalung

rice grains are lower than GI reported for brown Jasmine rice 70.3 (Jaisut, Prachayawarakorn, Varanyanond, Tungtrakul & Soponronnarit, 2008) and rice porridge (Srikaeo & Sopade, 2010). This confirms earlier suggestion that Sangyod Phatthalung rice is a reasonable candidate to moderate glycemic index (Hu et al., 2004).

4. Total phenolic content and α-amylase inhibition

Phenolic extracts from polished and unpolished rice contained various total phenolics with white rice the lowest in phenolic content and brown rice the highest (Table 3). This indicated that much of the phenolics were removed with the pericarp that was removed in the polishing process. Germination also led to some lowering of total phenolics which could be from the loss in processing water, thermal decomposition, possibly, interaction with other components (Walter, EnioMarchesan, Masson, Silva, Sartori & Ferreira, 2011).

Phenolic extracts were further studied in order to investigate the influence of phenolics on α-amylase inhibition comparing against three antioxidants (ferulic acid, ρ-coumaric acid and catechin). For the experiment with these controls, the inhibition of α-amylase activities was performed phenolic: starch ratio. The results showed an increase in α- amylase inhibition curve nearly with catechin, ferulic acid, and coumaric acid concentrations (Fig.3). The rice phenolic extracts were tested for amylase inhibition similarly but because different amounts of phenolics and hence different concentration of phenolics in the extracts differed, the experiments were therefore varied in phenolic:starch ratio (Table 3). The inhibition varied drastically from the sample with very low phenolics (2.44% inhibition) to samples with higher phenolics (15.6-16.3% inhibition) for extracts from brown rice (Table 3). These data

are superimposed against the three controls on Fig 3. It was concluded that phenolic components might have played an important role in α-amylase digestion of starch lowering GI and Hi in the cases of brown rice samples. But it could be argues that the starches might have different physic-chemical properties (e.g., number of double helices or crystallinity) and therefore they were tested by DSC.

Physico-chemical properties (DSC) of starch

Other factors may also vary and affect amylase digestion of starch among all rice samples studied. Since polishing of rice grains may have removed protein, lipids and modified some physical nature of the starches. In order to investigate the physicochemical effects, rice grains were milled, cooked (as in the digestion experiments) and frozen before tested for DSC endothermic transition analysis. The objective was to determine endothermic melting transition of amylopectin and amylase as well as any other endothermic starch-lipid complex.

The gelatinization temperature and transition enthalpy of cooked rice sample are shown in Table 4 and Fig 4. All samples showed three endothermic peaks, 52-62 ^{o}C, 75-86^{o}C, and 93-110^{o}C. The 52-62 ^{o}C peak was probably lipids- or protein-related transition; the 75-86 ^{o}C peak amylopectin melting transition; and the third peak at 93-110^{o}C amylose-lipid complex (AMC) and/or amylose melting transition (peaks for brown rice exhibited a broad multiple transition). These complexes have been earlier reported to be in this range (between 95 and 105^{o}C, Galloway, Biliaderis & Stanley, 1989; Karkalas & Raphaelides, 1996). It was found that the three rice samples exhibited some small ungelatinized or unmelted starch remained (endothermic peaks at 80^{o}C T_p). Comparing the DSC and starch digestion results, it was found that there was no correlation between endothermic starch melting

transitions (i.e., helix content) and starch digestibility by α-amylase suggesting that the helical structure of starch did not prevent the digestion in this case.

Type and content of phenolics in rice

HPLC spectra for all cooked and uncooked rice samples and the standard controls are shown in Fig. 5. The spectral peaks were identified for protocatechuic acid, *p*-coumaric acid, and ferulic acid and the contents of each are shown in Table 5. It was clear that Sangyod Phattalung rice contains protocatechuic acid (PCA) as the major phenolic component at levels approximately 1.1 and 1.5 mg/100 g dry sample for cooked and uncooked brown rice, respectively and 0.7 (cooked) and 0.85 (uncooked) unpolished germinated rice, respectively. The ρ-coumalic and ferulic acids were found to be more similar among samples although some significant difference was observed but the differences were relatively small. The results demonstrated that PCA was mostly lost through polishing whereas *p*-coumaric and ferulic acids were also lost but to a much less extent. Cooking resulted in a slight (5-10%) decrease in phenolics content.

