IN VITRO "RAPIDLY AVAILABLE GLUCOSE (RAG)" VALUE USE AS AN INDICATOR FOR GLUCOSE RESPONSE AFTER DIGESTION OF THAI RICE AND RICE PRODUCTS

NARUMOL RATANAKONPUN

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……..
Miss. Narumol Ratanakonpun,
Candidate

……..
Asst. Prof. Ratchanee Kongkachuichai,
Ph.D. (Food Science and Technology)
Major-Advisor

……..
Miss. Rin Charoensiri,
M.Sc. (Nutrition and Dietetics)
Co-Advisor

……..
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies

……..
Prof. Surat Komindr, M.D., F.R.C.P. (T),
Chair
Master of Science Programme in Nutrition
Faculty of Medicine, Ramathibodi
Hospital and Institute of Nutrition

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on
January 15, 2008

……..
Miss. Narumol Ratanakonpun,
Candidate

……..
Asst. Prof. Sudsai Trevanich,
Ph.D. (Food Science and Technology)
Chair

……..
Asst. Prof. Ratchanee Kongkachuichai,
Ph.D. (Food Science and Technology)
Member

……..
Miss Rin Charoensiri,
M.Sc. (Nutrition and Dietetics)
Member

……..
Assoc. Prof. Visit Chavasit,
Ph.D. (Food Science)
Director
Institute of Nutrition
Mahidol University

……..
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

……..
Prof. Rajata Rajatanavin,
M.D., F.A.C.E.
Dean
Faculty of Medicine,
Ramathibodi Hospital
Mahidol University

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Narumol Ratanakonpun

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NARUMOL RATANAKONPUN 4737733 RANU/M
M.Sc. (NUTRITION)

THESIS ADVISORS: RATCHANEE KONGKACHUICHAI, Ph.D. (FOOD SCIENCE AND TECHNOLOGY), RIN CHAROENSIRI, M.Sc. (NUTRITION AND DIETETICS)

ABSTRACT

The rate of starch digestion and absorption seem to be important factors for the health of consumer's, especially in diabetes patients. Therefore, the first objective in the present study was to validate *in vitro* enzymatic carbohydrate (CHO) digestion methods (rapidly available glucose (RAG) and slowly available glucose (SAG)) and whether they can be applied to estimate the glycemic index (GI) or glycemic load (GL) in various kinds of foods. The second objective was to screen and evaluate ninety four varieties of rice obtained from local markets in Thailand and new varieties (breeding lines from Kasetsart University) for low digestion CHO by *in vitro* RAG and SAG methods. Amylose content was determined by using colorimetry and resistant starch (RS) content was determined by using a RS kit. The third objective was to evaluate the correlation between various CHO fractions with RAG values in sixteen rice varieties using an *in vitro* RAG method. The fourth objective was to develop modified snack products from low RAG value rice flour. The last objective was to evaluate storage effects using steamed buns as a model on RAG and sensory acceptability at – 20 °C for 3 months.

Part I. The values between RAG and GL of fruit models had a significant positive correlation ($r = 0.687$ at $p < 0.01$), whilst SAG showed no significant correlation with GL. Nevertherless, data of RAG values in the selected fruits of the present study were close to the GL values in human studies.

Part II. There was large variation of non-glutinous rice for RAG values, ranging from 6.05 to 22.18 g/100g cooked, 15.72 to 42.71% for amylose and 0.02 to 2.16 g/100g for RS contents. Most cooked glutinous rice showed a high amount of RAG (>20g/100g), but was very low in RS (0.00-0.03 g/100g). The data showed a significantly negative correlation between amylose and RAG ($r = -0.247$, $p<0.05$) and a positive correlation for amylose and RS content ($r = 0.402$, $p<0.01$). Especially, milled Hom Mali Sichompoo, brown KD-BT 313-19-1-1 (harvested in dry season) and milled Hom Mali Thung kula Roi Et in particulars revealed low RAG levels which might recommend them for dietary management of diabetes.

Part III. Results of the selected sixteen rice varieties model showed that all RAG values of cooked glutinous rice (18.86 -27.98 g/100g) were higher than non-glutinous rice (13.53 – 17.77 g/100g) and cooked polished rice (15.27-17.77 g/100g) were higher than cooked brown rice (13.53-17.06 g/100g), except brown glutinous KDC18-3-7-1-11-0 gave a low amount of RAG (15.44 g/100g). Very low RS values were observed in all glutinous rice. A significantly positive correlation between RAG and RDS as well as RAG and SDI was observed with $r = 0.993$ and 0.815, respectively.

Part IV. Formulation of low RAG products by blending wheat flour with brown rice flour (non polished rice 313-19-1-1) in steamed buns, bread sticks, red bean filled buns and bread were replaced with 70%, 50%, 65% and 44% of rice flour, respectively. The RAG and RS values of each modified formula were significantly lower than the control formula ($p<0.05$).

Part V. Although the rate of RAG values of the steamed bun model at 0 mo were significantly lower than at 1 – 3 mo of storage, the sensory evaluation was accepted during all the storage period.

Conclusion Analysis of data indicated that many varieties of rice and four modified snack based flour products in this study might have a potential application as low or intermediate GI foods. *In vitro* methods were able to be used as screening tools for selecting the best varieties of rice and foods in order to explore or apply to further human study.

KEY WORDS: RICE/DIGESTIBILITY/AMYLOSE/RESISTANT STARCH/SNACK

143 pp.

การใช้ค่า RAPIDLY AVAILABLE GLUCOSE เป็นดัชนีชี้วัดการตอบสนองต่อระดับน้ำตาลที่เปลี่ยนแปลงในข้าวและผลิตภัณฑ์หลังจากการย่อย *(IN VITRO* "RAPIDLY AVAILABLE GLUCOSE (RAG)" VALUE USE AS AN INDICATOR FOR GLUCOSE RESPONSE AFTER DIGESTION OF THAI RICE AND RICE PRODUCTS)

นฤมล รัตนากรพันธุ์ 4737733 RANU/M
วท.ม. (โภชนศาสตร์)

คณะกรรมการควบคุมวิทยานิพนธ์: รัชนี คงคาฉุยฉาย, Ph.D. (FOOD SCIENCE AND TECHNOLOGY), ริญ เจริญศิริ, M.Sc. (NUTRITION AND DIETETICS)

บทคัดย่อ

อัตราการย่อยและการดูดซึมอาหารจำพวกแป้งเป็นปัจจัยสำคัญต่อสุขภาพของผู้บริโภค โดยเฉพาะผู้ป่วยเบาหวาน ดังนั้น วัตถุประสงค์แรกในการศึกษาครั้งนี้คือการทดสอบความใช้ได้ของวิธีการหาอัตราการย่อยแป้งเป็นน้ำตาลในหลอดทดลอง โดยวิธี rapidly available glucose (RAG) และ slowly available glucose (SAG) ซึ่งเป็นวิธีที่สามารถประเมิน glycemic index (GI) หรือ glycemic load (GL)ในตัวอย่างอาหารชนิดต่างๆได้ วัตถุประสงค์ที่สองเพื่อคัดกรองและประเมินพันธุ์ข้าวที่มีอัตราการย่อยแป้งเป็นน้ำตาลต่ำในข้าว 94 สายพันธุ์ที่ได้จากตลาดและมหาวิทยาลัยเกษตรศาสตร์ด้วยค่า RAG และ SAG รวมทั้งหาค่าอไมโลสด้วยเทคนิคคัลเลอร์ริเมตรี และหาค่า resistant starch (RS) โดยชุดทดสอบ วัตถุประสงค์ที่สามคือการหาความสัมพันธ์ระหว่างค่า RAG กับส่วนประกอบย่อยต่างๆของคาร์โบไฮเดรตในข้าว 16 สายพันธุ์ วัตถุประสงค์ที่สีคือการพัฒนาผลิตภัณฑ์อาหารว่างที่มีค่า RAG ต่ำ จากแป้งข้าว และ วัตถุประสงค์สุดท้าย คือเพื่อศึกษาผลของการเก็บรักษาต่อค่า RAG และการยอมรับทางประสาทสัมผัส ที่ -20 องศาเซลเซียส เป็นเวลา 3 เดือน

ส่วนที่หนึ่งพบว่า ค่า RAG และ GL ของผลไม้มีความสัมพันธ์กันอย่างมีนัยสำคัญในทางบวกที่ค่า $r = 0.687$ ($p<0.01$) และค่า RAG ที่ได้มีค่าใกล้เคียงกับค่า GL อย่างไรก็ตามไม่พบความสัมพันธ์ระหว่าง ค่า SAG กับ GL

ส่วนที่สองพบว่า ข้าวเจ้ามีค่า RAG อยู่ในช่วง 6.05 – 22.18 กรัมต่อ100กรัม อไมโลสอยู่ในช่วง 15.72 – 42.71% และ RS อยู่ในช่วง 0.02 – 2.16 กรัมต่อ100กรัม ส่วนข้าวเหนียวเกือบทุกสายพันธุ์มีค่า RAG สูง (>20 กรัมต่อ100กรัม) ในขณะที่มีค่า RS ต่ำมาก (0.00 – 0.03 กรัมต่อ100 กรัม) ในการศึกษานี้พบความสัมพันธ์อย่างมีนัยสำคัญในเชิงลระหว่างอไมโลสกับ RAG (-0.247, $p<0.05$) และในเชิงบวกระหว่างอไมโลสกับ RS ($r = 0.402$, $p<0.01$) สำหรับข้าวที่มีค่า RAG ต่ำ ได้แก่ ข้าวขัดหอมมะลิสีชมพู ข้าวกล้อง 313-19-1-1 (เก็บเกี่ยวในฤดูร้อน) และ ข้าวหอมมะลิทุ่งกุลาร้อยเอ็ด ซึ่งข้าวเหล่านี้อาจนำมาใช้แนะนำต่อผู้ที่ต้องการควบคุมระดับน้ำตาลในเลือดได้

ส่วนที่สามพบว่า ในตัวอย่างข้าว 16 สายพันธุ์ ข้าวเหนียวมีค่า RAG (18.86 -27.98 g/100g) สูงกว่าข้าวเจ้า (13.53 – 17.77 g/100g) และ ข้าวขัด (15.27-17.77 g/100g) มีค่า RAG สูงกว่าข้าวกล้อง (13.53-17.06 g/100g) ยกเว้น ข้าวเหนียวกล้องพันธุ์ KDC18-3-7-1-11-0 ซึ่งมีค่า RAG ต่ำ (15.44 g/100g) ส่วนค่า RS ในข้าวเหนียวมีค่าต่ำมาก สำหรับความสัมพันธ์ระหว่าง RAG กับ RDS และ RAG กับ SDI พบว่าเป็นไปในทางบวกอย่างมีนัยสำคัญที่ $r = 0.993$ และ 0.815 ($p<0.01$) ตามลำดับ

ส่วนที่สี่ การพัฒนาผลิตภัณฑ์โดยการแทนที่แป้งสาลีด้วยแป้งข้าวกล้องพันธุ์ 313-19-1-1 ในอาหารว่าง 4 ชนิดคือ หมั่นโถว ขนมปังขาไก่ ขนมปังไส้ถั่วแดง และขนมปังแซนวิช พบว่าสามารถทดแทนแป้งข้าวได้ 70%, 50%, 65% และ 44% ตามลำดับ และสูตรปรับปรุงทั้ง 4 ชนิดมีค่า RAG และ RS ต่ำกว่าสูตรปกติอย่างมีนัยสำคัญ ($p<0.05$)

ส่วนที่ห้า ค่าของ RAG ของหมั่นโถต้นแบบที่ 0 เดือนมีค่าต่ำกว่าหมั่นโถที่เก็บที่เวลา 1-3 เดือนอย่างมีนัยสำคัญทางสถิติและมีการยอมรับทางประสาทสัมผัสตลอดอายุการเก็บรักษา

สรุปได้ว่าในการศึกษานี้ ข้าวหลายสายพันธุ์รวมทั้งอาหารว่างที่ได้รับการดัดแปลงแล้วมีค่า GI อยู่ในระดับต่ำหรือระดับกลาง และวิธีวิเคราะห์อัตราการย่อยแป้งในหลอดทดลองอาจใช้เป็นเครื่องมือในการคัดกรองสายพันธุ์ข้าวและอาหารอื่นๆ เพื่อที่จะนำไปศึกษาในคนต่อไป

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LIST OF ABBREVIATIONS

AC	Amylose content
AMG	Amyloglucosidase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
^{O}C	Degree celcious
cal	Calorie
CHO	Carbohydrate
cm	Centimeter
CVD	Cardiovascular disease
DM	Diabetes mellitus
FG	Free glucose
g	Gram
hr	Hour
k	Kilogram
l	Liter
min	Minute
ml	Milliliter
MNT	Medical Nutrition Therapy
nm	Nanometer
GI	Glycemic index
GL	Glycemic load
GOD-PAP	Glucoseoxidase-peroxidase
RAG	Rapidly available glucose
RDS	Rapidly digestible starch
rpm	Round per minute
RS	Resistant starch
SAG	Slowly available glucose

LIST OF ABBREVIATIONS (Continued)

SDI	Starch digestible index
SDS	Slowly digestible starch
TDF	Total dietary fiber
TG	Total glucose
TS	Total starch
U	Unit
w/w	weight/weight

CHAPTER I
INTRODUCTION

Rice is the most important cereal crop and the staple food of over half the world's population. Normally rice is primary source of carbohydrate (CHO). After digested, CHO is changed to glucose. However, the rate of starch digestion and absorption seem to be important factors for consumer's health benefit, especially, non-insulin-dependent diabetes type II and hyperlipidemia. Numerous studies have been shown that slowly digested and absorbed CHO is favorable for dietary management of metabolic disorders particularly, diabetes and hyperlipidemia (1). Since blood glucose response is commonly used for estimating the glycemic index (GI), which relates to the response of a test meal comparing to that of a reference food, usually white bread or glucose solution. The GI value is obtained from the incremental postprandial blood glucose value after two hours of ingestion and divided by the corresponding blood glucose after ingestion of an equal CHO portion of the reference food (2). A food with low glycemic response is considered beneficial as nutritious food, especially for individual person who is suffering from impaired glucose tolerance (3). However, the methodology for evaluating GI in human study is very expensive and time-consume, moreover, a few samples can be done in the same time. In addition, the previous studies have been shown that the *in vitro* rapidly available glucose (RAG) and slowly available glucose (SAG) values were closely related to glycemic response in human studies (4, 5). Since the RAG value represents the amount of glucose (in g/100g food) that can be expected to be rapidly available for absorption after a meal while the SAG value relates to the food as eaten that includes both rapidly digestible starch (RDS) and free glucose (FG) (5, 6). Nowadays, the prevalence of degenerative diseases is more increasing, particularly, coronary heat disease, diabetes mellitus (DM) and renal disease and by the report of The World Health Organization estimates that more than 150 million people worldwide suffer

from DM and figures their double increase rate by 2025 (7) due to their change of life style through the diet and lack of exercise. Therefore, the development of the nutritious quality of foods and healthy products may be important for reducing the incidence of non-communicable diseases. Rice is not only the staple food of over half the world's population but also primary sources of CHO worldwide. Many researchers showed the GI of rice to be relatively high when compared to other starchy foods (8). Therefore, the development or search for a low GI rice variety and rice products can be very important for preventing or delaying the development of non-communicable diseases especially, the diseases that are linked to insulin resistance. The objectives of this study were to screen various rice varieties and develop low GI snack products from low GI rice flour and to estimate their GI by based on the amount of *in vitro* RAG, SAG, total glucose (TG), starch digestible index (SDI) and FG as following the methods of Englyst et al., (6).

CHAPTER II
OBJECTIVES

General objectives

1. To develop method for evaluating and screening on available glucose of new breeding and local various rice varieties using *in vitro* RAG and SAG.
2. To formulate 4 kinds of modified snack products that made from intermediate GI rice flour in order to provide nutritional quality and sensory acceptability.

Specific Objectives

1. To validate method for measuring RAG and SAG values of some kinds of fruits and other CHO foods model.
2. To evaluate the correlation between GL and RAG or SAG of some fruits.
3. To search various rice varieties, in which showed slowly starch digestion rate for recommendation to consumer.
4. To measure RAG, SAG, and AC of rice varieties and also to determine RS of some rice varieties from Kasetsart University.
5. To evaluate the correlation between the amounts of AC and RAG; amylose and RS contents of rice varieties.
6. To determine the amount of RAG, SAG, TG, FG, RDS, SDS, TS, RS and SDI values of selected sixteen rice varieties model.
7. To evaluate the correlation between the value of RAG, RDS, RS and SDI of selected sixteen rice varieties model.
8. To evaluate the correlation of RAG, RDS, TG, FG, TS and RS contents between the control and modified snack products as well as evaluate the sensory acceptability of modified snack products.

9. To determine the shell-life of modified steamed bun model by freezing at -20 °C for 3 months.

Expected Outcome

1. The study will provide beneficial nutritious products for health promotion food and health concern people.
2. The new breeding rice varieties that showed low RAG and/or GI are more value-added and the farmers get more income.
3. The food scientist can use these results as a basic research to develop other health foods.

CHAPTER III
LITERATURE REVIEW

3.1 Diabetes Mellitus (DM)

3.1.1 Pathophysiology

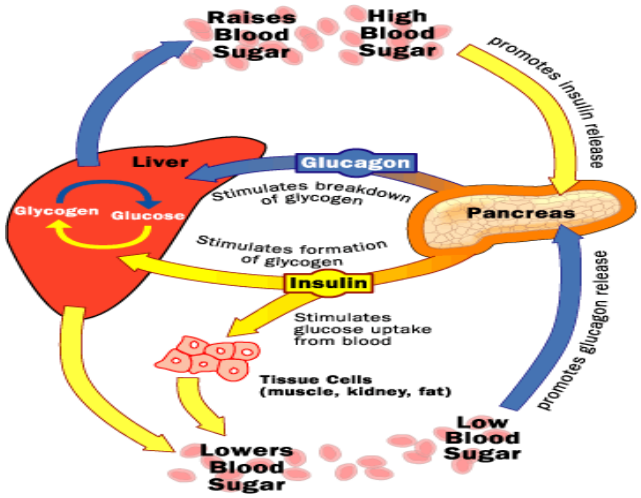

Figure 3.1 Pathophysiology of DM

The pancreas plays a primary role in the metabolism of glucose by secreting the hormones insulin and glucagon. Inadequate secretion of insulin, inadequate structure or function of insulin or its receptors results in impaired metabolism of glucose, carbohydrates, proteins and fats, characterized by hyperglycemia and glycosuria. Hyperglycemia is the most frequently observed sign of diabetes and is considered the etiologic source of diabetic complications both in the body and eye.

Glucagon is a hormone that opposes the action of insulin. It is secreted when blood glucose levels fall. Glucagon increases blood glucose concentration partly by breaking down glycogen in the liver. Following a meal, glucose is absorbed into the blood. In response to increased blood glucose levels, insulin is secreted causing rapid uptake, storage, or use of glucose by the tissues of the body. Unused glucose is stored

as glycogen in the liver. Between meals, when blood glucose is at minimal levels, tissues continue to require an energy source to function properly. Stored glycogen, via glucagon, is converted to glucose by a pathway known as glycogenolysis. Gluconeogenesis is the production of glucose in the liver from noncarbohydrate precursors such as glycogenic amino acids (9).

An additional complication of hyperglycemia is nonenzymatic glycosylation. Nonenzymatic glycosylation is the binding of excess glucose to the amino group of proteins in the tissues. As a possible result, at the level of the capillary membranes, altered cell function may lead to the development of microaneurisms, vascular loops, and vessel dilation, allowing blood leakage. Platelet aggregation secondary to these changes initiates tissue hypoxia. DM is a disease directly related to CHO, metabolism, and nutrition has always an integral role in its management. The contemporary term used to dietary prescriptions is medical nutrition therapy (MNT) for DM is published annually by the American Diabetes Association (ADA) (10).

3.2 CHO and Diabetes

Different types of CHO in food have also been shown to have an influence on the postprandial glycemic. Crapo et al., 1982 studied the acute effects of oral ingestion of fructose and sucrose sweetened in food products, dextrose and various CHO foods, such as cake, ice creams, potato, corn, and bread on serum glucose and insulin responses in normal subjects. The results showed that serum glucose and insulin responses to fructose used as a sweetener into food products were significantly lower than the responses to sucrose incorporated food products (11). In addition Chait, 1988 addressed that the consumption of simple sugars as well as diets containing large amount of sugar and glucose should be limited especially in and that diabetic patients. Since simple sugar and glucose are more rapidly absorbed, thus leading to more induce hyperglycemia than complex CHO (12). In addition, the regulation of blood glucose to achieve near-normal levels is a primary goal in the management of diabetes and thus dietary techniques that limit hyperglycemia following a meal are likely important in limiting the complications of diabetes while low CHO diets are not recommended in the management of diabetes. Although dietary CHO is the major contributor to postprandial glucose concentration, it is

important sources of energy and other nutrients, particularly fiber. Therefore, the National Academy of Science-Food and Nutrition Board recommended range of CHO intake around 45-65% of total calories. Both the amount (grams) of CHO as well as the type of CHO in a food influence blood glucose level. However, Sheard et al., 2004 suggested that the total amount of CHO consumed was a strong predictor of glycemic response, thus food exchanges or CHO counting were used to monitor the total grams of CHO for achieving glycemic control (13). While several investigators indicated that the different types of CHO in food have shown to have an influence on the postprandial glycemic. In 1981, Crapo et al., (14) studies the acute effects of oral ingestion of dextrose and various CHO foods, such as rice, potato, corn, and bread on serum glucose and insulin responses in diabetic subjects. The data showed that the same trend between serum glucose and insulin responses and the differences seen were most likely a function of some other physical or chemical characteristics of the natural food stuffs. Thus the control of blood sugar is important, diets enriched in the less glycemic starches, may have a therapeutic advantage for diabetic individuals. In addition, the new guidelines from Nutritional recommendations of DM treatment suggested that their diet should be revised recently. The new guidelines do not specifically restrict intake of sugars, although general recommendation are mode for including fiber, whole grains, vegetable and fruits (15), in addition to a focus on the role of the GI as a determinant of postprandial hyperglycemia and overall metabolic control. Furthermore, it has been argued that a high GI and GL diet would have greater adverse effects in persons with obesity, glucose intolerance, and insulin resistance, those recognized to be an increased risk for the development of type 2 DM (13).

3.3 Glycemic Index (GI)

The GI is a measure of the change in blood glucose following ingestion of CHO-containing foods. Some foods result in a marked rise followed by a more or less rapid fall in blood glucose, whereas others produce a smaller peak along with a more gradual decline in plasma glucose. The specific type of CHO (e.g., starch versus sucrose) present in a particular food does not always predict its effect on blood glucose as an equivalent amount of glucose or white bread. In general, CHO foodstuff can be

classified as falling into low-, medium-, or high- GI categories, but considerable variability in response can result for many reasons (16, 17, 18).

High	≥ 70
Moderate	55-69
Low	≤ 54

Factors influence glycemic responses (19) to foods, including the amount of CHO, type of sugar (glucose, fructose, sucrose, lactose), nature of the starch (amylose, amylopectin and RS) cooking and food processing (degree of starch gelatinization, particle size, cellular form), and food form, as well as other food components (fat and natural substance that slow digestion: lectins, phytates, tannins, and starch-protein and starch-lipid combinations). The GI of foods may have important implications for the prevention and treatment of the major causes of morbidity and mortality, including type 2 DM.

3.4 Glycemic Load (GL) (20)

GL of a typical serving of food is the product of the amount available CHO in that serving. In other words GL is more practical than GI of a food in terms of glucose in the circulation.

$$GL = \frac{GI \times \text{dietary CHO of serving (g)}}{100}$$

High	≥ 20
Moderate	11-19
Low	≤10

The research of the long term consumption of a diet with a high GL show a significant predictor of the risk developed in type 2 DM and cardiovascular disease (CVD). More recently, evidence has been accumulating that a low-GI diet might also protect against the development of obesity, colon cancer, and breast cancer (21, 22, 23).

3.5 Dietary management for diabetic patients

The management of diabetes includes dietary management, exercise and used hypoglycemic agent or insulin for glycemic control. Due to DM is metabolic disorder. So dietary management is attract because it is lower cost and directly effect to blood glucose and insulin level. In diabetic control, the choice of quantification and qualification of food improves glycemic control and decrease the risk of acute and long term complication particularly cardiovascular disease (23).

In the role of diet, particularly CHO the diabetes associations of many countries around the world reviewed their dietary recommendation and advised that diabetic patients should consume high CHO (complex CHO e.g. whole grains, fruits, vegetable) with low fat, low GI, high fiber and restrict simple sugars (24).

Table 3.1 Classification of food CHO (25)

Type	Components	Hydrolyzed and absorbed in the small intestine	Glycemic response
Sugars	glucose, fructose, sucrose, lactose	Mostly	Large
Sugar alcohols	Sorbitol, xylitol, lactitol, maltitol	Sparsely	0
Short-chain carbohydrates (SC)	Resistant (Fructo and galacto-oligosaccharides pyrodextrins, polydextrins)	No	0
	Maltodextrins	Yes	Large
Starch	RDS	Yes	Large
	SDS	Yes	Small
	RS	No	0
Non-starch polysaccharides (NSP)	Present as plant cell walls (dietary fiber)	No	0*
	Other NSP (Gums mucilage any isolated NSP)	No	0*

0* May affect the glycemic response to other carbohydrates.

The classification of food carbohydrates, their likely finish in the small intestine, whether they are included in two current measurements of dietary fiber, and the magnitude of the glycemic response (25).

It is therefore convenient to consider subdividing this class for nutritional purposes. The **Table 3.1** was lists RDS, SDS and RS fractions. The rate and extent of the digestion of starch is reflected in the magnitude and the duration of the glycemic response. A nutritional classification based on an *in vitro* measure of the rate and

extent of starch digestion is useful in predicting the likely glycemic response to foods (6).

3.6 RAG

The value of RAG achieve from hydrolyzed food by enzyme pancreatin amyloglucosidase (AMG) and invertase at 20 min of *in vitro* CHO digestible method as modifying the method of Englyst et al., 1992 and 1996 (5, 6). The RAG index was determined as a predictor of the potential glycemic response derived from the ingestion of these food items.

3.7 SAG

The value of SAG achieve from hydrolyzed food by enzyme pancreatin AMG and invertase between 20 and 120 min of *in vitro* CHO digestible method as modifying the method of Englyst et al., 1992 and 1996 (5, 6). The SAG index was determined as a predictor of the potential glycemic response derived from the ingestion of CHO food items.

3.8 The RAG and SAG fraction

The glycemic CHO fraction, which is available for absorption in the small intestine, is measured as the sum of sugars and starch, excluding starch RS. The glucose fraction can be divided into RAG and SAG, *in vitro* analysis which reflects the likely rate of release and absorption of glucose in the small intestine. Analytical procedures have been developed to characteristics of foods, which in turn reflect their likely physiological fate (6).

The hypothesis is the RAG fraction rapidly (within 20 minutes) released and absorbed and is a major determinant of the glycemic response. By contrast, the SAG fraction is released and absorbed slowly and is not expected to contribute to the glycemic response. The physio-chemical approach to characterizing CHO focuses solely on the contribution of the CHO component of foods in describing their GI values, and does not describe any of the numerous non-CHO nutrients that can also have an effect. They are mainly appropriate for the identification of the mechanisms responsible for GI value of different foods, and especially in distinguishing between

foods that have low GI values due to a high content of starch that is slowly digested (high in SAG). By contrast, caution is required when applying these measures to the prediction of glycemic responses to meals and diets, as this relationship will be confused by a combination of other meal mediated factors and the effects of within and between subject variations in gastrointestinal function and glucose tolerance.

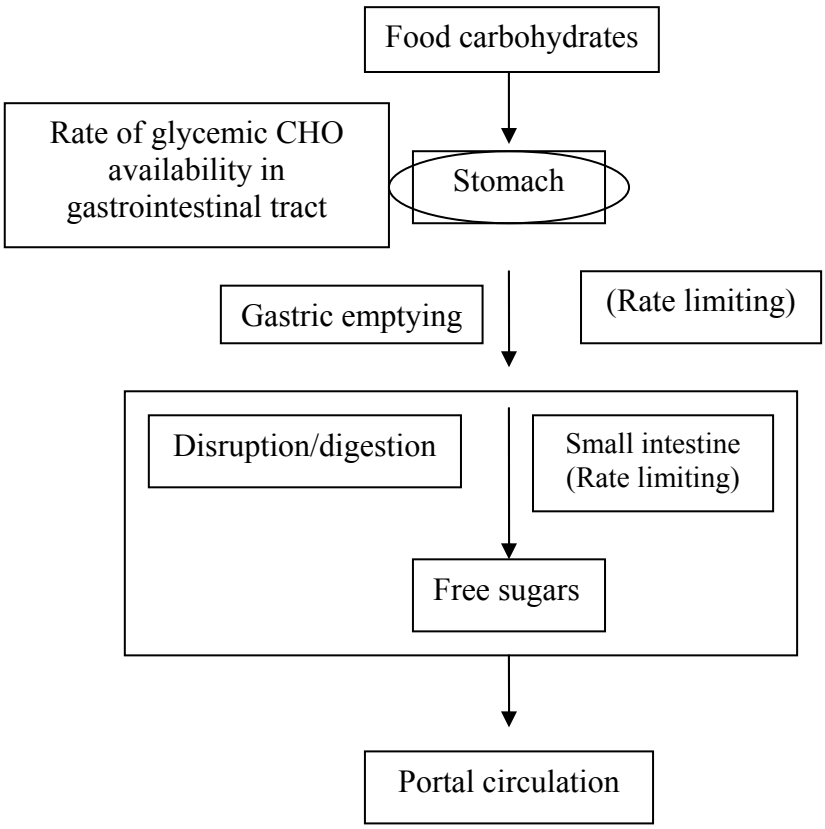

Figure 3.2 Rate of CHO availability (From Englyst et al., (6))

In 1999, Englyst et al., demonstrated that the *in vitro* measurement of the amount of RAG in foods could reflect the glycemic response (3), while Araya et al., 2002 showed that the amount of *in vitro* determination of CHO digestion rate were significant correlated with glycemic response in human study, this evidence indicates that *in vitro* CHO digestion rate can be a useful model to estimate the biological response of high CHO meal, in addition to its simple and inexpensive method (4). Likewise Englyst et al., 1996 showed that both RAG and RDS were highly correlated with GI (5).

The RAG, SAG and other CHO fraction may provide a tool for the assessment of "CHO quality", based on both the chemical and physio-chemical properties of CHO. Taking into account the CHO fractions contained in food should provide not only an indication of their likely GI value, but also suggest a food related mechanism to account for the effect. Caution is required when applying these concepts to meals and diets, as the basic relationships can be confounded by a combination of meal and subject specific factors.

During food processing, physio-chemical treatments may have a profound impact on the profile of starch digestibility. For cereal foods, alteration in moisture of the dough, baking time and temperature, pressure and mechanical treatments are important. A recent study has shown that a special biscuit manufacturing technique was the only process that allows the preservation of the SAG fraction over the different steps of the process, whereas in bakery products and ready-to-eat cereals, the SAG was progressively converted into RAG during the successive steps. In these various types of products, the food processing influences the extent of starch gelatinization. In bread, croissants, brioche, corn flacks and extruded ready-to-eat cereals, starch is totally gelatinized, while in some crackers and biscuits, starch moderately and lightly gelatinized, respectively. This is explained by the main physio-chemical parameters of the food processing. In bakery products and corn flakes, high temperature baking of the high moisture dough leads to starch alteration. In extruded ready-to-eat cereals, high mechanical shearing at high pressure is responsible for the main changes of the starch. In biscuits, however, very little water is available in the dough, which limits starch gelatinization in spite of high baking temperature. In crackers, moderate gelatinization of starch occurs because of the medium level of moisture of the dough prior to baking (26).

3.9 Research studies on RAG and SAG

- Englyst et al., 1999 indicated that the results from RAG *in vitro* digestibility rate of a test food significantly correlated to glycemic response in human studies (3).
- Araya et al., 2002 demonstrated that the results of RAG *in vitro* CHO digestion rate had not a significant correlation with the glycemic response in human studies. In contrast, when the amount of ratio of rapid digestion and slow digestion CHO at 120

min of hydrolysis was used to compare with the GI, a significant correlation was found (4).

- In 2003, Englyst and colleague revealed that foods with high amount of SAG values could be identified as low GI foods since their slow release of CHO might be claimed to be benefit for health (27).

- Englyst et al., 1996 addressed that the GI was an *in vivo* measurement based on the glycemic response to CHO containing foods and allowed foods to be ranked on the basic of the rate of digestion and absorption of the CHO that they contain. GI values were normalized to reference amount of available CHO and did not reflect the amounts of CHO present in foods. Such as food with low content of CHO might have a high GI values due to CHO rapidly digestion and absorption in the human lumen. These evidences were potentially confusing for the people who wished to control their blood glucose by selecting appropriate foods for their eating. Englyst and colleagues addressed that the RAG value was a direct calculation of the amount of glucose likely to be rapidly absorbed in the human small intestine and thus influences blood glucose and insulin levels. Therefore, in 1996, Englyst et al., studied the amount of RAG *in vitro* CHO digestion value and glycemic response on thirty-nine foods, the results revealed that the measurement of RAG *in vitro* CHO digestion rate of those foods had highly significant positive correlation with the GI values (5)

3.10 Starch

Starch is a mixture of two complex carbohydrates: amylose and amylopectin, both of which are polymers of glucose. Usually these are found in a ratio of 30:70 or 20:80, with amylopectin found in larger amounts than amylose. It is used by plants as a way to store excess glucose. The chemical formula for starch is $(C_6H_{10}O_5)n$, as it is a polymer of glucose. In terms of human nutrition, starch is by far the most important of the polysaccharides. It constitutes more than half the carbohydrates even in many wealthy diets, and much more in poorer diets. It is supplied by traditional staple foods such as cereals, roots and tubers. Starch is used in the manufacturing of adhesives, paper, textiles and as a mold in the manufacture of sweets such as wine gums and jelly beans. It is a white powder, and depending on the source, may be tasteless and odourless. The major resources for starch production and consumption worldwide are

rice, wheat, corn, and potatoes. Cooked foods containing starches include boiled rice, various forms of bread and noodles (including pasta). Starch can be hydrolyzed into simpler carbohydrates by acids, various enzymes, or a combination of the two. The extent of conversion is typically quantified by dextrose equivalent, which is roughly the fraction of the glycoside bonds in starch that have been broken (28, 29).

3.10.1 Amylose

Amylose is a linear polymer of glucose linked with mainly α (1→4) bonds. It can be made of several thousands of glucose units. It is one of the two components of starch, the other being amylopectin (30).

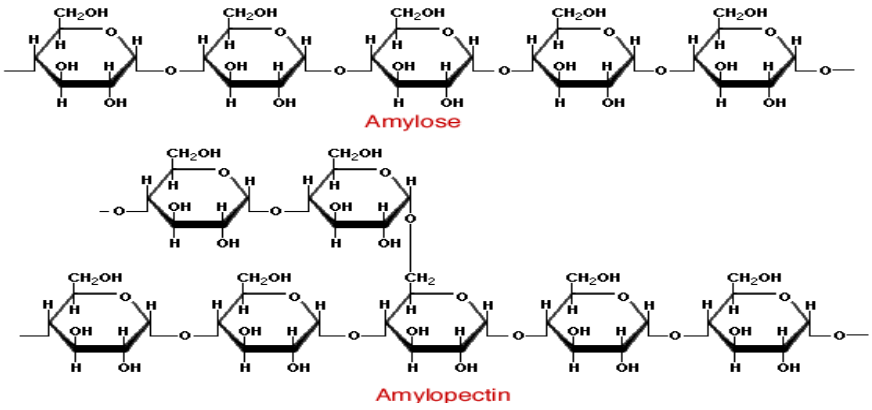

Figure 3.3 Sructure of amylose and amylopectin

Amylose starch is less readily digested than amylopectin. However it takes up less space so is preferred for storage in plants; it is how about 20% of the starch in plants is stored. The digestive enzyme amylase works on the ends of the starch molecule, breaking it down into sugars. High-amylose varieties of rice have a much lower GL which could be beneficial for diabetics.

3.10.2 Resistant Starch (RS)

RS is one of three types of dietary fiber, CHO that is not digestion in the small bowel and arrives undigested into the large bowel (31). Once there, it is fermented by bacteria and results in the formation of a number of beneficial volatile fatty acids

including butyrate, which promotes the production of healthy cells and fight colon cancer. RS also has a mild laxative effect and encourages the growth of healthy bacteria. It can be found in most CHO-rich foods but is high in cold cooked potatoes, unripe bananas, pasta, legumes and certain types of corn. Consumption of natural RS by humans has been shown to result in decreased glycemic response in healthy individuals (32), decreased glycemic response in diabetics, and increased insulin sensitivity in healthy individuals (33).

Classification and sources of resistant starch

RS can occur naturally in foods or in processed foods. It is categorized into four groups. Each of the four groups has a different structure.

RS1 is a tightly bound molecule wrapped in a fiber shell that does not allow the digestive enzymes access to the starch molecule. Legumes and whole or partly milled grains and seeds are sources of RS1.

RS2 molecule is termed raw ungeletinized starch because it is cooked or gelatinized. The RS2 has terminal glucose ends of starch structure wrapped tightly within its structure, resisting breakdown by amylase. Green bananas, raw potatoes and high amylose maize starch are sources of RS2.

RS3 is a retrograded starch molecule formed during heating and then cooling of the starch. This process called retrogradation produces crystalline potato, bread and corn flakes are sources of RS3.

RS4 is a chemically modified starch molecule that cannot be broken down since the modification process rendered the structure inaccessible for digestion by – amylase. Esterified or cross-bonded starches that are used in chemically synthesized foods are sources of RS4.

3.11 Rice

Rice (*Oryza sativa* L.) is the major cereal grain in Asia and Southeast Asia and the staple food of nearly a half of the world's population. White brown rice 313-19-1-1 will be used as the main ingredient for low GI buns since the data from human study showed intermediate GI (57%) in this rice (unpublished data).

Rice has given a wide range of results in GI value. The GI of white rice has range from as low as 54% to as high as 121% when bread (GI = 100%) is used as a

reference food. The rice with high AC is likely to have lower GI than those with low AC (34).

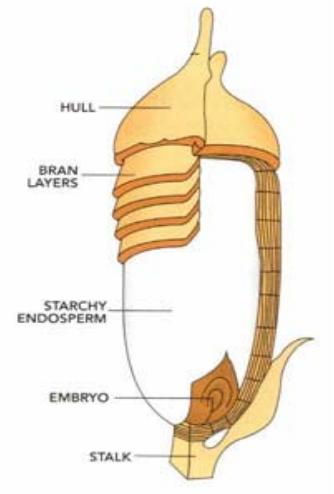

Figure 3.4 Structure of rice

Hull (35)

The most visible part or a rough rice grain is the husk. This is also known as the hull (**Figure 3.3**). This is formed from the two leaves of the spike let namely the palea covering the ventral part of the seed and the lemma covering the dorsal portion. Both parts are longitudinally joined together by an interlocking fold. This fold is a weak when a twisting force is applied to the grain. The upper end of the two hull sections transfer into the apiculus sections and finally ends in the pointed awn. At the lower part, where the grain is fixed on the panicle is a tiny leaf-shaped part called the sterile lemma and then the rachilla. Normally the panicle breaks off during threshing; however a small part of the pedicel frequently remains attached to the grain. The husk is formed mostly of cellulose and fibrous tissue and is covered with very hard glass-like spines or trichomes. The present of this make the husk abrasive and very hard thus, they give the grain a good protection against insects, microorganism, moisture and gases. The caloric value to the hulls is rather high and ranges from 3,000 to 3,500 kcal/kg making hulls an important source of energy in agriculture.

However, the most disturbing presence in rice hull is high proportion of silica which causes considerable damage to processing equipment through excessive wear of machine parts and interconnecting transfer facilities.

Pericap

When the hull is removed, a thin fibrous layer can be seen. This is called the pericarp, frequently known as the "silver skin". The layer is usually translucent or grayish in color. When the pericarp is not translucent, but reddish in color the gain is referred to as red rice. It is considered as an integral part of the brown rice kernel (caryopsis) but is easily removed in the whitening process. The main function of this layer is to serve as an additional protective layer against molds and quality deterioration through oxidation and enzymes due to the movements of oxygen, carbon dioxide and water vapor. The pericarp actually consists or 3 layers namely epicarp, mesocarp & cross layer. Immediately under the pericarp layer is the testa or sometimes called tegmen layer which is only a few cells in thickness but with less fibrous than the pericrap layer. This layer is rich in oil and protein but its starch content is very low. Sometimes this layer is considered as part of the seed coat but because of its oil content, it is normally considered as the outermost layer of the bran.

Bran

Immediately under the testa or tegmen layer is the bran layer or aleurone layer. This part is the main constituent removed in the whitening stage during milling. It has very low starch content but has a high percentage of fiber, oil, protein, vitamins and minerals. Because of its high oil content, the bran is easily affected by oxidation when the oxygen in the air comes in contact with oil (36). In the milling process, the higher milling degree indicates a greater percentage of bran removed. The degree of milling as determined by the quantity of the outer layer removed from the brown rice kernels. When rice is fully milled the vitamins (a complex), protein, mineral, and oil contents are lessened. This explains why persons with beriberi (Vitamin B1 deficiency) are advised to eat brown rice. This also probably explains why persons who eat well milled rice are prone to be protein deficient or even malnourished. Thus, it is not surprising that some dieticians recommend the eating of regularly milled or even under milled rice. Rice bran in the production of extracted cereal flour, bran and germ are separated from endosperm during the milling process. Rice bran is also a good source

of dietary fiber because the dietary fiber content is about 30 % and protein content is 17-21 %. Rice bran can absorbed either water or fat up to four times of its own weight. Furthermore, defatted rice bran can be produces to give bland flavor and excellent stability. Rice bran powder is also a source of antioxidant, namely gamma oryzanol which benefits in lowering blood cholesterol (37), and decreases the incident of atherosclerosis disease and had laxation time (38). In addition, Chunchomboon, 2007 (39) demonstrated that rice bran has the excellence sources of antioxidant activity and antioxidant contents such as anthocyanidins, polyphenol, beta-carotene and vitamin E while in 2007, Chatchawan et al., also indicated that the rice bran can be added to fried dough from rice flour for protecting or preventing the oxidative effects on fried dough during storage (40).

Embryo

The embryo is located at central bottom portion of the grain, where the grain has been attached to the panicle of the rice plant. This is the living organism in the grain which develops into a new plant. The embryo respires by taking in oxygen in the air, consumes food which comes from the starch in the grain itself while simultaneously releasing moisture and heat. This explains why grains during storage have the tendency to decrease in weight as a result of the loss in moisture and dry matter content in the endosperm. During milling, the embryo is removed resulting in an indented shape at one end of the milled rice grain.

Endosperm

When the husk, the pericarp, the bran and the embryo are removed, what remains is the endosperm. It mainly consists of starch with only a small concentration of protein and hardly any minerals, vitamins or oil. Because of its high percentage of carbohydrates, its energy value is high. In the central core of the grain the starchy cells are somewhat hexagonal in shape, but between the centers and outside they are elongated with the long walls radiating outwards from the center.

3.12 Major baking ingredients and their functions

Baking requires few ingredients, but each has a specific use. For this reason, it is important to understand the function of each ingredient.

Wheat flour (41)

Flour, which is milled from wheat or other grains, is the basic ingredient in the production of bakery goods. There are various types, mill brands, and qualities of flour. Wheat flour is unique among cereal products in that it can be made into cohesive elastic dough when it is mixed with water under appropriate conditions. Because of their physical characteristics, this dough will retain leaving gases throughout the various handing procedures necessary for making bread and rolls, and they can be made to yield finished products of low density with fine, uniform cell structure and a soft resilient response to chewing.

Hard flour is also called strong flour or bread flour. It contains a high protein content about 13-14%. It is heavy and creamy in color. It is milled from hard red winter or hard red spring wheat. Hard flour has high gluten content that has the ability to retain gas very well, resist mixing and stretch during yeast fermentation and baking in bread making.

Soft flour has low protein content about 8.5%. It is clear white in color and velvety to the touch. It sticks together when squeezed in the hand. It is milled from soft red spring wheat. This flour has low gluten content which has no ability to retain gas. The products made from it will be tendered with a delicate texture. Since it is used for cake, it is also called cake flour.

Mixed flour can be prepared by mixing soft and hard wheat flour in different proportions. All-purpose flour is an example of mixed flour. This flour has protein content about 10.5-11.0%. It can be used for making all types of bakery products especially cookies.