Comparing phenolics content against α--amylase inhibition data (Table 3) also shown in Figure 3 as horizontal lines, it can be seen that the inhibition of α--amylase did increase at elevated phenolics content.

Therefore, it was concluded that Sangyod Phattalung rice digestion can resulted in a lower GI due to the presence of phenolics (primarily PCA) which was the major contributor of α-amylase inhibition.

Conclusion

Unpolished Sangyod Phattalung rice in form of whole grains is hydrolyzed at rate slower than its flour counterpart. The reddish brown color of the pericarp is

shown to be rich of phenolics particularly PCA which (and together with other phenolics) exhibited α-amylase inhibition. Germinated brown rice also have similar effect but slightly less phenolics found due to some loss in the pre-treatment and germination process. Physico-chemical change in amylopectin and amylose helical formation and amylose-lipid complex were present in the three rice samples differently but did not correlate with α-amylase activities. Consuming brown Sangyod rice should help reduce starch digestion and consequently lower HI and GI. This is of great importance to weight control and diabetes control application.

Acknowledgment: This project was granted by Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand

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Table 1 Chemical composition of unpolished (brown) rice and unpolished (brown, germinated) rice of Sangyod Phatthalung rice (% dry basis)

	% dry basis		
	Polished rice	Unpolished rice	
	polished rice	(brown) rice	(germinated) rice
Macro nutrient			
Protein	7.81±0.02[c]	8.22±0.05[b]	8.30±0.01[a]
Lipid	0.51±0.03[c]	3.18±0.03[a]	2.80±0.13[b]
Ash	0.75±0.00[c]	1.36±0.01[a]	1.03±0.03[b]
Total starch	83.86±2.60[a]	73.05±4.76[b]	75.38±5.79[b]
Micro nutrient (ppm)			
Fe (iron)	12.28±0.06[b]	12.09±0.05[c]	19.69±0.05[a]
Zn (zinc)	20.93±0.29[c]	22.63±0.05[b]	31.53±0.11[a]
Na (sodium)	114.69±1.39[c]	162.99±2.25[b]	239.10±0.78[a]
Ca (calcium)	484.32±1.80[b]	482.64±4.40[b]	775.26±4.23[a]
Mg (magnesium)	513.30±2.77[c]	985.83±8.69[b]	1090.97±8.63[a]
K (potassium)	1192.43±22.06[b]	2118.14±17.02[a]	1226.17±18.67[b]
P (phosphorus)	1012.09±10.55[c]	2104.58±26.35[a]	2014.95±30.90[b]
Total phenolic (mg FA equiv/100g dry sample)	37.50±4.81[c]	385.42±4.17[a]	306.25±7.98[b]

Value are given as mean ± SD from triplicate
Different superscripts in the same row indicate significant different ($P<0.05$)

Table 2 *In vitro* glycemic index in grain and flour of Sangyod Phatthalung rice

	HI	GI	Interpretation
1. Flours			
White (polished)	70.9±1.45[a]	78.7±0.80[a]	High GI
Brown (unpolished)	68.0±1.72[b]	77.1±0.94[b]	High GI
Brown (unpolished) germinated)	67.9±0.97[b]	77.0±0.53[b]	High GI
2. Grains			
Polished rice	50.0±2.14[c]	67.2±1.17[c]	Medium GI
Brown (unpolished)	40.7±1.02[d]	62.0±0.57[d]	Medium GI
Germinated (unpolished)	42.5±1.62[d]	63.0±0.89[d]	Medium GI

Different superscripts in the same column indicate significant different ($P<0.05$)

Table 3 Total phenolic compounds and percentage of porcine α-amylase inhibition activity of Sangyod Phatthalung rice

	Phenolic content (mg/mL)	Starch content (mg/mL)	Phenolic and starch ratio	% inhibition
White (polished)	0.015 ± 0.002^{c}	10	0.15:1	2.44 ± 0.67^{b}
Brown (unpolished)	0.154 ± 0.002^{a}	10	1.54:1	15.62 ± 0.65^{a}
Brown germinated (unpolished)	0.123 ± 0.003^{b}	10	1.23:1	16.30 ± 0.66^{a}