Liquids

All the baked products contain a liquid. Water or milk is the usual liquids used in baking. Water gives a different texture to baked items. It is important because it can bind protein in the flour and form the gluten. Water may be hard or soft, depending upon the amount of minerals it contains. Very soft water weakens the gluten stands, causing them to collapse before the dough has risen to its full height. Professional bakers add some minerals to the water if the bakery is in an area with very soft water.

Milk products are important in baking. These products include liquid whole and skim milk, buttermilk, and dry milk solids. Besides adding nutritional value, milk assists gluten formation and gives a fiber, more velvety again. It also adds flavor and color as well as helps the product stay fresh longer (41).

Egg

Eggs perform several important functions. Eggs are essential because they maintain structure as well as aid in leaving and emulsifying, help shorten the product as well as add color and food value. Eggs are separated into 2 parts. One is egg yolk that is high in fat content and contain and important emulsifier, lecithin. Egg yolk is used for making cream and increasing the volume of products. The other is egg white that is involved in coagulation when contacting with heat and rapid beating (42).

Salt

Salt is needed in baked products and desserts to improve the flavor. To most people, food without salt is flat and uninteresting. Salt is also essential in producing a satisfactory yeast product since it helps regulate the activity of the yeast and strengthen gluten structure (42).

Shortening

Shortening is another word for the fat used in baking. Shortening is important because it affects the finished product in so many ways. It gives a tendered and lighter product, which rises evenly and has a smoother shape. It can also provide soft, tender, flakes in pastry-type products. This group of ingredients range from butter, margarine, lard and other shortening fats and oils (43, 44).

Sugar

Sugar is used in baking because of its sweet taste and other function including gluten tenderizing, creaming, texture, and color improvement. It also helps baked products stay fresh longer. There are several kinds of sugar. Each has a specific use. Fine graduated sugar is selected to use more than the other because it dissolves faster. Brown sugar is often called “soft sugar” because it is very moist compared to the granulated kind. Its color may vary from brown to dark brown (42).

Leavening agent

Leavening is the production or incorporation of gases in a baked product to increase volume and to produce shape and texture. These gases must be retained in

the Milk products are important in baking. These products include liquid whole and skim milk, buttermilk, and dry milk solids. Besides adding nutritional value, milk assists gluten formation and gives a fiber, more velvety again. It also adds flavor and color as well as helps the product stay fresh longer (45, 46).

Emulsifiers

Emulsifiers are surface-active agents promoting the formation and stabilization of emulsion. The products play many important roles in cookies and cakes formation. E.C.25K is one of the emulsifiers that are used for butter-type cake. It is a mixture of propylene glycol ester of fatty acid, vegetable fats, mono- and diglycerides. Its properties are assisting in combing liquid and fat, increasing the volume of cake, giving fine and light textures. Helping the cakes stay fresh longer and providing the moistened textures. The recommended amount of use is 12-15% of fat weight in the formation (45).

Gluten

The principal functional protein of wheat flour is gluten. Gluten has the important property that when it is moisture and worked by mechanical action, in form an elastic dough. It does this by forming linkages between protein molecules. These linkages form a three dimensional structure which provides strength to the dough. Gluten can be isolated from wheat flour by making dough with water then slowly manipulating this dough under a continuous stream of water.

Whole cereal flour

In the composition of whole cereal grains consists of bran, endosperm, and germ components. Whole meal flour can be making from wheat, rye, maize, rice, Barley, oat, and other cereal grains. One of the most common methods increasing food dietary fiber is to substitute whole wheat flour instead of highly extracted flour (46).

Sorbitol

D-Glucitol is known also as sorbitol, a sugar alcohol, present in many fruits especially in cherries and pears and in some fermented beverages such as cider (5-9 g/l). It is produced industrially mainly by hydrogenation of glucose derived from starch and from inverted sugar (sucrose). It is available in both liquid and crystalline form (48). The large quantities used in food manufacturing are produced by the

hydrogenation of glucose. Sorbitol is used as a sweetener in many types of products. Sorbitol is very stable, can withstand high temperature and does not participate in maillard (browning) reactions. The monosaccharide alcohols are slowly absorbed by facilitated diffusion. Compared with sucrose, their absorption is relatively low.

CHAPTER IV
MATERIALS AND METHODS

Experimental design

The study design was divided into five parts as following the objectives:

1. The first part of the present work was aimed to standardize the *in vitro* RAG method (RAG and SAG value) for evaluating the glucose release rate after enzyme digestion in various foods varieties, particularly of sugar alcohols, syrup, guar gum, fruits and other CHO foods.
2. The second aim was to determine and screen the amounts of RAG and SAG contents of various varieties of non glutinous and glutinous rice by using *in vitro* assay and to determine AC and RS in these rice.
3. The third purpose was to measure the amount of RAG, SAG, TG, FG, RDS, SDS, TS, SDI and RS contents of the selected sixteen rice varieties model shown low, medium and high RAG values and also was to evaluate the correlation coefficient between RAG, RDS, RS and SDI by using Pearson correlation.
4. The fourth purpose was to formulate the modified snack products made from rice flour and/or rice bran, red bean and black sesame and was also to evaluate on the RAG, SAG, TG, FG, RDS, SDS, TS, SDI and RS contents and to evaluate the correlation coefficient between RAG, RDS, TG, TS, RS and SDI by using Pearson correlation.
5. The lastly purpose was to evaluate the effect of storage model of a modified product during 3 months in defreeze refrigerator at -20ºC on the release of RAG and SAG rate and sensory acceptability.

4.1 Sample collection of various rice varieties

Rice samples; glutinous and non-glutinous rice (*Oryza sativa* L.) were

obtained from Center of Excellence for Rice Molecular Breeding and Product Development at Kasetsart University (KU), Kamphangsaen, Nakhon-Pathom 73140, Thailand and from local market and supermarkets in Thailand as the list names of various rice varieties in **Table 4.1** and **4.2**. The rice varieties (code-number K) were crossed breeding from wild rice *O.nivara* × Jao Hon Nin (BT) and the rice with code number KD were bred from KDML 105 (Khao Dawk Mali 105) × BT.

Table 4.1 List name of various rice varieties: KU

Pedigree	
***O. nivara* × BT**	
K291	K367
K310	K368
K334	K370
K337	K373
K342	K379
K349	K383
K363	K384
23-213 (Pin Kaset)	KD-BT 1000-11-1-24
Black glutinous KDC49-6-6-1-1-2	KD-BT 11-4-1-16
White glutinous KDC18-3-7-1-11-0	KD-BT 282-3-2-2
BT#1	KD-BT 313-19-1-1
BW1	KD-BT 909-10-1-1
BW3	KD-BT 909-21-1-39
Hom Pathumthani	KD-BT 909-21-2-3
IR71501 × Pin Kaset 3-1-14-20-2	Khao Dok Mali 105
IR71501 × Pin Kaset 3-1-22-6-1	*O. nivara*-BT #299
IR71501 × Pin Kaset 34-1-22-1-2	*O. nivara*-BT #91
IR71501 × Pin Kaset 34-1-3-1-2	Pin Kaset
Jao Hom Nin	

Table 4.2 List name of rice varieties from local market: Salaya market and supermarkets in Bangkok

Pedigree	
Benjakrayatip	Hom Mali Thung kula Amnatcharoen
Hom Mali Arawan	Hom Mali Thung kula Roi Et
Hom Mali Buriram	Hom Mali Thung kula Yasothon
Hom Mali Chiangrai (Middle)	Hom Mali Yasothon (old)
Hom Mali Chiangrai (new)	Khao Kaset (old)
Hom Mali Chiangrai (old)	Khao Tah Haeng (old)
Hom Mali Khemmarat	Leuang Pratew (Middle)
Hom Mali Nakhonratchasima	Leuang-orn (old)
Hom Mali Pathumthani (Middle)	Sang Yod
Hom Mali Sichompoo	Sao Hai 3 part (old)
Hom Mali Surin	Sao Hai Jekchei (old)
Hom Mali Surin (Middle)	Sao Hai Saraburi (Middle)
Hom Mali Surin (Middle)	Sao Hai Saraburi (old)
Hom Mali Thung kula (Middle)	Sung Yod Pattalung
Hom Mali Thung kula (new)	

4.2 Sample preparation for rice samples

4.2.1 Preparation of raw rice sample

The seeds were cleaned by hand to remove adhering dirt, damaged seeds and foreign materials. All rice grain samples were kept at room temperature. Raw seeds were ground in an electric coffee grinder (Princess model 2199). The powder samples were kept in polyethylene bag and stored at room temperature prior to moisture and amylose analysis.

4.2.2 Preparation of cooked rice sample

The rice samples were selected to evaluate RAG, SAG, TG and FG. All rice grain samples were commonly cooked as traditional consumption. Rice were prepared by using 50 ml centrifuge tube and water bath (Memmert). Before cooking, rice (1 portion) was washed with deionized (DI) water for 1 time and added with DI

water about 2 portions and then the washed rice was cooked using boiling water bath (Memmert) at 100 °C for 50 min and let cool at room temperature. The cooked rice sample was homogenized with Ultraturrax T25 (JANEE & KUNKEL: IKA-Labortechnik) before weighing for analyzing *in vitro* CHO digestibility.

4.3 Chemical analysis of rice samples

Rice samples were determined for moisture, amylose, RAG, SAG, TG and FG contents.

4.3.1 Percent moisture content

Moisture content was evaluated by drying the sample in a hot-air oven at 100 ± 5 °C until constant weight was obtained (AOAC, 2000). All analyses were done in duplicate.

4.3.2 Percent amylose content (AC)

AC was determined by iodine colorimetry base on the procedure of Juliano, 1971 and 1985 (49, 50)**.** All analyses were done in duplicate. Amylose was extracted from 100 mg of raw rice with 1 ml 95% ethanol and 9 ml 1 M NaOH. The samples were then heated at 100 °C in boiling water bath for 10 min, let them cool and then make up to 100 ml by deionized water and stand for overnight. Percent amylose was measured at wavelength 620 nm by Spectrophotometer (Spectronic Unicam, Unicam Helios Alpha & Beta). Standard solution from potato amylose (Amylose type III # A-0512: from potato, Sigma-aldrich) was used to prepare the standard curve. As seen in **Appendix A**.

4.3.3 Measurement for different types of starch of Thai rice varieties

Digestibility of starchy food was determined by using *in vitro* CHO digestible method modified from Englyst et al., 1992 (6), as seen in **Appendix B**. This method was based on measurement of the amount of glucose released at 20 min (RAG) and during 20-120 min (SAG) from a test meal by incubation with digestive enzymes. The amount of glucose when released at different time was used as an indicator of the CHO digestibility. The RAG and SAG value of each cooked rice sample and rice product were done in duplicates.

Enzyme and chemicals for *in vitro* digestion

The enzymes and chemical reagent that used for *in vitro* digestion included:

(i) Pepsin from porcine stomach mucosa (P-7000) from Sigma Chemical Co. St. Louis. Mo 63178 USA

(ii) Pancreatin from porcine pancreas (P-1750) from Sigma Chemical Co. St. Louis. Mo 63178 USA

(iii) Amyloglucosidase (AMG) from *Aspergillus niger* (A-7095) from Sigma Chemical Co. St. Louis. Mo 63178 USA

(iv) Invertase from Baker yeast (I-9274) from Sigma Chemical Co. St. Louis. Mo 63178 USA

(v) Glucoseoxidase peroxidase (GOD-PAP) (G-3660) from Sigma Chemical Co. St. Louis. Mo 63178 USA

(vi) Guar Gum (G-9752) from Sigma Chemical Co. St. Louis. Mo 63178 USA

(vii) D-glucose (Dextrose) from M & B chemical

4.3.3.1 *In vitro* measurement of RAG and SAG

In vitro measurement for the amount of RAG and SAG of all the samples in this study was modified from the method of Englyst et al., 1992 (6). Briefly, the samples were measured after incubation with invertase enzyme to hydrolyse sucrose, pancreatic α-amylase and AMG at 37 °C in capped tubes immersed horizontally in the shaking water bath (Memmert: SV-1422, Germany) with a speed calibrate to yield predetermined values for a reference material (white bread). All foods samples were homogenized with Ultraturrax T25 (JANKE & KUNKEL, IKA-Labortechnik). The sample weight required for analysis depend on the amount of CHO content of the test sample, which should be less than 0.3 g of CHO (3). The homogenized samples were immediately weighted between 0.2 and 2.0 g, depending on the amount of CHO in each sample into 50 ml screw-cap tube. The mixture pepsin solution was added in each tube and incubated at 37 ºC for 30 min. After that, 0.1 M sodium acetate buffer, five glass balls (1.5 cm diameter) and enzyme mixtures were added in each test tube and incubated with shaking water bath (horizontal) 37 ºC for 20 min. Then, 200 µl of hydrolysated sample was taken to the test tube containing 4 ml of 66% of ethanol

after that the sample tube was immediately placed into the shacking water-bath for further 100 min incubation. After a 120 min period, the hydrolysated sample was removed again for 200 µl. The hydrolysated sample taken at 20 min is designed as G20 (RAG; G20 used as indicator of glucose released from food digested with pepsin-and enzymes mixture after 20 min) while the sample at 120 min is designed as G120 (SAG: G120-G20 used as indicator of glucose released after further 100 min incubation). The hydrolysated samples at 20 and 120 min were diluted with deionized water (DI) and centrifuge at 1500 g for 5 min in order to remove the precipitate part**.** The 60 µl of diluted solution was taken and added into 96-well plate and the glucose-oxidase peroxidase kit (GOD-PAP) was immediately added into each well, then incubated in a water bath at 37 ºC for 30 min. After that, 6 M sulfuric acid was added to each sample and the samples were measured at 540 nm by UV/VIS spectrophotometer (TECAN: sunrise remote control microplate reader). As seen in **Appendix B**.

4.3.3.2 Measurement of TG

TG in each sample was measured as the method modified from Englyst et al., 1992 (6). It was obtained by gelatinization of the starch in the hydrolysated sample solution after 120 min (G120) in boiling water bath and treated with 2 M KOH at 0 ºC in ice-bath containing to disperse any retrograded amylose, followed by complete enzyme hydrolysis with AMG (50 AGU/ml). As seen in **Appendix B**.

4.3.3.3 Measurement of FG

0.1 M acetate buffer was added into each sample tube, and the sample was shaked or vortex-mixed vigorously to begin disrupting large particles. The tube was placed into a boiling water bath at 100 °C for 30 min, shaking occasionally to prevent aggregation. The tubes was removed and shaken vigorously again in shaking water bath at 37 °C. When equilibrated, added invertase, capped, and immersed horizontally in the shaking water-bath at 37 °C securing firmly. Incubated, with shaking, for 30 min. Took the tubes out of the bath and shaken vigorously. Removed one portion into a test tube containing ethanol and vortex-mixed (At this stage, continued with the main sample tube and returned to the FG portions when convenient). Diluted the

samples with DI and centrifuged at 1500 g for 5 min in order to remove the precipitation part. The 60 µl of diluted solution was then added into 96-well plate. Then the GOD-PAP was added to each well of sample, then incubated using water bath at 37 ºC for 30 min. After 30 min, added 6 M sulfuric acid to each sample and read at 540 nm by UV/VIS spectrophotometer (TECAN: sunrise remote control microplate reader). As seen in **Appendix C**.

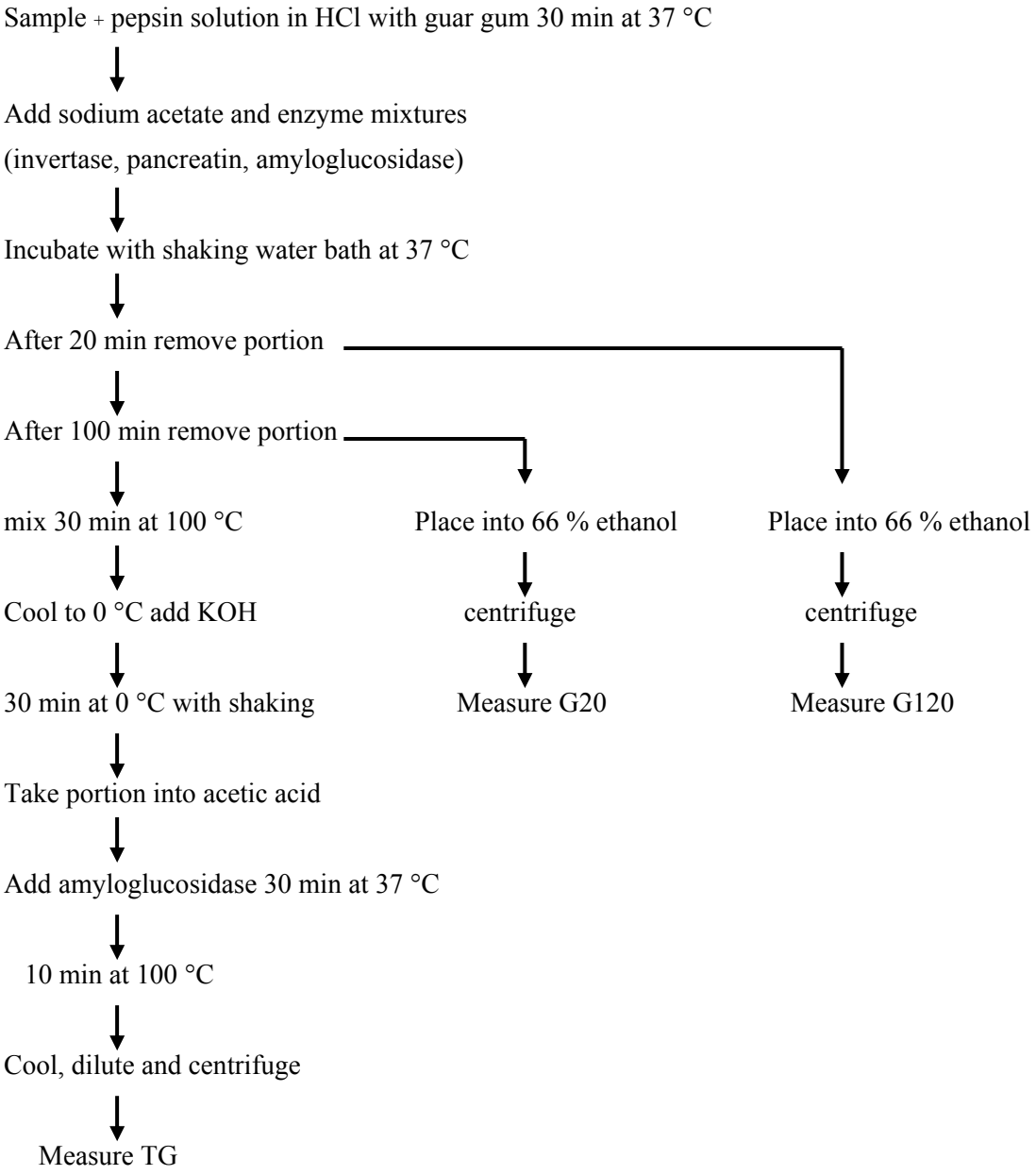

Figure 4.1 Procedure for measurement of RAG, SAG and TG contents in foods

4.3.3.4 Value of RAG, SAG, TG, FG, RDS, SDS, TS and SDI

Values for RAG, SAG, TG, FG, RDS, SDS, TS and SDI were calculated as the following formula which developed by Englyst et al., 1992 (5). As seen in the **Figure 4.1**.

RAG, SAG TG and FG were calculated as follow:

$$\% \text{ glucose} = \frac{At \times Vt \times C \times D}{At \times Wt} \times 100$$

- At = absorbance of test solution
- Vt = total volume of test solution
- C = concentration (in mg/ml glucose) of standard
- As = absorbance of standard
- Wt = weight (in mg) of sample taken for analysis, which may be corrected for moisture
- D = dilution factor

Calculation as content

RAG = G20

SAG = G120 – G20

RDS = (RAG – FG) × 0.9

SDS = SAG × 0.9

TS = (TG – FG) × 0.9

The 0.9 is a factor for correcting the weight of glucose to the weight of starch while compensating for the loss of one water molecule for every linkage of glucose (51).

SDI value represents the rate of starch digestion in food with the equal amount of TS content.

$$SDI = \frac{RDS}{TS} \times 100$$

4.3.3.5 Measurement of RS

RS in rice was analyzed by using a RS kit from Megazyme code K-RSTAR (Megazyme International Ireland Ltd, Ireland). In briefly, approximate about 100 mg of group rice sample were added pancreatic α-amylase containing 3 U/ml AMG to each tube. Tightly cap the tubes, mixed them on a vortex mixer and shaking horizontally in a shaking water bath at 37 °C 200 strokes/min for exactly 16 hr. The tube caps were removed and treated the contents with 4.0 ml of 99 % ethanol and mixed with a vortex. Centrifuged the tubes at 1,500 g 10 min (non-capped) and carefully decant supernatants and re-suspend the pellets in 2 ml of 50 % ethanol with a vortex mixer. Added 6 ml 50 % IMS (95% ethanol plus 5% methanol) mixed the tube and centrifuged again at 1,500 g for 10 min. Decant the supernatants were repeated this suspension and centrifugation step once more. The tubes were inverted on absorbent paper to drain excess liquid. Added a magnetic stirrer bar and 2 ml of 2 M KOH to each tube and re-suspended the pellets by stirring for approx. 20 min in an ice water bath over a magnetic stirrer. Added 8 ml of 1.2 M sodium acetate buffer (pH 3.8) to each tube with magnetic stirrer (Do not mix on vortex mixer and ensure vigorously with KOH). Immediately added 0.1 ml of AMG (3,300 U/ml) mixed well and place the tubes in a water bath at 50 °C. Incubate the tube for 30 min with intermittent mixing on a vortex mixer. Transfer 0.1 ml aliquots of duplicate sample solutions [(blank containing of 0.1 ml 0.1 M sodium acetate buffer pH 4.5 and 0.1 ml of standard glucose (Conc.1mg/ml)] and added 3.0 ml of GOPOD reagent and incubate at 50 °C for 20 min. Measured the absorbance of each solution at 510 nm against the reagent blank and standard. (As seen in **Appendix D**)

4.4 Quality Control (QC)

In-house control materials as ground dried white bread was used for the QC of analysis data. Homogenized sample white bread was prepared as an in-house control sample for RAG, SAG, FG and TG contents. It was kept in room temperature. Soybean flour was used as QC sample for protein, fat and ash determination in this study. Rice powder was used as QC sample of moisture and AC. The assigned value of each in-house QC was developed from 10 values of each content analysis on the different days. QC chart maintained the quality of analysis. The in house QC sample

was analyzed for RAG, SAG and TG in each set of unknown sample. The accepted value was within mean ± 2SD.