Values are given as mean ± SD from triplicate

Different superscripts of type of rice in the same column indicate significant different ($P<0.05$)

Table 5 Gelatinization temperatures and transition enthalpy from different flours of Sangyod Phatthalung rice

Peak	rice	transition temperatures (^{o}C)			ΔH (J/g)
		T_o	T_p	T_e	
First (Damage starch)	Polished (white)	51.93±0.18^{a}	58.06±0.06^{a}	62.42±0.51^{a}	0.20±0.05^{ab}
	Unpolished (brown)	51.67±0.03^{a}	58.00±0.29^{a}	62.24±0.35^{a}	0.06±0.01^{b}
	Unpolished (germinated)	39.41±0.71^{b}	50.17±1.78^{b}	62.45±0.49^{a}	0.44±0.14^{a}
Second (amylopectin)	Polished (white)	75.72±0.11^{a}	80.39±0.28^{a}	86.81±0.23^{a}	0.19±0.08^{a}
	Unpolished (brown)	75.87±0.11^{a}	80.44±0.61^{a}	86.06±0.31^{a}	0.04±0.01^{a}
	Unpolished (germinated)	75.57±0.17^{a}	80.61±0.46^{a}	85.64±0.44^{a}	0.11±0.05^{a}
Third (AMC)	Polished (white)	98.70±0.04^{a}	101.72±0.28^{a}	106.99±0.40^{b}	0.83±0.15^{a}
	Unpolished (brown)	93.93±2.94^{a}	104.94±1.02^{ab}	110.52±1.22^{b}	0.15±0.01^{b}
	Unpolished (germinated)	93.82±2.73^{a}	107.05±1.13^{a}	116.70±1.00^{a}	0.21±0.10^{b}

Value are given as mean ± SD from triplicate

Different superscripts of type of rice in the same column indicate significant different ($P<0.05$)

Fig. 1 Sangyod Phatthalung rice sample in the studies (a = polished rice flour, b = unpolished rice flour, c = germinated unpolished rice flour, d =polished rice grains, e = unpolished rice grains, f = germinated unpolished rice grains)

Fig. 2 Starch digestograms for cooked rice grains (A) and flours (B); increase of glucose contents during hydrolyses of rice grains and rice flours (C) and (D), respectively; standard (white bread (), white rice flour (),brown rice flour (),brown germinated rice flour (), white rice grain (), brown rice grain ( ) and brown and germinated rice grain ()

Fig. 3 Relationships between phenolic content and % inhibition porcine α--amylase activity of ferulic acid (), catechin ( ) and coumaric acid ()

Fig. 4 DSC thermograms of cooked and cooled white (polished, brown (unpolished), and germinated brown Sangyod Phattalung rice at about 90 percentage moisture content.

Fig. 5 HPLC chromatograms (290 nm.) of phenolic extracts from Sangyod Phattalung rice comparing polished (white), unpolished (brown) rice, germinated unpolished (brown) rice and also comparing the effect of cooking. Peaks: 1, protocatechuic acid; 2, *p*-coumaric acid; 3, ferulic acid.

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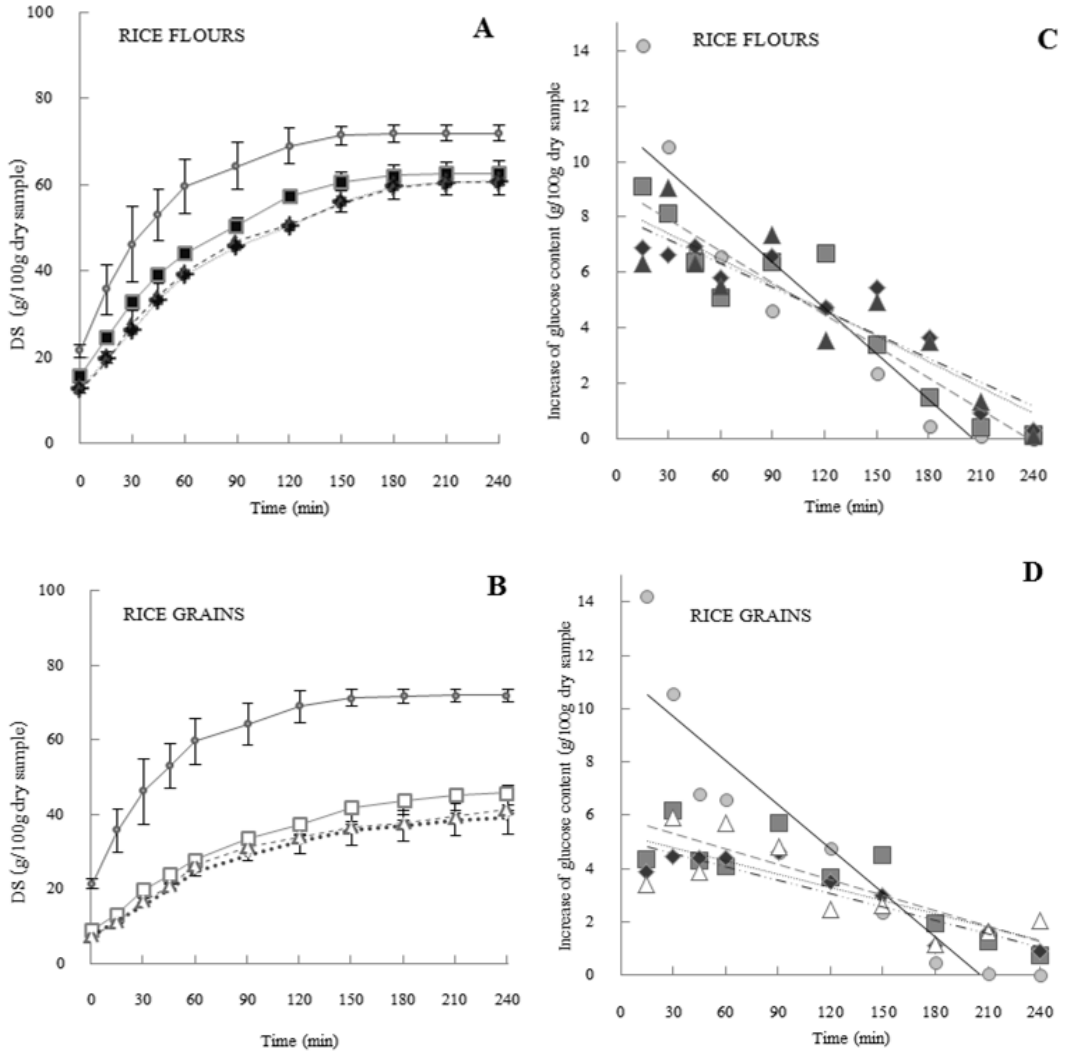


Fig 2 Starch digestograms for cooked rice grains (A) and flours (B); increase of glucose contents during hydrolyses of rice grains and rice flours (C) and (D), respectively; standard (white bread (—●—), white rice flour (- ■ -), brown rice flour (···◆···), brown germinated rice flour (- -★- -), white rice grain (—□—), brown rice grain (···◇···) and brown and germinated rice grain (- -△- -)