4.5 Sample collection for snack products

4.5.1 Preliminary Baking Trial

This trial aimed to determine the control formula for snack products namely, steamed bun, bread stick, red bean filled bun and bread. The control formulas of this study were selected from various conventional recipes of the cookbooks. Some recipes required appropriate adjustments prior to the production of final products. The recipe which gave the product with best characteristics i.e. texture, taste, color; was chosen as the final control formula. Such control formula was used as a reference, comparing with the modified snack products made from the rice flour and/or rice bran, boiled red bean and roasted sesame.

4.5.2 Preparation of rice flour from brown rice strain (313-19-1-1) and pigmented rice bran

Rice flour

In this study, rice strain (313-19-1-1) in the form of rice flour was used as the main raw material for the low GI modified products. Rice flour was used as an ingredient in four types of streamed bun, bread stick, filled bun and bread. It was ground into powder form in a miller attacked with a 0.08 mm (100-120 mesh) before mixing into the batter.

Rice bran

Pigmented rice bran was prepared from rice strain (rice berry variety), due to a good source of dietary fiber. The pigmented rice was eradicated dirtiness by panning and then ground with blender (Cucina HR 1791/6, PHILIPS)

Baking ingredients

The raw materials used for baking were all food-grade and purchased from local supermarkets and bakery stores in Bangkok area. The list of baking ingredients and the suppliers are shown in the sample preparation part.

4.6 Formulation of low RAG modified products

To modify low RAG products, a preliminary trial was carried out using rice flour (313-19-1-1) as a slowly CHO digestible ingredient. Rice flour was mixed into the modified products dough before cooking. The ratio of rice flour substituted for wheat flour was increased until unacceptability of pilot screening of 10 panelists by interviewing was indicated.

The particle size of rice flour after grounding in a miller attracted with a 100-120 mesh screen (0.08 mm.) are shown in the **Figure 4.2**.

Figure 4.2 Rice flour from 313-19-1-1 rice grain

4.7 Snack products preparation

For modified snack products, rice flour was used in order to replace some amount of wheat flour which used to prepare control formulas. There is no recommendation for percent replacement of rice flour in the snack products. Therefore, the percent replacement of rice flour in this study was different depending on the kinds of products and sensory acceptability. Additionally, to create and improve good characteristics and nutritious quality of the snack products, rice bran, boiled red bean and roasted black sesame were used as part of the ingredients to increase the amount of antioxidant and fiber contents in the products.

a) Steamed bun preparation

First, dry yeast and sugar were mixed warm water until they were dissolved. Wheat and rice flour was mixed and sifted. Dry ingredients were weighted and mixed at speed 1 by Kitchen Aid Mixer (Model 5kLNK530, USA). After that, wet ingredients such as water and sorbitol were slowly added and mixed in Kitchen Aid mixer from speed 1 to speed 2. The feel of the dough and its ability to form film on stretching and shear with regularity was judged as the optimum mixing time. The dough was fermented at 30 °C for 60 minutes. The dough was then punched down, sheeted with a rolling pin and molded by hands and placed on paper and then keeping of uniform size and rounded ends and proof for another 15-20 minutes. Steaming was performed with high heat for 15 minutes in steam pot. The recipe was tabulated in **Table 4.3**.

Table 4.3 The ingredients of steamed bun formula

Ingredient (g)	**Control**	**Modified**
Wheat flour (UFM Food Center Co., Ltd.)	50.0	14.56
Rice flour	-	33.98
Sugar (Mitr Phol®, Mitr Phol Sugar Co., Ltd.)	9.36	6.07
Salt (Prung Thip®, Nakhonratchasima)	0.12	0.16
Shortening (Olympic Kream®, Kedwanich Co., Ltd.)	3.74	4.85
Baking powder (Best Food®, CPC/Aji Co., Ltd.)	0.38	0.49
Yeast (Furmipan Co., Ltd.)	0.48	0.62
Wheat gluten	-	2.33
Rice bran	-	1.75
Water	14.97	29.13
Sorbitol	-	6.07

b) Bread sticks preparation

First, dry yeast and sugar were mixed in warm water until they were dissolved. Wheat and rice flour was mixed and sifted. Dry ingredients were weighted and mixed at speed 1 by Kitchen Aid Mixer (Model 5kLNK530, USA). After that wet ingredient as water was slowly mixed, and the speed of mixer was increased from speed 1 to speed 2. The feel of the dough and its ability to form film on stretching and shear with regularity was judged as the optimum mixing time. The dough was fermented at 30 °C for 60 minutes. The dough was then punched down, sheeted with a rolled pin and molded. To shape sticks, first shaped as small biscuits, rolled on board (where there is no flour) with hands until 6 inches in length, keeping of uniform size and rounded ends and proof for another 15-20 minutes. Baking was performed at 180 °C for 25 minutes. After baking, took the bread sticks out and placed on a slightly buttered surface into approximately. The recipe was tabulated in **Table 4.4**.

Table 4.4 The ingredients and the suppliers of bread stick formula

Ingredient (g)	Control	Modified
Wheat flour (UFM Food Center Co., Ltd.)	54.58	24.56
Rice flour	-	24.56
Sugar (Mitr Phol®, Mitr Phol Sugar Co., Ltd.)	1.97	1.75
Salt (Prung Thip®, Nakhonratchasima)	0.93	0.83
Shortening (Olympic Kream®, Kedwanich Co., Ltd.)	4.63	4.16
Yeast (Furmipan Co., Ltd.)	0.93	0.83
Boiled Red bean (Suvitcharoen®, Suvitcharoen Co., Ltd)	-	10.41
Roasted sesame (Raitip®, Raitunya Co., Ltd)	-	1.67
Water	37.00	31.22

c) Bread preparation

First, dry yeast and sugar were mixed in warm water until they were dissolved. Wheat and rice flours were mixed and sifted with wheat gluten powder. Dry ingredients were weighted and mixed at speed 1 by Kitchen Aid Mixer (Model 5kLNK530, USA). After that wet ingredients as water and sorbitol were slowly added and mixed in Kitchen Aid mixer from speed 1 to speed 2. The feel of the dough and its ability to form film on stretching and shear with regularity was judged as the optimum mixing time. The dough was fermented at 30 °C for 60 minutes. The dough was then punched down, sheeted with a rolling pin, molded by hands and placed in greased loaf pan (3.25 × 6 × 3.25 inch) for proofing 45 minute and then keeping of uniform size and rounded ends and proof for another 20-25 minutes.

Baking was performed at 180 °C 30 minutes. After baking, the bread loaf was let cool on the rack prior to slicing. The recipe was tabulated in **Table 4.5**.

Table 4.5 The ingredients and the suppliers of bread formula

Ingredient (g)	**Control**	**Modified**
Wheat flour (UFM Food Center Co., Ltd.)	53.22	22.53
Rice flour	-	17.75
Rice bran	-	2.24
Sugar (Mitr Phol®, Mitr Phol Sugar Co., Ltd.)	1.66	1.50
Salt (Prung Thip®, Nakhonratchasima)	0.67	0.60
Shortening (Olympic Kream®, Kedwanich Co., Ltd.)	5.24	4.70
Yeast (Furmipan Co., Ltd.)	0.93	0.84
Boiled ed red bean (Suvitcharoen®, Suvitcharoen Co., Ltd)	-	16.4
Roasted sesame (Raitip®, Raitunya Co., Ltd)	3.33	4.48
Water	34.43	25.35
UFM 505 (UFM Food Center Co., Ltd.)	0.53	0.47
Sorbitol	-	1.34
Wheat gluten	-	1.80

d) Red bean filled bun preparation

Dough

Dry yeast and sugar were mixed in warm water until they were dissolved. Wheat and rice flour were sifted with wheat gluten powder and UFM 505 (Improver). Dry ingredients were weighed and mixed at speed 1 by Kitchen Aid Mixer (Model 5kLNK530, USA). After that wet ingredients as water, egg and sorbitol were slowly added and mixed in Kitchen Aid mixer from speed 1 to speed 2. The feel of the dough and its ability to form film on stretching and shear with regularity was judged as the optimum mixing time. The dough was fermented at 30 °C for 60 minutes. The dough was then punched down, sheeted with a rolling pin, molded by hands and placed on dish.

Filling

The boiled red beans and rice bran portions were mixed and milled by home-use electric blender (Cucina HR 1791/6, Philipine). After that, mixed again in a brass pan with slightly heat until dry. Filling was molded by hands and weighted in each piece. In addition, sugar was substituted by sorbitol for reducing sugar content both in dough and filling. The recipe was tabulated **Table 4.6**.

Table 4.6 The ingredients and the suppliers of red bean filled bun formula

Ingredient (g)	**Control**	**Modified**
Dough		
Wheat flour (UFM Food Center Co., Ltd.)	11.3	7.69
Rice flour	-	14.3
Sugar (Mitr Phol®, Mitr Phol Sugar Co., Ltd.)	5.2	0.5
Salt (Prung Thip®, Nakhonratchasima)	0.5	2.3
Butter (Orchid®, The Thai Dairy Industry Co., Ltd.)	2.6	0.3
Yeast (Furmipan Co., Ltd.)	0.3	0.2
Egg	6.9	4.6
Water	8.7	5.2
UFM 505 (UFM Food Center Co., Ltd.)	0.3	0.2
Sorbitol	-	1.7
Wheat gluten	-	0.7

Table 4.6 The ingredients and the suppliers of red bean filled bun formula **(continued)**

Ingredient (g)	**Control**	**Modified**
Filled		
Sorbitol	-	10.4
Sugar (Mitr Phol®, Mitr Phol Sugar Co., Ltd.)	8.7	-
Salt (Prung Thip®, Nakhonratchasima)	0.1	0.1
Boiled red bean (Suvitcharoen®, Suvitcharoen Co., Ltd)	13.9	9.3
Rice bran	-	4.6
Water	41.6	20.9

4.8 Sensory Evaluation for Modified Healthy Products

The sensory evaluation questionnaires were used to evaluate the modified products after subject tasted.

Criteria

1. Consumer's acceptability before and after testing were tested by using 9 point hedonic scale.
2. Color, physical appearance and texture were evaluated by using 5 point just about right.

The sensory evaluation was performed by 25 panelists who were staff members and graduate students of Institute of Nutrition, Mahidol University at Salaya, Nakhon-Pathom, Thailand. The evaluation was performed under a daylight fluorescent lamp in an individual testing booth at the Sensory Food Science Laboratory of the Institute of Nutrition. For development of each snack formula, the acceptability in general appearance was measured by a 9-point hedonic scale, ranging from 1 ("dislike very much") to 9 ("like very much"); suitability of color, taste and

texture was measured by a 5-point "just-about right" scale (5 much too dark/elastic/hard, 3= just about right, 1= much too light/brittle/soft) (52). All products were considered acceptable overall, if their mean average score was about 6.5 ("like slightly"). The samples were prepared one day in advance before sensory evaluation. The samples were packed in a polypropylene plastic bag and store at room temperature. They were served on white plastic plates labeled with three-digit number codes selected from a random number table. Two samples were served to each panelist during the test; one sample at a time. The order of presentation was randomized. Panelists were asked to rinse their mouth with water between samples. The questionnaires used for sensory evaluation appear as shown in **Appendix E**.

4.9 Preparation of snack products

Snack products were dried in hot air oven at 60 °C overnight or until dried and homogenized by home-use electric blender (Cucina HR 1791/6, Philipine) before weighing for analyzing by *in vitro* CHO digestibility.

4.10 Chemical analysis of snack products

4.10.1 Measurement of different types of starch in snack products

Digestibility of starchy food was determined by using *in vitro* CHO digestible method modified from Englyst et al., 1992 (6) as seen in **Appendix B**. Both control snacks and modified snack samples were analyzed for RAG and SAG in five analyses each, excepted TG and FG were done in three analyses.

4.10.2 Nutritive analysis

Snack products were determined on proximate analysis for moisture, protein (N × 6.25), fat, ash, CHO, and energy contents. The analytical procedures were performed in duplicates according to the AOAC, 2003 (920.151, 930.30).

Proximate analysis (As seen in **Appendix F** to **H**)

- Moisture content was evaluated by drying the sample in a hot-air oven at 100 ± 5 °C until constant weight was obtained.
- Crude protein was analyzed using Macro-Kjeldahl technique with a conversion factor 5.7 as to converse nitrogen to protein.

- Crude fat was determined by solvent extraction in a Soxhlet Apparatus (continuous extraction method using Soxhlet System HT6, Tecator, Sweden).
- Ash was determined by incinerating sample in a muffle furnace at 550 °C.
- CHO content, in this study, was calculated by subtracting the percentage of crude protein, fat, ash and moisture from 100.
- Energy contents were obtained by calculation of protein, CHO, and fat, with conversion factor 4 for protein and CHO, and 9 for fat, respectively.

4.11 The effect of freezing storage model on RAG and SAG

In this study, a snack product (steamed bun) was selected for evaluating the storage model within 3 months. The sample was kept in zip locked polypropylene bag and placed in a refrigerator at -20 ºC. The evaluation of RAG and SAG were performed every month as well as the sensory acceptability of the product on 0, 1 and 3 months.

4.12 Statistical Analysis

The screening RAG, SAG and AC were carried out in duplicate and all the results in present study were reported as average of duplicate analysis. Data of modified products and storage model were presented as mean ± standard deviation. Statistical analysis was performed with the used of Statistical Package for the Social Sciences (SPSS) for windows software, version 13 (SPSS Inc., Chicago, Illinois, USA). The significant difference among RAG and SAG in each type of rice and grounds of breeding were classified as low, medium and high levels by the Quartile deviation. The mean values of RAG and AC of type and source were detected significance different by Independent sample T-test or Man-Whitney U test. Correlation of RAG, SAG and their GL of fruits and their AC of Thai rice were analyzed by Pearson's correlation coefficient in bivariate correlation. Differences were considered significant if p-value ≤ 0.05. For part of sensory evaluation, mean values of the sensory acceptability scores of each snack product and steamed bun storage in freezing temperature were evaluated for significant difference at 5% level of probability by using the Independent sample T-test or one-way analysis of variance (One-Way ANOVA). When significant differences in One-Way ANOVA were

detected, means were compared using the Duncan's multiple comparison tests.

CHAPTER V
RESULTS

The present study was divided into five parts. The first part was to validate *in vitro* enzymatic CHO digestion RAG and SAG method whether they can be applied to estimate the GI or GL in various kinds of foods.

PART I

5.1 The amount of RAG and SAG values of the various varieties of CHO model

The amount of RAG and SAG values of various food models was reported as glucose releases in gram per 100g. **Table 5.1** gave the ranking of food model by the amount of RAG and SAG values. Lowest amount of RAG value was found in sugar alcohol (sorbitol) and guar gum with 0.35 and 0.70 g/100g for RAG and 0.39 and -0.31 g/100g for SAG, respectively. In addition, the accompaniment with decreasing in the RAG values in food model were followed by potato starch (9.78), mungbean noodle (9.71) and pigmented rice bran (4.52). Whereas high amounts of RAG values were found in mungbean starch (27.19) and black sticky rice boiled with sugar (Khaw Nieaw Dum Peuag: 15.90).

Table 5.1 RAG and SAG values of various samples[1]

Food model	RAG (g/100g)	SAG (g/100g)
Guar gum	0.70	- 0.31
Sorbitol	0.35	0.39
Pigmented rice bran	4.52	8.81
Mungbean noodle	9.71	0.10
Potato starch	9.78	22.83
Mungbean starch	27.19	36.99
White sticky rice boiled with sugar	10.94	1.29
Black sticky rice boiled with sugar	15.90	1.66

[1]Values are a mean of duplicate analysis. (g/100g wet weight)

5.2 RAG and SAG values of orange juices and sugar cane syrups

Results of RAG and SAG values of orange juices and sugar cane syrups are expressed as glucose in g/100g or in g/100ml, as seen in **Table 5.1** and in **Appendix J**. In fresh orange juice, 25% and 100% orange juices and various concentrations of sugar cane syrup, the amount of glucose release after enzyme digestion of CHO (RAG and SAG) of all the orange juices and sugar cane syrups ranged from 1.47 to 43.38 g/100g for RAG and -1.74 to 6.36 g/100g for SAG. The highest amount of RAG and SAG were found in sugar cane syrups especially in high concentration (50%).

Table 5.2 RAG and SAG values of orange juices and sugar cane syrups[1]

Food model	RAG (g/100g)	SAG (g/100g)
25 % Orange juice	3.77	1.83
100 % Orange juice	2.45	0.23
Fresh orange juice	1.47	0.71
12.5 % syrup	11.10	-1.74
25 % syrup	21.59	4.39
50 % syrup	43.38	6.36

[1]Values are a mean of duplicate analysis. (g/100g wet weight)

5.3 RAG and SAG values of various fruit varieties

The results of RAG and SAG of various fruit varieties are expressed as glucose release after enzyme digestion, as summarized in **Table 5.3**. Wide variation of RAG and SAG were found in fruits ranged from 1.03 to 8.44 and -0.02 to 9.51 g/100g, respectively. When the quartile deviation was used, the RAG and SAG of fruits were divided into three levels, particularly in high (> 6.10 g/100g for RAG and > 4.10 g /100g for SAG), medium (1.64 - 6.10 mg/100g for RAG and 0.18 - 4.10 mg/100g for SAG), and low level (< 1.64 mg/100g for RAG and < 0.18 mg/100g for SAG). **Table 5.3** showed the ranking of fruits by values of the RAG and SAG (g/100g) and from the lowest to highest values. Orange showed the lowest RAG value (1.03), followed by guava (1.41) and dragon fruit (1.64). Medium content of RAG values were found in ripe and unripe mango (2.15 and 2.15), longan (3.14), banana (3.42), ripe papaya (5.14) and pineapple (6.10). High RAG value was found in grape (8.44). Whilst, the SAG was used as indicator of glucose release after 120 min of enzyme digestion was found to be high in unripe mango (9.51), longan (4.87) and longan in syrup (4.10) when was compared to other fruits of this study.

Table 5.3 RAG, SAG and GL values of 10 fruit varieties[1]

Fruit model	RAG (g/100g)	SAG (g/100g)	GL (ref) per 100 g
Orange	1.03	0.41	3[2]
Guava	1.41	1.24	2[3]
Dragon fruit	1.64	-0.02	5[3]
Mango ripe	2.15	2.16	7[3]
Mango unripe	2.21	9.51	-
Longan	3.14	4.87	8[3]
Banana	3.42	2.41	10[2]
Papaya ripe	5.14	0.63	8[2]
Pineapple	6.10	0.18	7[2]
Longan can	6.24	4.10	-
Grape	8.44	0.16	9[2]

[1]Values are a mean of duplicate analysis. (g/100g wet weight)
[2]Result from Kaye Foster-Powell and colleagues was published in Am J Clin Nutr. 2002
[3]Calculated by using GI from the study of Somnuk (unpublished).

5.4 Correlation between the RAG and SAG values of various fruits model with GL from reference data

When comparing the rate of RAG, SAG and GL (reference data) values between various fruits, as seen in **Table 5.3**, the RAG and GL values had significant positive correlation with $r = 0.687$ at *p value* less than 0.05 whilst the SAG value gave no significant correlation ($r = 0.251$) with GL, as shown in **Figure 5.1** and **5.2**. However, the results of the correlation coefficient between RAG and SAG value of various fruits model, it revealed again that no significant correlation between RAG and SAG values was observed in the fruit model (**Figure 5.2**).

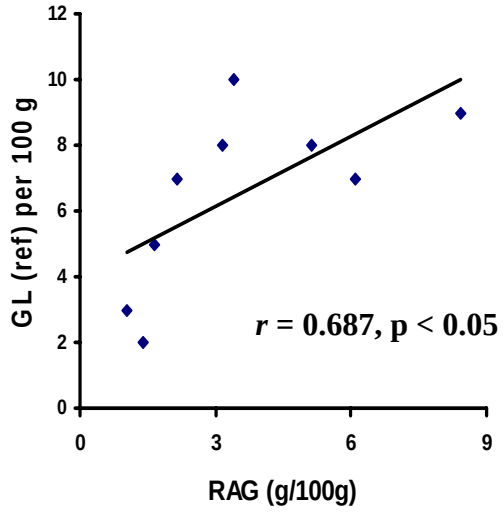

Figure 5.1 Pearson Correlation Coefficients between the RAG and GL values of fruit model

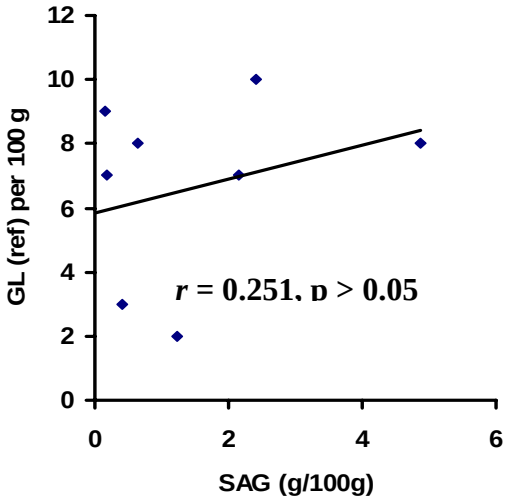

Figure 5.2 Pearson Correlation Coefficients between the SAG and GL values of fruit model

Analysis data from the first part indicated that high significant correlation between the amount of RAG and GL values was observed as seen in **Figure 5.1** (r = 0.687 $p < 0.01$). Moreover, results of RAG values in the selected fruits of present study also gave nearly close to the GL value obtained from Powell and Miller published in 2002 (53) and Somnuk (unpublished data) (54), as seen in **Table 5.3**. With the correlation between RAG and GL, the RAG values can be used as an

indicator for screening glycemic response in various foods and food products. While measuring of SAG no correlation with GL can help to predict the rate and extent of CHO digestion**.**

PART II

The second part was to screen and evaluate of low digestion CHO by *in vitro* RAG and SAG method in ninety four varieties of rice obtained from local markets in Thailand and new varieties breeding line from KU. AC was determined by iodine colorimetry method and RS content was measured by using RS kit.