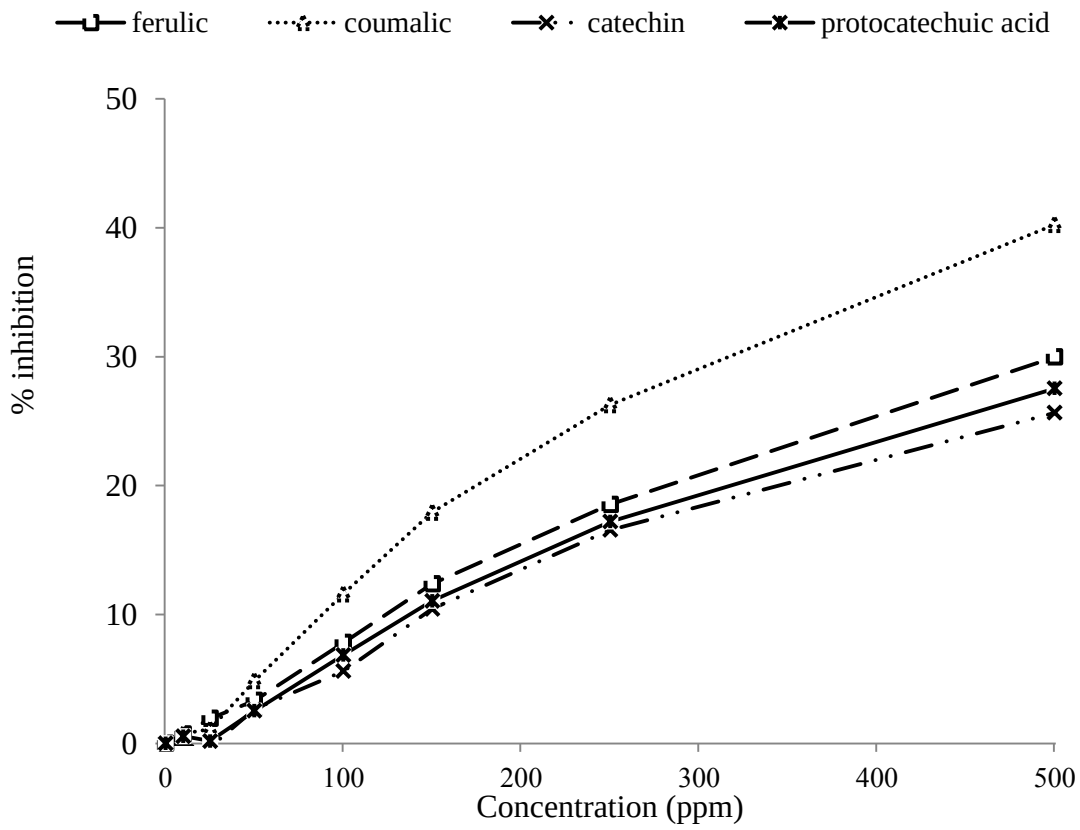


Fig. 3 Relationships between phenolic content and % inhibition porcine α-amylase activity of ferulic acid, catechin and coumalic acid.