5.5 RAG, SAG, RS and AC values of Thai rice varieties

The RAG, SAG and AC of 33 varieties of non glutinous and glutinous rice were given in **Table 5.4**, and 61 new rice varieties (breeding lines) produced by crossing wild rice *O.nivara* with Chao hom nin (BT), and Khaw Dokmali 105 (KDML 105) with BT were given in **Table 5.5**. Wide variation of the RAG, SAG and AC values ranged from 6.05 to 24.97 mg/100g, 0.34 to 14.66 mg/100g and 7.38 to 42.71%, respectively. The quartile deviation (in **Table 5.4** and **5.5**) was used to set the ranking amounts of RAG and SAG contents into three levels, particularly in low (< 14.01 g/100g and < 2.41 g/100g, respectively), medium (14.01 to 18.88 g/100g and 2.41 to 6.96 g/100g, respectively), and high level (> 18.88 g/100g and > 6.96 g/100g, respectively). Lowest amounts of RAG contents was found in Hom Mali Sichompoo (6.05), following by Hom Mali Thung kula Roi Et, Leuang-orn (old), Hom Mali Khemmarat, Hom Mali Chiangrai (middle) and Hom Mali Thung kula (Amnatcharoen), Sang Yod Patthalung, Hom Mali Thung kula Yasothon, Hom Mali Chiangrai (new), Hom Mali Nakhonratchasima and so on (6.05 – 14.08 g/100g), as seen in **Table 5.4**. The medium level (14.01-18.88%) were found in brown BT#3, K373, K363, BW3 and so on, as shown in **Table 5.4**. The greatest RAG values (19.07 - 21.42%) were found in Hom Mali Surin (old) and Hom Mali Burirum, respectively, as well as the lowest of SAG value was observed in these rice varieties. The highest SAG was found in Hom Mali Khemmarat (14.66%), followed by Hom Mali Thung kula Amnatcharoen, Hom Mali Thung kula (new), Hom Mali Thung kula Yasothon, Hom Mali Chiangrai (new) and Leuang-orn (old) ranging from 11.37 –

12.79%, while lowest SAG value was found in Khaw Benjakrayatip (0.49 %), as seen in **Table 5.4**. From the analysis data of RAG and SAG values in all the glutinous rice in this study, it was revealed that the greatest amount of RAG values as expected due to the characteristic of waxy rice; low amylose values (8.76 – 10.50%) and high in GI. AC of local rice varieties ranged from 15.72 to 41.55% for non-glutinous and 8.76 to 10.50% for glutinous rice while brown non-glutinous rice (Sang Yod variety) was the lowest (8.59 %). Leuang Pratew (middle), Sao Hai Jekchei (old) and Mixed Sao Hai 3 part (old, from three sources of area growth) were found to provide the highest amylose values (40.38- 41.55 %), followed by Sao Hai Saraburi (old and middle), Khao Kaset (old), Khao Tah Haeng (old) and Hom Mali Pathumthani (Middle) (32.99 – 39.30 %), as seen in **Table 5.4.**

Table 5.4 Ranking of RAG, SAG and AC values of twenty-nine varieties of non glutinous rice and four glutinous rice bought from local market and supermarkets in Thailand [1]

Name of rice variety	RAG (g/100g)	SAG (g/100g)	AC (%)
Milled Hom Mali Sichompoo	6.05	9.58	20.54
Milled Hom Mali Thung kula Roi Et	8.04	9.03	20.15
Milled Leuang-orn (old)	9.17	11.37	35.26
Milled Hom Mali Khemmarat	9.74	14.66	19.93
Milled Hom Mali Chiangrai (middle)	10.12	10.17	19.15
Milled Hom Mali Thung kula Amnatcharoen	11.40	12.79	18.61
Milled Sang Yod Patthalung	11.46	5.13	21.35
Milled Hom Mali Thung kula Yasothon	11.76	11.58	20.53
Milled Hom Mali Thung kula (new)	11.91	12.01	18.62
Milled Hom Mali Yasothon (old)	12.35	8.18	23.17
Milled Hom Mali Chiangrai (new)	12.48	11.52	17.94
Milled Khao Tah Haeng (old)	12.93	6.66	35.88
Milled Hom Mali Surin (middle)	13.38	9.11	25.82
Milled Sao Hai Saraburi (middle)	13.83	5.60	39.20
Milled Leuang Pratew (middle)	13.89	5.20	41.55
Milled Hom Mali Nakhonratchasima	14.08	7.37	25.58

[1]Values are a mean of duplicate analysis. (g/100g wet weight)

Table 5.4 Ranking of RAG, SAG and AC values of twenty-nine varieties of non glutinous rice and four glutinous rice bought from local market and supermarkets in Thailand [1] **(continued)**

Name of rice variety	RAG (g/100g)	SAG (g/100g)	AC (%)
Milled Benjakrayatip	14.05	0.49	15.72
Milled Hom Mali Arawan	14.69	5.28	23.82
Milled Sao Hai Jekchei (old)	14.96	5.84	41.19
Milled Sao Hai Saraburi (old)	15.05	4.17	39.30
Milled Khao Kaset (old)	15.31	6.93	36.44
Milled Hom Mali Pathumthani (Middle)	15.54	4.04	32.99
Milled Hom Mali Thung kula (Middle)	15.77	3.90	24.54
Milled Hom Mali Chiangrai (old)	15.93	9.02	24.31
Milled Sao Hai 3 part (old)	17.36	6.64	40.38
Brown Hom Mali Surin	17.42	0.83	23.23
Brown Sang Yod	18.05	6.89	8.59
Milled Hom Mali Surin (old)	19.07	2.11	26.27
Milled Hom Mali Buriram	21.42	0.80	24.74
Milled glutinous Maechun	23.76	3.74	8.76
Milled glutinous Sanpahtawng	24.86	0.56	10.50
Milled glutinous Maechun (old)	24.87	2.93	8.76
Milled glutinous RD 6	24.97	1.18	10.50

[1]Values are a mean of duplicate analysis. (g/100g wet weight)

Analysis data of RAG, SAG, RS and AC from the new breeding lines of various rice varieties obtained from Center of Excellence for Rice Molecular Breeding and Product Development at KU were given in **Table 5.5**. Low amounts of RAG were found in brown KD-BT 313-19-1-1 (harvest in dry season), following by brown K368, 310, 367, 334, and 349, brown KD-BT 313-19-1-1 (harvest in wet season), brown IR71501 × Pin Kaset 3-1-22-6-1 and brown rice K383 (6.13–14.37 g/100g). Medium RAG values were found in brown 23-215 (Pin Kaset) (KD × BT), brown KD-BT 1000-11-2-26, brown K367, brown KD-BT 909-21-1-41, brown IR71501 × Pin Kaset 3-1-14-20-0 (14.46 – 15.76 g/100g). It was noted that the amounts of RAG in the same brown rice variety 313-19-1-1 which harvested in dry season (6.13%) was lower than that harvested in wet season (13.53%), in addition, the SAG data also showed that SAG of brown rice 313-19-1-1 in dry season was higher (11.97%) than rice was harvested in wet season (7.77%). This result needs further research study to be able to explain why the same rice variety harvested in different seasons give different RAG and SAG values. In **Table 5.5**, AC ranged from 16.37% (milled *O.nivara*-BT #91) to 42.71% (IR71501 × Pin Kaset 34-1-22-1-0). Lowest amount of AC were ranged 7.38 to 9.30 % in all the glutinous rice. From the present study, it is surprising that white glutinous (brown) KDC18-3-7-1-11-0 (AC 8.52%) which crosses breeding from Hompama with Kum Doi Chang revealed low amounts of RAG (15.44 g/100g). In general, glutinous rice varieties contained high amounts of RAG and had high GI values (estimated GI 68-109.2) (75). The content of RS was low in all cooked rice varieties ranged from 0.07 – 2.16 g/100g for cooked brown rice and 0.02 – 1.14 g/100g for cooked polished rice varieties. Especially, there are two new breeding rice varieties, the brown and milled rice #34-1-22-1-0 and #34-1-3-1-0 and another one was a brown rice KD-BT11-4-1-18, gave the highest amount of RS. These new rice varieties showed not only had the greatest amount of RS (0.78 -2.16 g/100g) but also highest in the amount of AC, as seen in **Table 5.5**, whereas the RAG values in both varieties were in the intermediate and high level (15.63-18.92 g/100g), respectively.

Table 5.5 Ranking of RAG, SAG, RS and AC values of the new breeding lines on various rice varieties from KU

Name of rice variety	RAG (g/100g)	SAG (g/100g)	RS[4] (g/100g)	AC %
Brown KD-BT313-19-1-1 [1] (harvest in dry season)	6.13	11.97	NA	16.58
Brown K368 [2]	9.19	8.24	0.27	21.70
Brown K310 [2]	10.67	9.69	0.36	34.79
Brown K367 [2]	12.56	6.34	1.01	17.96
Brown K334 [2]	13.01	7.38	0.16	18.01
Brown K349 [2]	13.06	2.15	0.38	18.27
Brown KD-BT313-19-1-1 [1] (harvest in wet season)	13.53	7.77	0.17	17.59
Brown IR71501 × Pin Kaset3-1-22-6-1	13.65	9.40	0.20	25.15
Brown K383 [2]	14.37	5.79	0.13	23.87
Brown 23-215 (Pin Kaset)	14.46	8.71	0.33	19.23
Brown KD-BT1000-11-2-26 [1]	14.55	5.31	0.17	17.98
Brown KD-BT909-21-2-5 [1]	14.64	6.24	0.09	18.83
Brown BT#3 (Jao hom nin)	14.93	4.87	0.13	18.88
Milled KD-BT 313-19-1-1 [1] (harvest in wet season)	15.27	4.36	NA	19.51
Brown White glutinous KDC18-3-7-1-11-0 [3]	15.44	7.04	NA	8.52
Brown IR71501 × Pin Kaset34-1-22-1-0	15.63	8.11	1.13	40.20
Brown K373 [2]	15.71	3.60	0.36	35.69
Brown IR71501 × Pin Kaset3-1-14-20-0	15.76	4.29	0.19	23.74
Brown K363 [2]	15.91	0.74	0.24	33.42
Brown KD-BT909-21-1-41 [1]	16.27	5.44	0.38	21.69

Values are a mean of duplicate analysis. (g/100g wet weight)
[1]Khaw Dok mali 105 (KDML 105) × Jao hom nin
[2]*O.nivara* (wild rice) × BT
[3] Kum doi chang × Hom pama
[4]RS were determined by Megazyme RS kit (code K-RSTAR);
NA: not analyzed

Table 5.5 Ranking of RAG, SAG, RS and AC values of the new breeding lines on various rice varieties from KU **(continued)**

Name of rice variety	RAG (g/100g)	SAG (g/100g)	RS[4] (g/100g)	AC %
Milled IR71501 × Pin Kaset34-1-22-1-0	16.74	1.82	1.14	42.71
Brown KD-BT282-3-2-4 [1]	16.75	0.84	0.23	19.30
Brown KD-BT909-10-1-3 [1]	16.84	3.23	NA	18.27
Brown Hom Pathumthani	16.90	2.75	0.33	24.00
Brown IR71501 × Pin Kaset34-1-3-1-0	17.06	1.42	0.99	38.13
Milled 23-215 (Pin Kaset)	17.11	7.66	0.06	21.89
Brown Pin Kaset	17.13	2.59	0.19	24.53
Milled IR71501 × Pin Kaset3-1-14-20-0	17.29	3.39	0.17	22.17
Brown *O.nivara* - BT#299 [2]	17.54	3.87	0.07	20.89
Milled IR71501 × Pin Kaset34-1-3-1-0	17.77	2.87	0.78	37.81
Milled KD-BT909-21-1-41 [1]	17.90	4.24	0.03	21.06
Milled Pin Kaset	17.96	4.10	0.11	23.24
Milled IR71501 × Pin Kaset3-1-22-6-1	18.05	3.07	0.21	24.36
Brown BW3	18.11	2.90	0.13	21.22
Brown K379 [2]	18.12	0.91	0.37	22.72
Brown Khao Dok Mali 105 (KDML 105)	18.18	2.03	0.13	20.53
Milled Khao Dok Mali 105 (KDML 105)	18.28	2.49	0.03	19.49
Milled BW3 [3]	18.32	4.88	0.04	20.83
Milled KD-BT909-21-2-5 [1]	18.32	4.82	0.05	20.76
Brown K370 [2]	18.37	6.08	1.09	19.95
Brown K384 [2]	18.53	2.06	0.37	24.93
Milled *O.nivara* - BT#299 [2]	18.66	2.64	0.04	19.15

Values are a mean of duplicate analysis. (g/100g wet weight)
[1] Khaw Dok mali 105 (KDML 105) × Jao hom nin
[2] *O.nivara* (wild rice) × BT
[3] Kum doi chang × Hom pama
[4] RS were determined by Megazyme RS kit (code K-RSTAR);
NA: not analyzed

Table 5.5 Ranking of RAG, SAG, RS and AC values of the new breeding lines on various rice varieties from KU **(continued)**

Name of rice variety	RAG (g/100g)	SAG (g/100g)	RS[4] (g/100g)	AC %
Milled KD-BT282-3-2-4 [1]	18.66	1.14	0.04	18.03
Brown Black glutinous KDC49-6-6-1-1-2 [3]	18.86	5.49	NA	7.90
Brown KD-BT11-4-1-18 [1]	18.92	0.44	2.16	18.96
Milled BT#3	18.95	3.61	0.04	20.87
Brown BW1	18.99	2.85	0.19	20.48
Milled KD-BT1000-11-2-26 [1]	19.09	1.44	0.05	18.97
Milled BW1	19.11	4.61	0.05	18.41
Brown *O.nivara* - BT#91 [2]	19.20	3.36	0.11	16.79
Milled Hom Pathumthani	19.28	0.62	0.09	22.56
Milled *O.nivara* - BT#91[2]	19.39	2.82	0.02	16.37
Milled KD-BT11-4-1-18	19.48	2.14	0.04	18.24
Milled White glutinous KDC18-3-7-1-11-0 [3]	19.75	6.40	NA	9.30
Brown K342 [2]	19.95	3.59	0.28	35.94
Milled KD-BT909-10-1-3 [1]	20.16	1.86	0.05	20.34
Milled Jaohom Nin	20.20	1.47	0.03	17.86
Brown Jaohom Nil	20.40	0.34	0.14	19.51
Milled Black glutinous KDC49-6-6-1-1-2 [3]	20.89	4.35	NA	7.38
Brown K337 [2]	21.54	2.62	0.14	26.39
Brown K291 [2]	22.18	0.59	0.17	19.87

Values are a mean of duplicate analysis. (g/100g wet weight)
[1] Khaw Dok mali 105 (KDML 105) × Jao hom nin
[2] *O.nivara* (wild rice) × BT
[3] Kum doi chang × Hom pama
[4] RS were determined by Megazyme RS kit (code K-RSTAR);
NA: not analyzed

From **Table 5.6**, in comparison between RAG content between polished and brown rice varieties obtained from Center of Excellence for Rice Molecular Breeding KU, it was found that brown rice had significantly lower amounts of RAG values

(16.33 ± 2.88 g/100g) than polished rice (18.38 ± 1.16 g/100g) as well as significantly higher AC in brown rice (23.41 ± 6.51%) was also observed. When compared the RAG values of polished local rice varieties bought from local markets and the new breeding lines, it revealed that the RAG values was significantly lower in the local varieties, with approximately 13.40 ± 3.28 g/100g cooked rice, particularly, Hom Mali Sichompoo than the new breeding variety (18.38 ± 1.16 g/100g cooked rice), as seen in **Table 5.6**. However, the new breeding variety number 313-19-1-1, produced from cross breeding between BT with KDML 105 which was harvested in dry season, shown the lowest value of RAG (6.13 g/100g), as seen in **Table 5.5.**

Table 5.6 Comparison the RAG and AC values among polished rice, brown rice obtained from Center of Excellence for Rice Molecules and Product Development, KU and polished rice bought from local markets[1]

Rice	n	RAG (g/100g)	AC %
Polished rice (KU)	21	18.38 ± 1.16^{c}	22.13 ± 6.38^{a}
Brown rice (KU)	36	16.33 ± 2.88^{b}	23.41 ± 6.51^{a}
Polished (local)	27	13.40 ± 3.28^{a}	27.15 ± 8.40^{b}

[1]Values represent mean ± standard deviation of in duplicates analysis.
RAG values within same column means followed by letters "a", "b" and "c" ($p < 0.05$) shows statistical differences between polished (KU and local market) and unpolished rice (KU, respectively by using independent t-test
AC within same column means followed by letter "a" and "b" ($p < 0.05$) shows statistical differences between polished (KU and local market) and unpolished rice (KU, respectively by using Man-Whitney U test

5.6 Correlation coefficient between AC, RAG or RS values of various Thai rice varieties

Table 5.7 showed the Pearson-correlation coefficient between AC and RAG; AC and RS which were obtained from the regression analysis. The data of present study revealed that the AC had a significantly negative correlation coefficient with RAG value ($r = -0.247$; *p value* < 0.05) while a significantly positive correlation ($r =$

0.402; *p value* < 0.01) was found between AC and RS contents (**Table 5.7**).

Table 5.7 Correlation coefficient between the AC and RAG or RS contents of various Thai rice varieties

		r
AC and		
	RAG	-0.247*
	RS	0.402**

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

5.7 Classification of Thai rice varieties on apparent AC according to Juliano publication in 1985b (51)

Juliano in 1979 and 1985b classified various rice varieties into 6 groups according to the percentage of AC. The percentage of AC were classified by 1-2 % as waxy (glutinous), followed by 2-12 % as very low, 12-20 % as low, 20-25 % as intermediate; 25-30 % as high AC and more than 33 % as very high AC (51, 52). In the present study, there was wide variation of AC in various rice varieties ranged from 7.38 to 42.71%, as seen in **Table 5.4** and **5.5**. As expected, the very low AC (7.38 to 10.5%) should be found only in glutinous rice. But the data of present study found that one of non glutinous rice strain (brown), named “Sung Yod” showed very low amount of AC (8.59%), as seen in **Table 5.8 - 5.12**.

Table 5.8 List names of rice varieties with very low AC

Very low AC (2-12%)
Milled Black glutinous KDC49-6-6-1-1-2
Brown Black glutinous KDC49-6-6-1-1-2
Brown White glutinous KDC18-3-7-1-11-0
Brown Sang Yod
Milled glutinous Maechun
Milled glutinous Maechun (old)
Milled White glutinous KDC18-3-7-1-11-0
Milled glutinous Sanpahtawng
Milled glutinous RD 6

Table 5.9 List names of rice varieties with low AC

Low AC (12-20%)
Milled Benjakrayatip
Milled *O.nivara*-BT#91
Brown *O.nivara*-BT#91
Brown KD-BT313-19-1-1 (harvest in wet season)
Milled Jao Hom Nin
Milled Hom Mali Chiangrai (new)
Brown K367
Brown KD-BT1000-11-2-26
Brown K334
Milled KD-BT282-3-2-4
Milled KD-BT11-4-1-18
Brown K349

Table 5.9 List names of rice varieties with low AC **(continued)**

Low AC (12-20%)
Brown KD-BT909-10-1-3
Milled BW1
Milled Hom Mali Thung kula Amnatcharoen
Milled Hom Mali Thung kula (new)
Brown KD-BT909-21-2-5
Brown BT#3
Brown KD-BT11-4-1-18
Milled KD-BT1000-11-2-26
Milled Hom Mali Chiangrai (Middle)
Milled *O.nivara*-BT#299
Brown 23-215 (Pin Kaset)
Brown KD-BT282-3-2-4
Milled Khao Dok Mali 105 (KDML 105)
Brown Jao Hom Nin
Milled KD-BT313-19-1-1 (harvest in wet season)
Brown K291
Milled Hom Mali Khemmarat
Brown K370

Table 5.10 List names of rice varieties with intermediate AC

Intermediate AC (20-25%)
Milled Hom Mali Thung kula Roi Et
Milled KD-BT909-10-1-3
Brown BW1
Milled Hom Mali Thung kula Yasothon
Brown Khao Dok Mali 105
Milled Hom Mali Sichompoo
Milled KD-BT909-21-2-5
Milled BW3
Milled BT#3
Brown *O.nivara*-BT#299
Milled KD-BT909-21-1-41
Brown BW3
Milled Sung Yod Pattalung
Brown KD-BT909-21-1-41
Brown K368
Milled 23-215 (Pin Kaset)
Milled IR71501 × Pin Kaset3-1-14-20-0
Milled Hom Pathumthani
Brown K379
Milled Hom Mali Yasothon (old)
Brown Hom Mali Surin
Milled Pin Kaset
Brown IR71501 × Pin Kaset3-1-14-20-0
Milled Hom Mali Arawan
Brown K383
Brown Hom Pathumthani

Table 5.10 List names of rice varieties with intermediate AC **(continued)**

Intermediate AC (20-25%)
Milled Hom Mali Chiangrai (old)
Milled IR71501 × Pin Kaset3-1-22-6-1
Brown Pin Kaset
Milled Hom Mali Thung kula (Middle)
Milled Hom Mali Buriram
Brown K384

Table 5.11 List names of rice varieties with high AC

High AC (25-33%)
Brown IR71501 × Pin Kaset3-1-22-6-1
Milled Hom Mali Nakhonratchasima
Milled Hom Mali Surin (middle)
Milled Hom Mali Surin (old)
Milled K337
Milled Hom Mali Pathumthani (Middle)

Table 5.12 List names of rice varieties with very high AC

Very high AC (> 33%)
Milled K363
Milled K310
Milled Leuang-orn (old)
Brown K373
Milled Khao Tah Haeng (old)
Brown K342
Milled Khao Kaset (old)
Milled IR71501 × Pin Kaset34-1-3-1-0
Brown IR71501 × Pin Kaset34-1-3-1-0
Milled Sao Hai Saraburi (Middle)
Milled Sao Hai Saraburi (old)
Brown IR71501 × Pin Kaset34-1-22-1-0
Milled Sao Hai 3 part (old)
Milled Sao Hai Jekchei (old)
Milled Leuang Pratew (Middle)
Milled IR71501 × Pin Kaset34-1-22-1-0

PART III

The third part was to evaluate the correlation between various CHO fractions with RAG values in sixteen rice varieties model shown as low, medium and high RAG level by using *in vitro* RAG method.

5.8 RAG, SAG, TG, FG, TS, RDS, SDS, RS and SDI values of some cooked rice varieties model

Table 5.13 summarized the data of RAG, SAG, RDS, SDS, TG, FG, TS, RS and SDI values of 16 new breeding varieties of both non glutinous and glutinous rice. The values of RDS, SDS, TS, RS, and SDI were calculated from the amounts of RAG and SAG as glucose releasing after 20 and 120 minutes incubation with pancreatin,

AMG and invertase. All of the RAG, RDS and SDI values of cooked glutinous rice were higher than non-glutinous rice. The values of cooked polished rice were higher than that of cooked brown rice. TG and RDS of all the various rice varieties were ranged from 18.55 to 37.29 and 10.49 to 23.28 g/100g cooked rice, respectively, whereas non-glutinous brown rice KD-BT1000-11-2-26 (24.10 %), milled KD-BT313-19-1-1 (28.80 %) and brown 34-1-3-1-0 (37.29 %) was also shown high amount of TG, as seen in **Table 5.13**. The observation on the amounts of RAG values of those varieties shown in medium levels due to their containing medium and high value of RS (0.11 to 0.99 g/100g cooked rice). The finding of present study was in agreement with Schakel et al., in 2008 who suggested that there were many factors such as, RS, fiber, fat, type of sugar, refining grain or reducing particle size of starch, and dairy protein influence on GI values (57). Cooked glutinous rice of present study showed the greatest amount of TG, RDS, TS and SDI as well as the lowest amount of RS (0.00 – 0.03 g/100g cooked rice), as seen in **Table 5.13**.

Table 5.13 Ranking CHO fraction values of brown and polish of non glutinous and glutinous rice (g/100g as eaten) [1, 2]

Name of rice variety	RAG	SAG	TG	FG	RDS	SDS	TS	RS[3]	SDI[4]
Brown KD-BT313-19-1-1 (wet season)	13.53	7.77	20.11	1.87	10.49	6.99	16.42	0.17	63.90
Brown KD-BT1000-11-2-26	14.55	5.31	24.10	1.91	11.38	4.78	19.97	0.17	56.98
Brown KD-BT909-21-2-5	14.64	6.24	20.08	1.78	11.57	5.62	16.47	0.09	70.24
Milled KD-BT313-19-1-1 (wet season)	15.27	4.36	28.80	1.64	12.27	3.92	24.45	0.11	50.19
Brown White glutinous KDC18-3-7-1-11-0	15.44	7.04	23.98	0.45	13.49	6.34	21.18	NA	63.70
Brown IR71501×Pin Kaset34-1-22-1-0	15.63	8.11	20.94	0.56	13.56	7.30	18.35	1.13	73.92
Milled IR71501×Pin Kaset34-1-22-1-0	16.74	1.82	18.55	0.59	14.54	1.64	16.16	0.14	89.97
Brown IR71501×Pin Kaset34-1-3-1-0	17.06	1.42	37.29	0.99	14.46	1.28	32.67	0.99	44.26
Milled IR71501×Pin Kaset34-1-3-1-0	17.77	2.87	20.65	0.70	15.36	2.58	17.96	0.28	85.53
Brown Black glutinous KDC49-6-6-1-1-2	18.86	5.49	25.08	0.39	16.62	4.94	22.22	NA	74.79
Milled White glutinous KDC18-3-7-1-11-0	19.75	6.40	26.20	0.86	17.00	5.76	22.81	NA	74.54
Milled Black glutinous KDC49-6-6-1-1-2	20.89	4.35	26.54	0.77	18.11	3.92	23.19	NA	78.08
Milled glutinous Udonthani (Local market)	27.26	0.32	27.17	1.78	22.93	0.29	22.85	0.03	100.34
Milled glutinous Maechun (Local market)	25.54	0.27	26.63	1.13	21.97	0.24	22.95	0.02	95.73
Milled glutinous Esan (Local market)	27.98	-0.70	26.31	2.21	23.19	-0.63	21.69	0.03	106.90
Milled glutinous Sunpathong (Local market)	27.03	0.61	27.50	1.17	23.28	0.54	23.70	0.00	98.24

[1]Values are a mean of duplicate analysis. NA: not analysed
[2]TG, total glucose; FG, free glucose; including that from sucrose; TS, total starch; RS, resistant starch;
RDS, rapidly available starch; SDS, slowly digestible starch (calculated as the sum of glucose from RDS and FG).
[3]RS content was determined by Megazyme RS kit code K-RSTAR.
[4]SDI, starch digestible index

5.9 Correlation coefficient between RAG, RDS, RS and SDI contents of Thai Rice Varieties Model

Table 5.14 gave the correlation coefficients obtained by Pearson regression. All samples (sixteen varieties model) showed a significant positive correlation between RAG and RDS and SDI (r = 0.993, p < 0.01; r = 0.815, p < 0.01) and between RDS and SDI (r = 0.819, p < 0.05). SDI was inversely related with RS (r = -0.502, p < 0.05) in this sample model.

Table 5.14 The correlation coefficient between RAG, RDS, RS and SDI contents of the selected sixteen rice varieties

		r
RAG and		
	RDS	0.993**
	SDI	0.815**
RDS and		
	SDI	0.819**
SDI and		
	RS	-0.502*

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

PART IV

The fourth part was to formulate and evaluate the modified food products made from rice flour and/or rice bran, red bean and black sesame for decreasing the RAG values after enzyme digestion of CHO (CHO digestion rate).

5.10 Preliminary baking trial

Various conventional recipes for snack products were determined and selected as the control formula. These recipes required appropriate adjustment and re-evaluation in order to obtain the snack products with desirable characteristics. Bread

stick and bread were bought from cafeteria (Mahidol University) and Institute of Nutrition at Mahidol University, respectively, used as control formulas. These control formulas were used for product application testing of the rice flour.

For formulation of rice flour from application testing in snack products in this study, the brown rice grain no. 313-19-1-1 was selected to prepare as rice flour for developing a low glycemic response (low RAG) of rice products. Since previous study in human on the glycemic response of diabetics type II fed with brown rice number 313-19-1-1, the result showed that brown rice no. 313-19-1-1 had intermediate GI (53-58% of GI) and was able to reduced serum LDL-cholesterol and triglyceride levels in the subjects (unpublished data). This finding was in agreement with our data which revealed that brown and polished rice number 313-19-1-1 were low-intermediate RAG values (13.53 and 15.27 g/100g), as seen in **Table 5.5.**

5.11 Preliminary study for preparation of rice flour products in the present study

Rice flour was obtained from brown rice grain no. 313-19-1-1 which was ground into powder form by a miller (I-149-Rtch GmbH, Germany) attacked with a 0.08 mm (100-120 mesh) before mixing into the batter. After rice flour was applied in snack products, the products characteristics appeared to be satisfactory. It was found that rice flour could partially substitute wheat flour up to 70 %, 50 %, 65 % and 44 % for steamed bun, bread stick, red bean filled bun and bread respectively. Beyond these levels, there were production problems as well as unacceptability in product characteristics, for example, poor structure, texture and strong floury odor. Hence, formulated rice flour incorporation above the mentioned levels were not selected for sensory screening test.

Therefore, all the modified rice products, namely stream bun, bread stick, red bean filled bun and bread, which prepared from rice flour no. 313-19-1-1 were tested the sensory evaluation using volunteer panelists in order to increasing the good texture and acceptability. The results from preliminary shown that the steamed bun had more compact/heavier texture, as well as the loss of elasticity and the structure tended to collapse when substituted wheat flour with rice flour higher than 70 %. The

denser product was detected in steamed bun from high level of rice flour incorporation. Similar results were found in red bean filled bun and bread rice flour higher than 65 % and 44 % of the wheat flour, respectively. All of products showed more fragile and denser texture than that of the control formulas. In addition, modified steamed bun, bread stick, red bean filled bun and bread had higher interior moisture than the control formulas. Therefore, in this study, the liquid portion and some ingredient contents such as egg, yeast, gluten and sugar were increased or reduced from original formula in order to increase the scores of sensory acceptability.

5.12 Sensory evaluation of control product and modified product recipes by using in-house consumer test

After preliminary test, all of snack products prepared from rice flour with the highest possible level of rice flour incorporation to find the best formula of each product were evaluated by 25 panelists by using 9-point hedonic scale for evaluating overall acceptability and general appearance and 5-points just about right for evaluating color, texture, sweet and salt suitability (in-house consumer test according to the method described in materials and methods part). Snack products were considered acceptable overall if their mean average score was about 6 (“like slightly”).

Table 5.15 The sensory evaluation of steamed bun using 70% rice flour compared with streamed bun control formula[1, 2]

Formula	General appearance[3]	After testing			
		Overall acceptability[3]	Color suitability[4]	Sweet suitability[4]	Texture suitability[4]
Control	7.46 [a] (1.03)	7.31 [a] (1.26)	3.00 [b] (0.00)	3.00 [b] (0.40)	3.12 [a] (0.33)
0 month	6.96 [a] (1.30)	6.65 [a] (1.23)	3.23 [a] (0.43)	2.50 [a] (0.58)	2.96 [a] (0.66)
Freeze 1 month	6.80 [a] (1.17)	6.73 [a] (1.51)	3.15 [a] (0.54)	2.54 [a] (0.58)	3.12 [a] (0.52)
Freeze 3 month	7.00 [a] (0.98)	6.96 [a] (1.28)	3.12 [a] (0.52)	2.54 [a] (0.58)	3.04 [a] (0.53)

[1]Mean (SD) from complete randomized design, n = 25
[2]Values within the same column with difference superscripts are significant different by non parametric using independent and Duncan's multiple comparison test at $p < 0.05$.
[3]Nine-point hedonic scale (9=like extremely, 5=neither like nor dislike, 1=dislike extremely)
[4]Five-point just-about-right scale (5=much too dark/elastic/hard, 3=just-about-right, 1=much too light/brittle/soft)

Figure 5.3 Modified steamed bun was made from rice flour including rice bran and replaced one-half of sugar with sorbitol

Table 5.15 presented the sensory acceptability result of reduce sugar steamed bun contained 70 % rice flour, one-half the amount of sorbitol substituted for sugar and 1.75 % rice bran of all ingredients in order to provide fiber property. To obtain good characteristics of the product, some ingredients were increased or decreased. No significant differences ($p > 0.05$) were found in the score of appearance, overall acceptability, and texture among all samples except the score of color and sweet of control. Comments from the panelists included the floury odor and heavy texture. However, the steamed bun made from 70 % rice flour was still accepted by the panelists with score 6.96 for general appearance and 6.65 for over all acceptability ("like slightly" and "moderately"). The photograph of steamed bun is illustrated in **Figure 5.1**. In addition, **Table 5.15** was also shown the result of sensory evaluation scores of the modified steamed bun during 3-month storage. There were no significant differences in general appearance with score 6.80 to 7.00, overall acceptability 6.65 to 6.96, color 3.12 to 3.23, sweet 2.50 to 2.54 and texture suitability scores (2.96 to 3.12) during three months of storage condition, except the control steamed bun which had significantly lower color score (3.00), notwithstanding, all the modified steamed bun could have a shelf life of at least 3 months without alteration in their sensory quality from those control ones, except dark color and less sweet suitability than the control.

Table 5.16 In-house consumer test of bread stick using 50% rice flour by weight compared with control formula

Formula	General appearanceP[3]	After testing			
		Overall acceptability[3]	Color suitability[4]	Salt suitability[4]	Texture suitability[4]
Control	7.12[a] (1.07)	6.77[a] (1.03)	3.12[a] (0.33)	3.08[a] (0.39)	3.50[a] (0.51)
Modified	6.85[a] (0.97)	7.04[a] (1.31)	3.23[a] (0.51)	2.96[a] (0.60)	3.27[a] (0.53)

[1]Mean (SD) from complete randomized design, n = 25
[2]Values within the same column with difference superscripts are significant different by non parametric using independent and Duncan's multiple comparison test at $p < 0.05$.
[3]Nine-point hedonic scale (9=like extremely, 5=neither like nor dislike, 1=dislike extremely)
[4]Five-point just-about-right scale (5=much too dark/elastic/hard, 3=just-about-right, 1=much too light/brittle/soft)

Figure 5.4 Modified bread stick made from rice flour

Table 5.16 gave the sensory acceptability results of bread stick made from 50 % rice flour of the whole flour by weight compared with the control recipe with added gluten, red bean and sesame, and reduced sugar by using sorbitol as sugar replacement in order to create good characteristics and properties. Some ingredients were increased or decreased in order to obtain good characteristics of the product. The results revealed that there were no significant differences ($p>0.05$), in general appearance, overall acceptability, color, flavor and texture where the score of acceptability was higher than that of the control formula. Comments from the panelists were only on the floury odor of the rice bread stick. In spite of this, it was well accepted by the panelists indicated by sensory acceptability scores above 7 ("like moderately"). The photograph of rice bread stick is illustrated in **Figure 5.2.Table**

5.17 In-house consumer test of red bean filled bun using 65% rice flour by weight compared with control formula[1,2]

Formula	General appearance[3]	After testing			
		Overall acceptability[3]	Color suitability[4]	Sweet suitability[4]	Texture suitability[4]
Control	7.36 [a] (0.91)	7.48 [a] (1.08)	3.04 [a] (0.20)	3.24 [a] (0.44)	3.40 [a] (0.58)
Modified	6.60[b] (1.29)	7.08 [a] (1.26)	3.16 [a] (0.37)	2.72 [b] (0.54)	3.64 [a] (0.57)

[1]Mean (SD) from complete randomized design, n = 25
[2]Values within the same column with difference superscripts are significant different by non parametric using independent and Duncan's multiple comparison test at $p < 0.05$.
[3]Nine-point hedonic scale (9=like extremely, 5=neither like nor dislike, 1=dislike extremely)
[4]Five-point just-about-right scale (5=much too dark/elastic/hard, 3=just-about-right, 1=much too light/brittle/soft)

Figure 5.5 Modified red bean filled bun made from rice flour and rice bran

The results of sensory test of red bean filled bun using rice flour were shown in **Table 5.17**. No significant difference was detected in any sensory attributes ($p>0.05$), excepted only in sweet flavor. However, the preference of the panelists towards the red bean filled bun was like moderately even at the replacement of 65 % rice flour for wheat flour in the formula of bun shell. The filled used in the formula was without oil and sugar, partially replaced with 100% sugar alcohol (sorbitol) for sugar by weight, and used 33.33% rice bran for red bean substitution and increase or decrease some ingredients in order to create good characteristics which was accepted for an in-house consumer test, as comparing with the control formula as seen in the procedure of material and method. The photograph of red bean filled rice bun is shown in **Figure 5.3**.

Table 5.18 In-house consumer test of bread using 44% rice flour by weight compared with control formula [1, 2]

Formula	General appearance[3]	After testing			
		Overall acceptability[3]	Color suitability[4]	Sweet suitability[4]	Soft suitability[4]
Control	7.12 [a] (1.09)	7.20 [a] (0.82)	2.92 [a] (0.40)	2.76 [a] (0.52)	3.04 [a] (0.20)
Modified	7.12 [a] (0.24)	6.60 [a] (1.44)	3.44 [b] (0.51)	3.12 [b] (0.67)	2.92 [a] (0.76)

[1]Mean (SD) from complete randomized design, n = 25
[2]Values within the same column with difference superscripts are significant different by non parametric using independent and Duncan's multiple comparison test at $p < 0.05$.
[3]Nine-point hedonic scale (9=like extremely, 5=neither like nor dislike, 1=dislike extremely)
[4]Five-point just-about-right scale (5=much too dark/elastic/hard, 3=just-about-right, 1=much too light/brittle/soft)

Figure 5.6 Modified bread made from rice flour and rice bran

Results from the in-house consumer test (sensory evaluation) for bread made from 44 % rice flour of the whole flour by weight was accepted and then the formula was used to prepare rice bread. The gluten, rice bran, red bean and sesame were added in order to create good characteristics and properties. For reducing sugar content, a sorbitol was used for sugar replacement. Some ingredients were increased

or decreased in order to get good characteristics. There were no significant differences in any sensory attribute ($p>0.05$) with score of general appearance (7.12 for modified bread) and 6.60 of overall acceptability, except for color and sweet was significantly higher than control bread. Moreover, some a few panelists commented about the unpleasant odor, dense, loss of elasticity and floury mouth feel of rice bread. The photograph of rice bread is illustrated in **Figure 5.4**.

5.13 Chemical analysis of snack products

Result of proximate analysis of steamed bun, bread stick, red bean filled bun and bread per 100 g sample was summarized in **Appendix K.** Their nutrient composition were calculated based on 100 g or the serving size of commercial products which ranged from 25 - 50 g, as shown in **Table 5.19.**

5.13.1 Nutritive value of products

Nutrient composition of modified rice flour base products *per* serving are shown in **Table 5.19**. Nutritive values of the products varied depending on the ingredients used in the preparation sample. The protein content of the present modified product ranged from 2.5 to 4.7 %, while total fat ranged from 2.3 to 4.0 % and energy varied from 72 to 155 kcal. Among these products, red bean filled bun showed the highest amount of nutritive values.

Table 5.19 Nutritive analysis of rice products ***per serving*** wet weight [1, 2]

Products (per serving)	Protein (g)	Fat (g)	CHO (g)	Energy (Kcal)	TDF
Steamed bun (40 g)	2.8	2.5	20.1	115	0.5
Bread stick (30 g)	4.0	4.0	21.0	136	1.0
Red bean filled bun (50 g)	4.7	3.5	26.1	155	1.0
Bread (25g)	2.5	2.3	10.4	72	1.0

[1]Values are a mean of duplicate analysis.
[2]Factor of 4, 4 and 9 were used to calculate the energy from protein, CHO and fat respectively.

5.13.2 RAG, SAG, TG, FG, TS, RDS, SDS, SDI and RS values of modified rice flour products

Data in **Table 15.22** and **Appendix L** presented RAG, SAG, RDS, SDS, TG, FG, TS, RS and SDI of all control and modified snack products made from rice flour. There were significant differences ($p<0.05$) on RAG values between each control and modified rice flour product. When comparing the RAG values of all the products between control and modified product, the higher RAG rate (29.48-62.34%) were detected in all the control products ($p<0.05$). In comparison, the RAG ranking values among 4 modified rice base flour product as g/100g as eaten were found lowest in the modified filled bun (26.80%), followed by the modified bread (33.07%), modified steamed bun (35.27%) and modified bread stick (40.32%), respectively.

TG contents were found to be 38.86 to 71.78% for the control products and 37.85 to 68.53 % for the modified products. While all of them had low FG content as 1.88 to 3.28% for the modified products and 1.69 to 4.75% for the control. With the exception of red bean filled bun which shown the highest amount of FG in control (9.08%) whilst the lowest value was found in control bread (1.69%) and modified steam bun (1.88%). All control and rice base flour products of this study contained low RS values, ranging from 0.07 to 0.52 %, especially in all control products (0.07 to

0.37%), which shown lower RS values than the modified products (0.15 – 0.52%), as seen in **Table 5.20**. The greater amount of RS contents was found in the modified bread stick, red bean filled bun and bread, with ranging from 0.42 to 0.52 g/100g.

In addition, the amount of RDS and TS were found significantly higher values in all control products than the modified products, with ranging from 18.36 to 54.37 % and 26.80 to 62.86%, respectively (**Table 15.22**). When considerable on RS content, it revealed that the amounts of RS of all modified products were higher than the control ones, especially the modified red bean filled bun (0.52%) which was somewhat higher than other rice products, whilst showed the lowest amount of RAG or the rate of glucose releasing, as seen in **Table 5.22**. The lower RAG of the modified products than the control ones could be explained as the results of low RAG ingredients in these products such as red bean (16.87%), sesame seed (0.32%) and rice bran (4.52%), including high RS of the rice flour # 313-19-1-1 (RS 3.47%), as seen in **Table 5.21** which was higher than wheat flour and was used to replace the wheat flour in the products.

Table 5.20 Comparison on the amount of RAG and RS values among control and modified food products[1]

Product	Formula	RAG (g/100g)	RS (g/100g)
Steamed bun	control	45.64	0.07
	modified	35.27	0.15
Bread stick	control	62.34	0.26
	modified	40.32	0.45
Red bean filled bun	control	29.48	0.37
	modified	26.80	0.52
Bread	control	45.41	0.13
	modified	33.17	0.42

[1]Values are a mean of duplicate analysis. (g/100g wet weight)

Table 5.21 RAG, SAG and RS values of some ingredient used in the modified rice base flour products preparation[1]

Ingredient	**RAG (g/100g)**	**SAG (g/100g)**	**RS (g/100g)**
Red bean	16.87	3.77	0.73
Sesame seed	0.32	0.17	9.92
Wheat flour	33.24	31.37	0.08
Wheat flour *	40.00	38.87	1.8
Brown rice 313-19-1-1 flour	40.92	16.61	3.47
Rice bran	4.52	13.33	NA

[1]Values are a mean of duplicate analysis. (g/100g wet weight)
*data from Englyst et al., 1992
NA: not analyzed

Table 5.22 CHO fraction of control and modified rice flour products (g/100g as eaten)*[1]

Product		RAG	TG	FG	RDS	TS	RS[2]
Steamed bun	control	45.64[b] (2.18)	49.88[b] (3.17)	4.75[b] (0.25)	36.80[b] (1.96)	40.62[a] (2.86)	0.07[a]
	modified	35.27[a] (1.23)	44.42[a] (1.57)	1.88[a] (0.21)	30.06[a] (1.11)	38.29[a] (1.41)	0.15[b]
Bread stick	control	62.34[b] (1.65)	71.78[a] (4.58)	1.93[a] (0.22)	54.37[b] (1.49)	62.86[a] (4.13)	0.26[a]
	modified	40.32[a] (1.33)	68.53[a] (2.09)	2.88[b] (0.38)	33.69[a] (1.20)	59.09[a] (1.88)	0.45[b]
Red bean filled bun	control	29.48[b] (1.39)	38.86[a] (1.98)	9.08[b] (0.08)	18.36[a] (1.25)	26.80[a] (1.78)	0.37[a]
	modified	26.80[a] (0.93)	38.37[a] (3.40)	2.88[a] (0.01)	21.53[b] (0.84)	31.94[b] (3.06)	0.52[b]
Bread	control	45.54[b] (2.32)	45.96[b] (1.44)	1.69[a] (0.23)	39.46[b] (2.09)	39.84[b] (1.30)	0.13[a]
	modified	33.07[a] (0.41)	37.85[a] (1.42)	3.28[b] (0.10)	26.81[a] (0.37)	31.11[a] (1.28)	0.42[b]

[1]Values within the same column with difference superscripts are significant different by one way ANOVA and Duncan's multiple comparison test at $p < 0.05$.
*TG, total glucose; FG, free glucose; including that from sucrose; TS, total starch, RDS, rapidly available starch; (calculated as the sum of glucose from RDS and FG) [2]RS (resistant starch) content were determined by Megazyme RS kit,

5.14 The correlations coefficient between RAG and various starch fractions

Table 5.23 gave the correlation coefficients between RAG and RDS, RS, TG, FG, and TS which were obtained by the regression analysis. All the rice flour products of this study shown significant positive correlations between RAG and TG, TS and RDS (r = 0. 749 to 0.948, $p < 0.01$) whilst FG and RS was significantly inverse related with RAG (r = - 0.440, $p < 0.05$ and -632, $p < 0.01$, respectively).