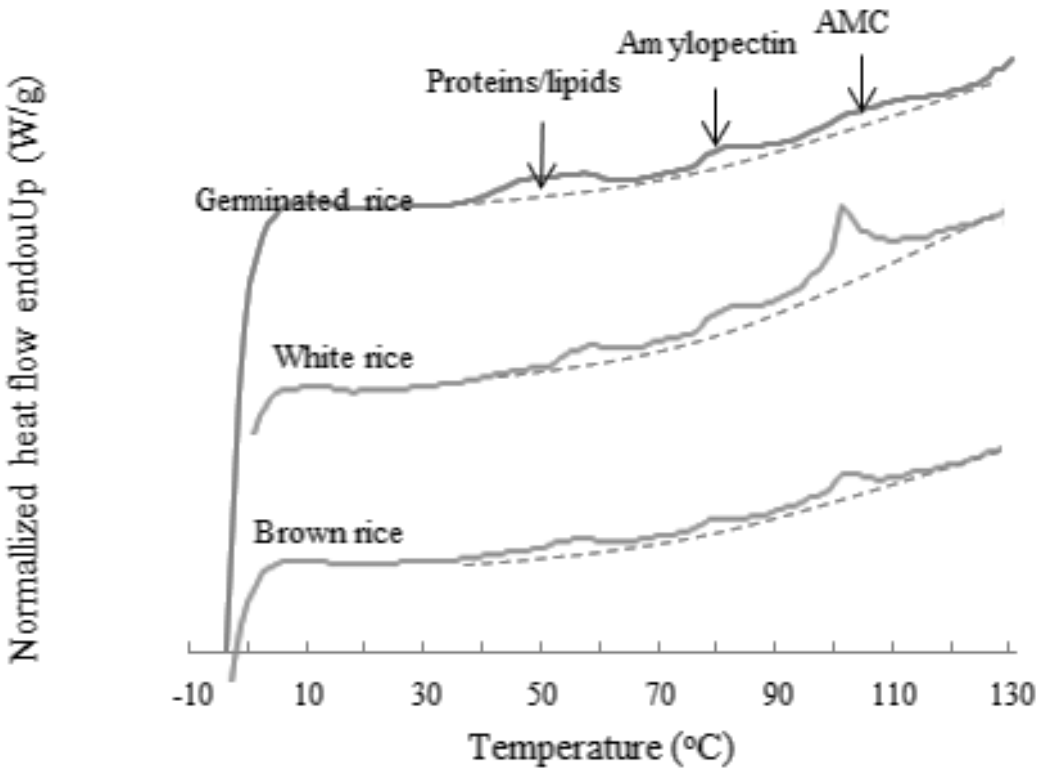
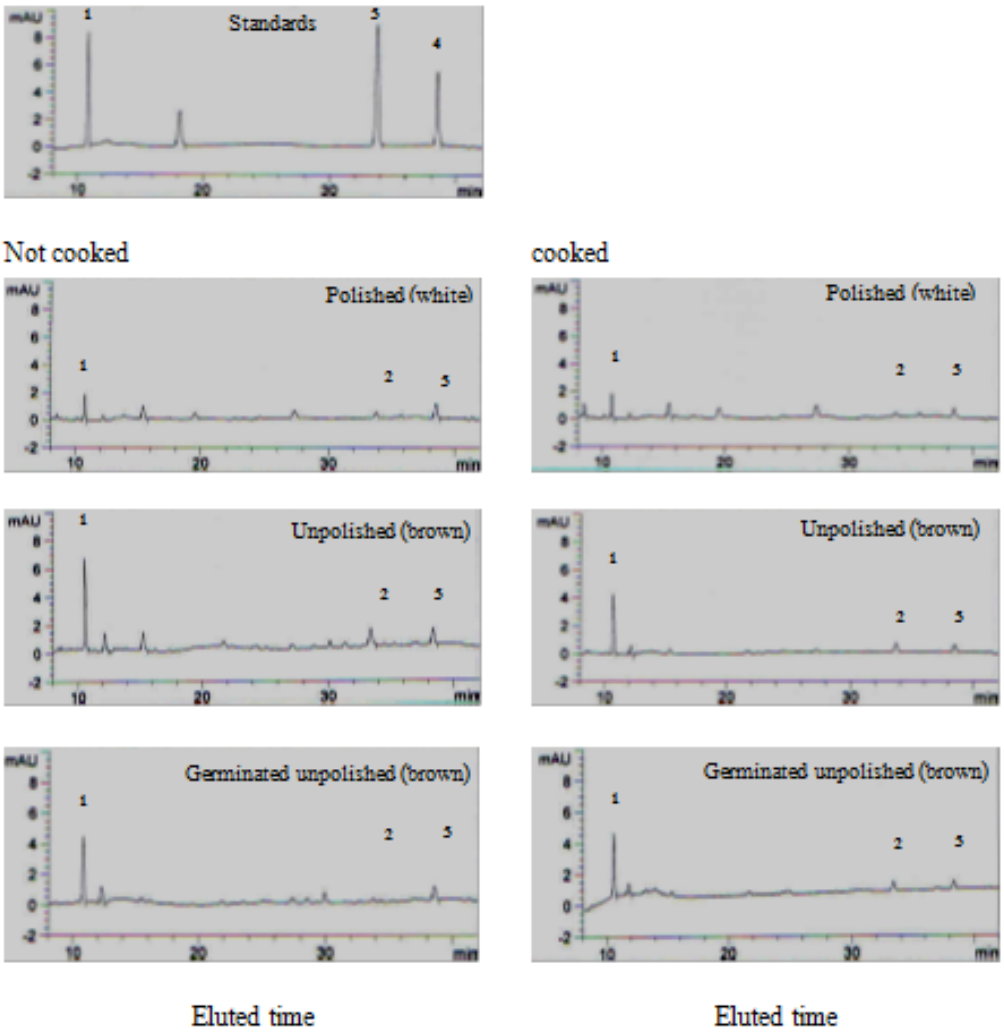


Fig. 4 DSC thermograms of cooked and cooled white (polished), brown (unpolished), and germinated brown Sangyod Phattalung rice at about 90 percentage moisture content.



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