Table 5.23 Correlation between RAG and RDS, RS, TG, FG and TS contents of rice flour products

	r
RAG and	
RDS	0.948**
RS	- 0.632**
TG	0.749**
FG	-0.440*
TS	0.762**

** $p < 0.01$, * $p < 0.05$

Part V

The fifth part was to evaluate the effect of storage of steamed bun as a model on RAG value and sensory acceptability at – 20 °C for 3 months.

5.15 Effect of storage of steamed bun products during 3 months in defreeze of refrigerator on the amounts of RAG and SAG values

After storage for 3 month in de-freezed-refrigerator (-20 °C), the data on RAG and SAG of steamed bun made from 70 % rice flour by weight against the control recipe were shown in **Table 5.24**. The amount of RAG of the control steamed bun was significantly higher than modified steamed bun at $p<0.05$. In comparison, the rate of RAG after CHO digestion of modified steamed bun at 0 month was considered significantly lower than 1 month up to 3 month of storage. However, when comparing each storage time of steamed bun with the control, the RAG values of all

modified steamed bun (at 1, 2 and 3 month) were significantly lower than the control but significantly higher than the steamed bun at 0 month. However, their glucose release rate after enzyme CHO digestion in the modified product (RAG) showed no significant difference during the storage time.

Table 5.24 RAG and SAG values of modified steamed bun during 3-month storage (g/100g as eaten) [1]

Steamed Bun	RAG (g/100g)
Control	45.64 [c] (2.18)
Modified 0 month	35.27 [a] (1.23)
Modified 1 month	40.38 [b] (2.01)
Modified 2 month	40.82 [b] (1.66)
Modified 3 month	39.67 [b] (1.74)

[1]Values within the same column with difference superscripts are significantly different by One Way ANOVA and Duncan's multiple comparison test at $p < 0.05$.

CHAPTER VI
DISCUSSION

PART I

This study was a part of the project of Integrated Biotechnology in developing rice strains for high value added and nutrition enrichment. This part aimed to investigate *in vitro* CHO digestibility in various cooked rice varieties obtained from new breeding lines and bought from local markets in Thailand. The various carbohydrates in Thai rice, such as RS, AC and amylopectin contents, had affected the rate digestibility which had implications in many diseases (58, 59).

The development of an *in vitro* enzymatic method by Englyst et al., 1992, 1996 (5, 6) was used as a screening tool for evaluating the CHO digestion rate in foods and meals after hydrolyzed TS by enzyme within reasonable time which must be completely disrupted all of structures of starch particles. In *in vivo*, the rates of digestion and characteristics of starch influenced by particular structures of food when eaten. Thus, Englyst assay which measures the rate and extent of digestion *in vitro* demands the samples to be analyzed as eaten by using extensive homogenized sample. They found that digestibility by mincing was similar to that of starch hydrolysis, and mincing gave a smaller range of standard deviation than that of chewing in each case. Thus, mincing was chosen for treating the samples in this experiment. The glass balls were added into the sample tube during incubation in order to provide a milling reaction. The addition of guar gum was to increase the viscosity of the sample mixture and extend the consistency of hydrolysis of the starch granules. However, it had little effect on the disruption of particles in food, and it was not interfered the method. The invertase was to convert all sucrose into its composing monomers. Free glucose was released from food by Englyst assay and then GOD-PAP kit follow by o-dianisidine (developing color) was used for determination of free glucose.

Values for RAG and SAG in foods were reported to reflect the rate of starch digestion *in vivo* (3, 5, 24). This method is commonly used for determined the *in vitro* digestibility CHO because it was inexpensive, useful for routine analysis and no interferes from extrinsic factor which does not require specialized equipment. In addition, the study of Ells et al., (2005) (61) confirmed that starch characterized as high versus low RDS by the Englyst procedure in differently patterns of metabolism postprandial in healthy young female volunteers. The findings suggested that consumption of low RDS could improve glycemic control, prevent and treat on diabetes and complications of metabolic syndrome. The *in vitro* measured RDS and the *in vivo* assessed GI showed strong correlation (5) thus, this parameter is complementary tools which now can be suggested as potentially important indices of the CHO links with human health.

Therefore, the objectives of present study were to analyze the CHO contents and identify any CHO fraction that could be measured *in vitro* and used as a meaningful predictor of the glycemic response of food.

6.1 The RAG and SAG values of various samples, orange juices and sugar cane syrups

The RAG value of sorbitol was very low (0.35 g/100 g wet weight) because sorbitol is a sugar alcohol which can be slowly metabolized in the body. Unlike glucose, sorbitol has the hydroxyl group instead of the aldehyde group. Therefore, it limits the capacity of absorption by the small intestine (62). Guar gum also had very low RAG which was 0.70 g/100 g wet weight since guar gum is a water soluble fiber and not digested into glucose (63). The RAG value of mungbean noodle (17.48 g/180g) in the present study was closely to the value from GL (17.55 g per serving (180 g)) which reported by Chan (64). In contrast, the RAG value of orange juices (3.8 8 – 9.88 g/250 ml) was lower than the GL value of 9.4 - 15.11 per serving (250 ml) which reported by Mendoson and Jenkin in 1981 (65). However, there are many factors which are known to affect on the GL value in fruits such as genetic, environmental condition and seasonal variation (66, 67, 68, 69, 70).

6.2 The correlation between RAG and GL values of fruits

The GI concept is based on ingestion of a fixed amount of available carbohydrates usually 50 g (71). However, it had a limitation in practical in everyday use. For example, watermelons and carrots had adverse effect on health because of their high GI rank but eating in small amounts could cause little or no increase in postprandial glycemia (74). Therefore, the serving size of food should be concerned in recommendation as well. GL is the product of the GI which involves with size of typical serving, therefore, GL concept could solve the weak point of the GI concept. Hence, in this study, the association of RAG and GL was evaluated between the RAG values in this study and the GL values determined by Powell and colleagues (53) and the GL values calculated from GI assessed by Somnuk's unpublished data (54). The results showed significant positive correlation with $r = 0.687$ ($p < 0.05$). Similar to many studies, they found high correlation between *in vitro* CHO using Englyst assay and the *in vivo* GI in many kinds of food samples. In 1996, Englyst and co-worker found that the correlation between the GI values and those for RAG of 39 starchy food was $r = 0.760$ ($p < 0.01$) (5). In case of glycemic response, Englyst and colleagues (1999) reported that RAG and glycemic response of starchy foods had strong association ($r = 0.981$, $p < 0.01$) (3). According to study of Englyst et al., in 2003 (27), it was noticed that RAG value was strongly correlated with GI in cereal foods. No information was found in any publications with regard to the RAG values in fruits and the inter-relationship between RAG and GL. Nevertheless, this study had shown the high correlation between the RAG and GL with $r = 0.687$ ($p < 0.05$).

In case of ripe mango and unripe mango which showed similar RAG values of glucose released (2.15 and 2.21 g/100 g as eaten) from starch particles after 20 min of incubation with hydrolytic enzymes but the SAG in unripe mango was higher than ripe mango due to different in physio-chemical property of both conditions. According to the publication of Wolvever et al., 1993, GL was classified into three levels; ***Low*** level is ≤ 10, ***Medium*** is 11-19 and ***High***; ≥20. When look at over all data of RAG values, the amounts of RAG in selected fruits in this study were similar to the GL of fruits in other studies (≤ 10) which was classified to be in low level of GL, as seen in **Table 5.3**, except banana. Thus the *in vitro* CHO digestibility after

enzyme hydrolysis (RAG and SAG) showed similar values to the values obtained from GL thus the *in vitro* model was applied to use as a screening tool for the measurement of GL in various rice varieties and rice flour products.

PART II

6.3 The RAG, SAG, AC and RS values of various rice varieties

Rice is one of the primary dietary sources of CHO worldwide, is of particular interesting when assessing variability in CHO digestibility. In addition, the chemical and physical property of rice after digestion are more and more important for therapeutic diets such as GI, percent AC, amounts of RS, and the forming of gelatinization, etc has been widely recognized in recent years as some factors to affect blood glucose response. The results of *in vitro* CHO hydrolysis method from various studies and our preliminary data from method validation in the first part of the study suggested that the method could be use to predict *in vivo* glycemic response (6, 73). The objective of the second part was to evaluate RS and AC in various rice varieties and to estimate their glycemic response based on a model established by Englyst et al., 1992 and 1996.

The results of RAG and SAG contents were measured by Englyst assay as tabulated in **Table 5.4** to **5.5**. When comparing the analysis data of RAG and SAG in cooked rice of present study with Rashmi et al., 2003, (74) Englyst et al., 1996, (5) and Aarathi et al., 2003 (75) the mean of RAG and SAG contents did not completely agree with previous studies (**Table 6.1**), especially the SAG values of polished rice reported by Rashmi & Urooj, 2003 (14.2-30.6 g/100g cooked rice) and RAG reported by Aarathi, et al., 2003 (RAG 26 g/100g cooked rice) which was quite differed from our results (0.6-12.0 g/100g cooked rice for SAG and 6.1-21.4 g/100g cooked rice for RAG). The differences among mean of some RAG and SAG values in present study and previous publication might cause by differences from the variety, environment conditions (fertilizer, growing season, growing area, water and soil) which could strongly affect on the chemical and physicochemical properties, granule size during the process of starch gelatinization. Our suggestion could be supported by Panlasigui, et al., 1991 (76) who found a high gelatinization temperature, a high minimum cooking time and a low volume expansion upon cooking to be good predictors of a low glycemic response.

Table 6.1 Comparison of the results obtained in this study with other publications.

Reference	RAG (g/100g as eaten)		SAG (g/100g as eaten)	
	Milled	**Brown**	**Milled**	**Brown**
Englyst et al., 1996 (5) (long grain rice)	19.3	16.3	6.2	10.2
Englyst & Hudson, 1996 (24) (long grain rice)	19.0	16.0	6.2	10.2
Rashmi & Urooj, 2003 (74)	12.8-21.0	-	14.2-30.6	-
Aarathi et al., 2003 (75)	26.6	-	10.6	-
Present study	6.1-21.4	6.1-22.2	0.6-12.0	0.3-14.66

AC is one of the most important determinants of rice quality. Rice that high in AC shows high volume expansion and high degree of flakiness (77). According to report of Powell and Miller, 1995 indicated that the GI tend to be higher in cooked low amylose rice than cooked intermediate-and high amylose rice (78). In addition, Yamasaki et al., 2007 (79) studied on influence of rice with different AC on postprandial glycemic response and demonstrated that the higher AC was able to lower the postprandial glycemic and insulinemic response in type 2 DM while Denardin et al., 2007 revealed that animals fed with low AC showed higher postprandial blood glucose response than did the animal fed with high or intermediate AC (80). In contrast, Panlasigui et al., 1991 suggested that AC alone should not be considered as a decisive factor for starch digestibility and glycemic response, since rice cultivars with similar AC can differ in physio-chemical properties, particularly, gelatinization. As Panlassigui et al., 1991 (76) mention above might supported our results which found that some varieties of rice with high amounts of AC had higher RAG values than the low AC varieties, as seen in **Table 5.4** and **5.5**.

AC of raw various rice varieties was determined by iodine colorimetry by the procedure of Juliano 1985 (50). The results were summarized in **Table 5.4** to **5.5**. The AC of rice varieties ranged from 7.38 to 42.71 % which some results were not

completely similar to other publications. For example, Rashmi and Urooj (74) indicated that amylose values of steaming rice found ranging from 10.8 – 15.3 g/100 g wet weight, whereas Cheng et al., 2006 studied in 44 rice varieties found AC around 14.8 – 21.4 % (81) which was similar to our report.

RS content was determined by Megazyme RS kit (code K-RSTAR), as shown in **Table 5.5**. RS is defined as the fraction of starch that escapes digestion in the small intestine and that is fermented in the large intestine. RS is slowly digestible and is likely to be negatively correlated with GI thus RS can be used as a mean for slow release of glucose which helps for decreasing postprandial glucose and insulin responses. This behavior had benefits in controlling blood glucose and insulin releasing (55). The amounts of RS contents of all rice in the present study were quite low with ranging from 0.02 to 2.16 g/100g cooked rice. The greatest amounts of RS found in brown K370, brown and milled IR71501 × Pin Kaset 3-1-14-20-0 and brown KD-BT 11-4-1-18 with ranging from 1.09 – 2.16 g/100g cooked rice. In comparison of RS of various rice varieties between analysis data in present study and the previous studies were found that RS values of brown and milled cooked rice varieties ranged from 0.07 – 2.16 g/100 g wet weigh and 0.02 – 1.14 g/100 g wet weight, respectively. This finding is in agreement with the result of Rashmi & Urooj (2003) (74) and Aarathi et al., (2003) (75). Rashmi & Urooj and Aarathi et al. who reported that RS contents ranged from 0.5-9.6 g/100 g fresh basis and 0.5 g/100 g fresh basis, respectively. Moreover, in 1996, Englyst & Hudson (24) reported that RS in both polished and brown rice were not detected (0.0 g/100g wet weight). Differences in the contents of RAG, SAG, RS and AC in rice varieties may be due to different types of rice (milled or brown rice and glutinous or non glutinous rice), method of processing, rice genotype, soil, water, climate and environmental cultivation that might influenced to the carbohydrate fraction of rice (66, 67, 68, 69, 70). Jenkin et al., 1981 (82) proposed the levels of GI values for classifying starchy foods on the basis of their GI response. GI is calculated as the area under the postprandial plasma glucose response curve of a test meal compared with a reference meal equivalent to 50 or 100 g available CHO. Later, Englyst et al., 1992 (6) proposed the RAG index as a predictor of the glycemic response to a meal. RAG values are given directly to calculate the total amount of glucose likely to be released into the blood stream from a

portion of food eaten. Analysis data indicates that most selected varieties of rice sold in local markets and supermarkets in Thailand and new breeding lines varieties both brown or milled rice might be classified as low to intermediate RAG value, with except for some non-glutinous rice and glutinous rice strains such as milled Hom Mali Buriram, brown and milled Jao Hom Nin, brown and milled KD-BT 909-10-1-3, K337 and 291 and all selected glutinous rice which stood out with the greatest RAG values (20.16 – 27.03 g/100g cooked rice), as seen in **Table 5.4, 5.5** and **5.12** and these rice might be classified as a group of high GI foods. Therefore, rice which showed low and intermediate amounts of RAG values might be expected to have low and intermediate glycemic and insulinemic response such as various rice varieties from the local markets and supermarkets e.g. Hom Mali Sichompoo, Hom Mali Thung kula Roi Et , Leuang-orn (old), Hom Mali Khemmarat, Hom Mali Chiangrai (middle) and Hom Mali Thung kula Amnatcharoen, Sang Yod Patthalung, Hom Mali Thung kula Yasothon, Hom Mali Chiangrai (new) and Hom Mali Nakhonratchasima and new breeding varieties e.g. brown KD-BT 313-19-1-1 (harvest in dry season), brown K368, 310, 367, 334 and 349, brown KD-BT 313-19-1-1 (harvest in wet season), brown IR71501× Pin Kaset 3-1-22-6-1 and so on, as see in **Table 5.4** and **5.5**. Moreover, the comparison on the results of RAG values in cooked brown 313-19-1-1 (13.53 g/100g) with the results of glycemic response from human study (DM type 2) indicated that the RAG value obtained from *in vitro* CHO digestion was in agreement with the results from human study. In addition, when comparing the amounts of RAG values between two varieties brown rice #313-19-1-1 and brown rice Jao Hom Nin # 1000, it was found that RAG value of brown rice # 313-19-1-1 (13.53 g/100g cooked rice) was somewhat lower than brown rice Jao Hom Nin (14.55 g/100g cooked rice) and lower values of RAG was also found in milled rice # 313-19-1-1 (15.27 g/100g cooked rice) than milled Jao Hom Nin (20.20g/100g cooked rice). Our finding was similar to the data from human study in type 2 DM study which showed intermediate GI of brown rice 313-19-1-1 and Jao Hom Nin (58 and 62% GI, respectively) while polished rice of both varieties provided about 72 and 74% GI (reported by Integrated Biotechnology in developing rice strains for high value-added and nutrition enrichment, 2004).

In generally, high AC has a pronounced effect on the GI as it has been showed by other report (73), since AC had an obvious impact on the starch degradation and starch digestibility while various investigator suggested that RS is slowly absorbed in the small intestine resulting in decreased postprandial glucose and insulin responses and is likely to negatively correlate with the GI (6, 55). However, analysis data in the present study was found that only 2 varieties of rice (brown and milled rice IR71501 × Pin Kaset 34-1-22-1-0 variety and brown and milled IR71501 × Pin Kaset 34-1-3-1-0 variety had high contents of RS (0.78 – 1.14 g/100g) and AC (37.81 – 42.71%) and also revealed intermediate rate of RAG releasing after enzyme digestion (15.63-17.77%). This finding agreed with the results of Eggum et al., 1993 (86) and Frei et al., 2003 (73) that the waxy and low AC rice cultivars had very low RS and high GI values. In addition Frei et al, 2003 addressed that AC had an impact on starch degradation thus it was used to be predict the glycemic response. However, Analysis data of present study found that some variety of rice had similar AC but the RAG and RS concentrations were quiet different, as seen in **Table 5.5**. These finding was in agreement with Panlasigui et al., 1991 (76) which suggested that the AC of rice alone should not be considered as a decisive factor for starch digestibility and glycemic and insulinemic response, since rice cultivars with similar amylose values can differ in physiochemical properties especially gelatinization and the ratio of AC and amylopectin contents. The GI or RAG value was generally high in glutinous rice, with exception for the new breeding variety “brown white glutinous KDC18-3-7-1-11-0” with an intermediate rate of RAG releasing after enzyme digestion (15.44 g/100g cooked rice), the cause of lower value of RAG in this variety could not be explained and need further exploration in human study. However, non-glutinous rice such as milled Hom Mali Sichompoo (6.05), following by milled Hom Mali Thung kula Roi Et, Leuang-orn (old), Hom Mali form Khemmarat, Chaingrai (middle), Thung kula Amnatcharoen, and so on or new breeding varieties; brown # 313-19-1-1 produced crossing from KDML 105 and Jao hom nin, BT (white color), brown K 367, 310, 334, 349 and brown rice IR71501 × Pin Kaset3-1-22-6-1 and brown white glutinous KDC18-3-7-1-11-0, as seen in **Table 5.4** and **5.5** might be assumed to be potentially useful for providing low or intermediate GI in the diet for normal people or diabetic person.

PART III

6.4 The RDS, SDS, TS and SDI values of 16 cooked rice varieties model

The measurement of *in vitro* RDS, SDS, TS and SDI provides valuable predictions of the rate and extents of starch digestion in the human small intestine. The measurement of *in vitro* RAG provides value for direct amount of glucose likely to be rapidly absorbed, thus it influences blood glucose and insulin levels. These values, including RAG, RDS and SDI, can be used to compare between types of food in equal weight basis. Therefore, it is necessary to know the amounts of those contents which are important indicators for the consumer to plan their food diet.

The RDS, SDS, TS and SDI values of non glutinous polished rice model ranged from 12.3 to 15.4 g/100 g, 1.6 to 3.9 g/100 g, 16.2 to 24.5 g/100 g and 50.2 to 90.0, respectively. The RDS values were similar to many studies, such as Rashmi et al., in 2003 (7.0-16.9 g/100g), Rosin et al., in 2002 (11.7 g/100g) and Englyst & Hudson in 1996 (17.4 g/100g). While the RDS value of Aarathi et al., in 2003 (75) (20.0 g/100g) was higher than that of the present study. The SDS values of this study were lower than the study of Aarathi et al., in 2003 (9.6 g/100g), Rashmi et al. (74) in 2003 (12.8 – 21.5 g/100g), Rosin et al., in 2002 (14.2 g/100g) and Englyst & Hudson in 1996 (24) (5.6 g/100g). The TS values of this study were similar to the study of Rashmi et al., in 2003 (23.0 g/100g), but the values differed from the study of Aarathi et al., in 2003 (30.2 g/100g). In case of SDI values, they were greater than the study of Rashmi et al., in 2003 (19.0 – 43.0).

The RDS, SDS and TS values of non glutinous brown rice ranged from 10.5 to 14.5 g/100 g, 1.3 to 7.3 g/100 g and 16.4 to 32.7 g/100 g, respectively. Compared with Englyst & Hudson study in 1996, the RDS values (14.6 g/100 g) were similar to the results in this study, whereas they were higher than the results of Rosin et al., study in 2002 (6.9 g/100g). Rashmi et al., in 2003 and Englyst & Hudson in 1996 measured SDS in this rice and found that the values of SDS in both studies were similar to that of the present study. The TS of non glutinous brown rice value demonstrated by Rashmi & Urooj (2003) was 23.8 g/100 g which was similar to the results of this study, as seen in **Table 6.2.** The differences among means of some data of RDS, SDS, TS and SDI in the present study with other publications might come

from the different in pedigree, cultivar, variety, environmental condition, climate season, soil, water and fertilizer.

However, when look at the data of RAG, RDS, SDS, RS and SDI of the selected white glutinous rice (glutinous Udonthani, Maechun, Esan and Sunpathong (**Table 5.12**) model, it revealed that RAG (25.54 – 27.98 g/100g as eaten), RDS (21.97-23.28 g/100g as eaten) and SDI (95.73 – 100.34 g/100g as eaten) were higher than other rice varieties as well as lower amount of SDS and RS was observed. The results might indicate that high values of RDS and SDI may attribute to more rapidly complete digestion of starch in those glutinous rice varieties. In addition, many publications indicated that RS levels influenced to postprandial plasma glucose and insulin response as well as affected on the amounts of RAG values thus this suggestion supported our finding that all the glutinous rice samples showed the highest level of RAG values due to the lowest values of RS (83, 84, 85). Whilst the results revealed that high values for both RDS and RAG were similar to the reports of Sharavathy et al., 2001 (86). High RDS indicated more increasing rate of complete digestion of starch in that rice as a result of induced RAG releasing after enzyme digestibility at 20 min, in **Table 5.12** while inter-relationship between other starch fractions such as RS, SDI and SDS with RAG, showed wide variation in those content, especially in rice which produced from crossing line, it difficult to explain since the data of present study was not consistency. The cause of inconsistency of our data might come from the genetic of rice varieties themselves because rice in the present study, especially non-glutinous rice produced from crossing glutinous and non-glutinous cultivars, thus rice in new breeding line might still have dominant genes which mixed between the glutinous and non-glutinous characteristics, might strongly influence on our analysis data.

Table 6.2 Comparison of the results obtained in this study with other publications.

Reference	RDS (g/100g as eaten)		SDS (g/100g as eaten)		TS (g/100g as eaten)		SDI	
	milled	**brown**	**milled**	**brown**	**milled**	**brown**	**milled**	**brown**
Aarathi et al., 2003 (75)	20.0	-	9.6	-	30.2	-	-	-
Rashmi & Urooj 2003 (74)	7.0-16.9	-	12.8-21.5	-	23.0	23.8	19.0-43.0	-
Rosin et al. 2002 (87)	11.7	6.9	14.2	8.6	-	-	-	-
Englyst & Hudson 1996 (24)	17.4	14.6	5.6	9.2	-	-	-	-
Present study	12.3-15.4	10.5-14.5	1.6-3.9	1.3-7.3	16.2-24.5	16.4-32.7	50.2-90.0	44.3-85.5

PART IV

Preliminary Baking Trial

It has been known that starch used as major ingredients in most snack food of the world for many years. However, during the past 20 years, the eating habit and life style pattern of most people has changing from a traditional practice to a more westernized one. Over consumption of energy especially from carbohydrate and less vegetables and fruits were observed. This leads to a rising incidence and mortality rate of chronic degenerative decrease among Thai population (Ministry of public health). Nowadays, more attention has been placed in healthy lifestyle especially desirable healthy diet. Many information from both experimental and epidemiological studies showed that low digestibility starchy food was consequent with their potential to reduce the risk of many chronic diseases such as diabetes, heart disease, obesity and cognitive disease (88, 89, 90, 91, 92, 93).

6.5 Formulation of rice flour and rice bran and application testing in snack products

Snacks are selected products for developing the low RAG products by replacing some wheat flour with rice flour (brown 313-19-1-1 rice) and/or rice bran, red bean and black sesame. Rice bran is known as a good source of vitamins and dietary fiber (38, 94). It also has high antioxidant, especially γ-oryzanol which is used for treating hyperlipoproteinenia (95). There were many researches reported that legumes and cereal had not only low GI but also influence to reduce blood glucose and plasma insulin. However, incorporation of rice flour and/or rice bran may cause poor characteristics.

Hence, in this study, rice flour was used for partially replacement of white wheat flour with rice flour and addition of rice bran, red bean and sesame in steamed bun, bread stick, red bean filled bun and bread. The replacement level was determined in accordance to the previous studies which involved the incorporation of rice flour and rice bran as the ingredients in snack products.

The levels of rice flour substitution in snacks were increased until the appearance of products could not be accepted. The incorporation levels of rice flour

in each mixed flour were 70 %, 50 %, 65 % and 44 % in steamed bun, bread stick, red bean filled bun and bread respectively.

The dough of all snack products was drier and less elastic resulting in a fragile structure in bread stick and easier collapse of surface in bun and bread after steaming or baking. The dilution effect of gluten in the dough by rice flour as well as cut the gluten strands caused the dough less elastic (96). Similar result was found in the study of Sudha et al. (2005) (94) which increase in the rice bran level leaded to more increase in water absorption. Fiber bread was prepared by Hung et al., (2005) (97). The fiber bread dough became weaker and lost elasticity with high incorporation of other flour due to greater amount of AC in the mixture. (97). The study of Waring (1998) (96) also indicated that addition of other flour in the bread formula caused the dough to be slightly drier, thus additional water could improve the texture and physical properties of the products. In order to improve the dough and product characteristics, water and gluten were added in the formulas. As mention in study of Waring (1998) (96), gluten in the range of 10-15% of flour weight was applied, and some extra water was added as well.

Not only addition of some ingredient but also the procedure was modified (97). The mixing and fermentation time of all products was extended. The doughs of all snacks were fermented (final proofing) for 10-15 minutes longer than the control bread. This agreed with the study of Wang (2002) which mentioned that the interactions between gluten and fiber prevent free expansion of wheat dough during the proofing stage (99).

Some product were added red bean and black sesame which are suitable to use because of that are legume and grain which are improving to low digestible and absorption (100).

6.6 Sensory Evaluation

The sensory acceptability scores of the in-house consumer test confirmed that the amount of addition ingredients slightly impacted ($p<0.05$) to general appearance, color suitability or sweet suitability in snack products. Nevertheless, the panelists could not detect the difference of the acceptability between the product added other ingredients and the control. In addition, after testing, the differences in each sensory attribute were more noticeable as indicated by the lower sensory acceptability scores in snack products from rice flour excepted bread stick. It was higher overall acceptability score significant difference than that of control products.

As seen in **Table 5.14** and **5.17,** the score of color suitability were detected significant difference to compare steamed bun and their storage condition and bread with their control. They were still accepted by the panelists. Since these modified products was added pigmented rice bran, but the red bean filled bun was added that of in filling. In agreement with the study of Sudha et al., in 2007 suggested that increasing rice bran to reflect the darkness and reduced the surface smooth. Above 30 % incorporation of rice bran in the formulation biscuits had hard dark crumb color, dry mouthfeel and very hard texture (94).

Sweet suitability score of all modified products excluded bread stick were significant difference to the control recipes. Because some sugar of the modified recipes were reduced and/or replaced with sorbitol which decreased sweetener for healthy human or diabetes.

No significantly differences ($p>0.05$) were found for the texture suitability score of all snack products. Although, panelists noted that it was less crunchy or had stronger floury odor than control formula. The wheat gluten was selected because of it can improve texture which rice flour was made non elastic texture. It is quite obvious that even at 50 % level of rice flour of bread stick, were still accepted by the panelists. The acceptability scores for bread stick were above 7 (like moderately) and no significant differences were detected in any attributes ($p > 0.05$). Comments from the panelists toward products were fragile and tender which is in agreement with the study of Waring (96) who formulated fiber cookies.

There was significant reduction ($p<0.05$) in sensory preference in the aspect of general appearance score of only rice flour enriched red bean filled bun. Because of,

all snack products, used shortening except red bean filled bun are combination with butter since it had better taste. Shortening is more suitable for creaming than butter (101). Thus, the final product in steamed bun, bread stick and bread were influenced more air which is incorporated and characteristics.

In addition, sensory panelists gave the remarkableness that rice flour products should have mouthfeel and odor nature within the products and some rated the color score as darkness. This is because the panelists were familiar with the products with unpolished rice flour and perceived that it should be darker color and some product were added black rice bran that are ingredient.

This study was indicated that rice flour substitution with 44 – 70 % and addition some rice bran inclusion level could be utilized in snack products with minimal defected in eating quality.

6.7 Rice Based Flour Snack Products and the Correlations between RAG and Various Starch Fractions

Health benefits have been reported for the consumption of carbohydrate that are slowly digested (low GI) or not digest in the small intestinal RS and fiber). The beneficial effects for improving the glycemic control in diabetes by decreasing in blood insulin or lipid metabolism and potential decrease in the risk for development of diabetes were observed (58, 102). Powell and Miller, 1995 indicated that the different sources of carbohydrate and different types of starch affect the postprandial glucose and insulin responses in both normal people and others with impaired glucose tolerance. Therefore, if the amount of GI can be detected or screen for those carbohydrate foods, it might be more beneficial to people, particularly, who should avoid of rapidly glucose and insulin response. In generally, starch is the main glycemic CHO in starch foods, according to the rate and extent of starch digestion *in vitro*, starch has been classified into three major fractions. First is rapidly digestion starch, the portion of starch digested with enzyme as the intestinal condition within the first 20 minutes of incubation, the second is SDS, the portion of starch digested from 20 to 120 minutes, and the lastly part is RS, the remaining portion that cannot be further digested. In addition, Englyst, 1996, 1999 and 2003 (5, 6, 24) demonstrated that GI in human study had highly significant positive correlation with *in vitro* RAG.

Englyst and Hudson (1996) explained that the measurement of RAG provides values for direct calculation of the amount of glucose likely to be rapidly absorbed, and thus to influence blood glucose and insulin level while the measurement of RDS, SDS and RS *in vitro* provides valuable predictions of the rate and extent of starch digestion in the small intestine. Therefore the study of this part was to use *in vitro* starch digestion for evaluating the rate of glucose releasing for snack products developed for low glycemic response. Analysis data showed that there were significant differences ($p<0.01$) on the RAG and RDS values between each control and modified products. Lower RAG and RDS values of all the modified products than the control ones mean than that the modified products would be more slowly digested and absorbed in the small intestinal and would induce insulin release and increase blood glucose more slowly than the control products. According to Englyst and Hudson in 1996 (24), low RAG values were associated with low levels of FG and a low proportion of starch measuring as RDS. In the present study, all modified products had significantly lower RAG values that the control products and were also associate with significantly lower RDS ($r = 0.984$), but not for the modified red bean filled bun which had lower RAG but contained higher RDS value than the control (21.53 vs 18.36 g/100g). This may be because the modified red bean filled bun contained the same amount of TG as the control but had significantly lower amount of FG (2.88 vs 9.08 g/100g) due to the replacement of sorbitol for sucrose in its ingredients. However, in the present study, correlation coefficient between RAG value and FG value showed a negative correlation and our finding is in agreement with Englyst et al., 2003 (27). In general, there are strong indicators that the large amount of RAG was derived from starch and FG in the diet and lead to preliodic elevated plasma glucose and insulin concentration (103). However, the finding of FG in present study was inconsistent with this suggestion because even though the RAG value was somewhat positively correlated with FG in some products, but not for modified bread which showed higher value of FG than the control whereas the rate of RAG releasing was lower, see in **Table 15.22**. The lower RAG both modified products even though higher FG might be the effect of the low GI, high dietary fiber and RS of the ingredients in he modified products (as seen in **Table 5.21**) that may help to decrease the rate of glucose releasing, especially red bean which contained in the ingredient of both products with approximately 10 %

red bean in bread stick and 17 % in bread. When considering on the amount of TG, total starch (TS) and RAG values, the results of correlation coefficient between RAG and TG; RAG and TS of the rice flour products indicated positive correlation with regression nearly 75% for TG ($r = 0.749$, $p < 0.01$) and more than 76 % for TS ($r = 0.762$, $p < 0.01$). The positive correlations between RAG and TG or TS mean that when the products contain high amounts of TG or TS, the RAG values of these products would be expected to be high. This was true for all the control products since control bread stick containing the highest TG and TS and then provided the highest RAG values, followed by control steamed bun, bread, and red bean filled bun, however, the results were somewhat inconsistent among all the modified products. Modified bread stick also contained higher amount of TG or TS but lower in RAG values when compared to other control products the contained lower TG or TS as seen in **Table 15.22**. This might be because the ingredients of both modified products contained red bean which could reduce the rate of RAG releasing. Various researches indicates that red bean had low GI (GI = 31 %) as well as GL (per serving) and had the lowest RAG value (55, 26). This also might be the reason for the lowest RAG values of both control and modified red bean filled bun when compare to other control and modified products since these products contained some considerable amount of red bean in their ingredients, as seen in **Table 5.22**. Moreover, the lower RAG values of all modified products than the control might come from the main ingredient of all modified products which were replaced with 35-65 % brown rice flour instead of wheat flour. The replacement of intermediate GI rice flour # 313-19-1-1 (105) for wheat flour in the ingredients of the modified products might contribute to the decrease of RAG values when compared to the controls due to the higher RS (3.47 g/100g) and total dietary fiber (3.54 g/100g) of the rice flour when compared to the wheat flour (0.08 and 2.7 g/100g, respectively), as seen in **Table 5.22**. Moreover the addition of rice bran and sesame seed also aid to reduce the RAG values since they had lower RAG, higher dietary fiber and RS (excepted rice bran) than wheat flour, especially sesame seed had very high RS (9.92 g/100g RS). Therefore, according to the ingredients of the modified products in this study (**Table 521**), it is not surprising that all modified rice base flour products had lower RAG values and contained higher amount of RS (0.15 – 0.52 g/100g) than the control products (0.07 to 0.37 %).

According to Englyst in 1996 and Jenkins in 2000 (5, 105), RS is defined as the starch and starch degradation products not absorbed in the small intestine and pass into the large intestine human. The amounts of RS in food were depending on various factors particularly, the sources of starch and type of food processing (5, 106). In the present study, the result from the correlation coefficient between RAG and RS, as seen in **Table 5.20** showed significant inversely related RS and RAG at $p < 0.01$. That means, if food had high amount of RS, the low RAG value was observed. The review of Asp and Bender reported that foods with a low glycemic often had a high content of RS, e.g. bean (107). Our explanation was supported by the various research which indicates that RS is not digested and absorbed in the small intestine and it does not directly increase blood glucose and insulin responses in overweight women and in people with impaired glucose tolerance and in people type 2 DM (84, 108, 109). Behall et al., suggested that people need at least 5 to 6 g of RS per day to be able to observe the beneficial effect on insulin response and also foods with a low GI often have a high content of RS. In addition, Saguilan, et al., 2007 indicated that high RS in cookies products (8.42% RS) was significantly lower in the percentage of starch hydrolysis than the control cookie (1.48 % RS) (84). Again, Kabir et al., 1998 (111) and Garcia-Alonso et al., 1999 (112) indicated that resistant starch consumption has been related to reduce postprandial glycemic and insulinemic responses. Moreover, brown rice flour as a main ingredient in the modified products contained higher amount of total dietary fiber (TDF) than wheat flour, as seen in **Appendix N**, therefore the replacement of rice flour for wheat flour would increase the amount of TDF in the modified products and make the products more beneficial to people health. Dietary fiber is widely recognized as an important part of the treatment and prevention of diabetes, colorectal cancer, gastrointestinal disorders, heart disease and obesity (113, 114). Various researches demonstrated that dietary fiber decreased serum insulin and plasma glucose concentration (103). Jimenez-Cruz et al., in 2004 indicates that dietary fiber is one of the food factors that affect the role of digestion of foods and the consumption of dietary fiber (soluble fiber) may also improve glucose tolerance in people with diabetes (115). However, in 1990, Wolever did not found any affect of soluble fiber on the GI but insoluble fiber was significantly related to the GI response (116).

CHAPTER VI
CONCLUSION

For method validation, the results of RAG values of some fruits model were strongly significant correlation with GL values $p < 0.05$. In addition, analysis data of sorbitol and guar gum showed low RAG values similar to literatures which found low glycemic response. Especially, the RAG value in brown rice # 313-19-1-1 (intermediate RAG; 13.53 g/100g cooked rice) also showed similar value to glycemic response (intermediate level with GI 58%) studied in type-2 diabetes. Therefore, *in vitro*, RAG method of Englyst et al., can be used as a screening tool due to its high correlated with the GI value, simple and inexpensive method.

The RAG content of various rice varieties were ranged with quartile deviation. Low level of the RAG values were in milled Hom Mali Sichompoo, brown KD-BT 313-19-1-1 (harvest in summer), milled Hom Mali Thung kula Roi Et, milled Leuang-orn, brown K368, milled Hom Mali Khemmarat, milled Hom Mali Chiangrai (middle), brown K310, 334, 349 and brown rice IR71501 × Pin Kaset3-1-22-6-1 (<14.01 g/100g). The intermediate level of RAG was in brown K383, brown 23-215, milled Hom Mali Arawan, brown BT#3, milled Sao Hai Jekchei (old), milled Sao Hai Saraburi (old), brown white glutinous KDC18-3-7-1-11-0, milled Hom Mali Thung kula (Middle), brown KD-BT 909-10-1-3, brown Hom Pathumthani, milled 23-215, (14.01-18.88 g/100g), as seen in **Table 5.4** and **5.5**. Therefore, rice varieties which showed low and intermediate amounts of RAG values might be expected to give low and intermediate glycemic and insulinemic response. As mentioned above, all the rice varieties which revealed low and intermediate level of RAG might be assumed to be potentially useful to provide low or intermediate GI diet for normal people or diabetic to select for their consumption. All the cooked glutinous rice of present study was not only high in the amounts of RAG (>20 g/100g cooked rice) but also very low in RS (0.00-0.03 g/100g cooked rice) and AC (<10.50%). Significant inversely correlation

between RAG and AC was observed whilst positive correlation was found with RS at $p < 0.05$.

Analysis data indicates that many varieties of rice and four modified snack based flour products in this study might have a potential application to a low or intermediate GI. However, *in vitro* method could be only used as a screening tool for selecting the best varieties of rice and foods in order to explore or apply to further human study.

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APPENDIX

APPENDIX A

Determination of amylose

Principal:

One hundred milligrams of sample was weighed accurately in duplicate and extracted with 1 ml of 95 % ethanol and 9 ml of 1M NaOH. The sample was heated for 10 min in a boiling water bath to geletinized the starch; cooled and trnsferred with deionized water; mixed well and let stand for one night. Five milliliters of the starch solution was pipetted into 100 ml volumetric flask, 1 ml of 1 M acetic acid and 2 ml of iodine solution (mixture of iodine and potassium iodide) added and measured absorbance with spectrophotometer at wavelength 620 nm. Amylose content was determined by reference to standard curve.

Reagent:

1. 95% Ethanol
2. 1 M NaOH
3. 1 M Acetic acid (Merck # 100063)
4. Iodide solution
   (Iodide Merck # 4761 & Potassium iodide: Ajex Finechem # A409)
5. Ethyl alcohol
6. Amylose type III: from potato (Sigma # A-0512)

Mixture solution

2.0 % KI and 0.2 % I_2: Weight 2.0 g Iodine and 20 g KI and dissolve with DI water to 1000 ml and mix for overnight or until the mixture solution clear.
Glacial acetic acid 1 M: 60.05 ml glacial acetic acid dilute to 1 L with DI water

Procedure:

1. Weighed 100 mg of whole-grain milled rice in duplicate, into a 50 ml Erlenmayer flask.

2. Added 1 ml of 95 % ethanol and 9 ml of 1 M NaOH.
3. Heated for 10 min in a boiling-water bath, cooled transferred with several water, into a 100 ml volumetric flask; brought up to volume with deionized water and mixed well.
4. Pipette 5 ml of starch solution into a 100 ml volumetric flask.
5. Added 1 ml of 1 M acetic acid and 2 ml of iodine solution (0.2 g iodine and 2 g potassium iodide in 100 ml of aqueous solution)
6. Made up the solution with distilled water, shaken and let stand for 20 min.
7. Measured absorbance at 620 nm.

Standard curve:

1. Weight 40 mg of staredard amylose (from potato) and added 1 ml ethyl alcohol and 9 ml 1M NaOH.
2. Heat for 5-10 min in a boiling-water bath, cooled and made up to 100 ml.
3. Pipette 1, 2, 3, 4 and 5 ml in 100 ml volumetric flask.
4. Acidified with 1 M acetic acid (0.2, 0.4, 0.6, 0.8 and 1 ml respectively) and treat as above.
5. Measure absorbance at 620 nm.

Preparing working blank and standards of amylose

Vol. of standard (ml)	Vol. of 1 M acetic acid (ml)	Vol. of iodine solution (ml)	Final Volume (ml)
0 (blank)	1	2	100
1	0.2	2	100
2	0.4	2	100
3	0.6	2	100
4	0.8	2	100
5	1	2	100

APPENDIX B

Determination of Rapidly available glucose (RAG), slowly available glucose (SAG) and total glucose (TG)

Reagent:

1. Amyloglucosidase (from *Aspergillus niger* 300 U/mL: Sigma # A7095)
2. Invertase (from Bakers yeast: Sigma # I9247)
3. Pancreatin (from porcine pancreas: Sigma # P1500)
4. Pepsin (from porcine stomach mucosa: Sigma # P7000)
5. Glucose-oxidase peroxidase (GOD-PAP) (Sigma # G3660)
6. Sodium acetate trihydrate ($CH_3COONa.3H_2O$) (Merck # 106267)
7. Guar gum (Sigma # G9752)
8. Benzoic acid (0108G USA) (FSN # 505-153-8233)
9. D-glucose (M&B # G15/18/67)
10. Calcium chloride ($CaCl_2$) (Merck # 102083)
11. Glacial acetic acid (CH_3COOH) (Merck # 100063)
12. 37% Hydrochloric acid (HCl) (Merck # 100317)
13. Sulfuric acid (H_2SO_4) (Merck # 100731)
14. Sodium hydroxide (NaOH) (Merck # 106498)

Procedure:

Prepare reagent solution

Sodium acetate buffer (0.1 M)

1. Dissolve 13.6 g sodium acetate trihydrate ($CH_3COONa.3H_2O$) in 250 ml saturated benzoic acid solution, and make to 1 l with water.
2. Adjust to pH 5.2 with 0.1 M acetic acid.
3. To stabilize and activate enzymes, add 4 ml 1 M $CaCl_2$ per liter of buffer.

Glucose standard solution

1. Weight 5 g D-glucose to the nearest 0.1 mg.
2. Make up to 200 ml with sodium acetate buffer to give a 25 mg/ml solution.

Enzyme solution I

1. Dilute 1.85 ml 300 U/ml amyloglucosidase
2. Weigh 3.0 g pancreatin into 50 ml centrifuge tube and suspend 20 ml distillation water.
3. Add stirrer and stir magnetically or 10 min, then centrifuge or 0 min at 1500 g.
4. Take 19 ml supernatant from tube and mix with 6 m dilute

Measure RAG and SAG

1. Weigh samples (containing < 0.3 g carbohydrate) into 50-ml polypropylene centrifuge tubes Add 5 ml freshly prepares pepsin–guar gum solution (5 g/l pepsin and 5 g/l guar gum in 0.05 M HCl).
2. Cap tubes and vortex mix and place into a water bath at 37 ºC for 30 min.
3. Add 10 ml 0.5 M sodium acetate (equilibrated to 37 ºC) to each tube to form a buffer at pH 5.2.
4. Add 5 glass balls cap and shake and place in the 37 ºC water bath to equilibrate.
5. Remove tubes from the 37 ºC water bath
6. Add 2.5 ml enzyme mixture (pancreatin, invertase and amyloglucosidase) and immediately cap and mix gently before take into the 37 ºC shaking water bath.
7. Start at this time until 20 min (G20) and 120 min (G120) portions are collect (0.2 ml) and add to 4 ml absolute ethanol.
8. Dilute with DI water, add 120 µl glucose-oxidase peroxidase kit and placed in the 37 ºC water bath to incubate 30 min.
9. Add 120 µl 6 M sulfuric acid and measure at 540 nm by microplate reader.

Measure of TG

Continue tubes from the shaking water-bath of determination RAG and SAG.

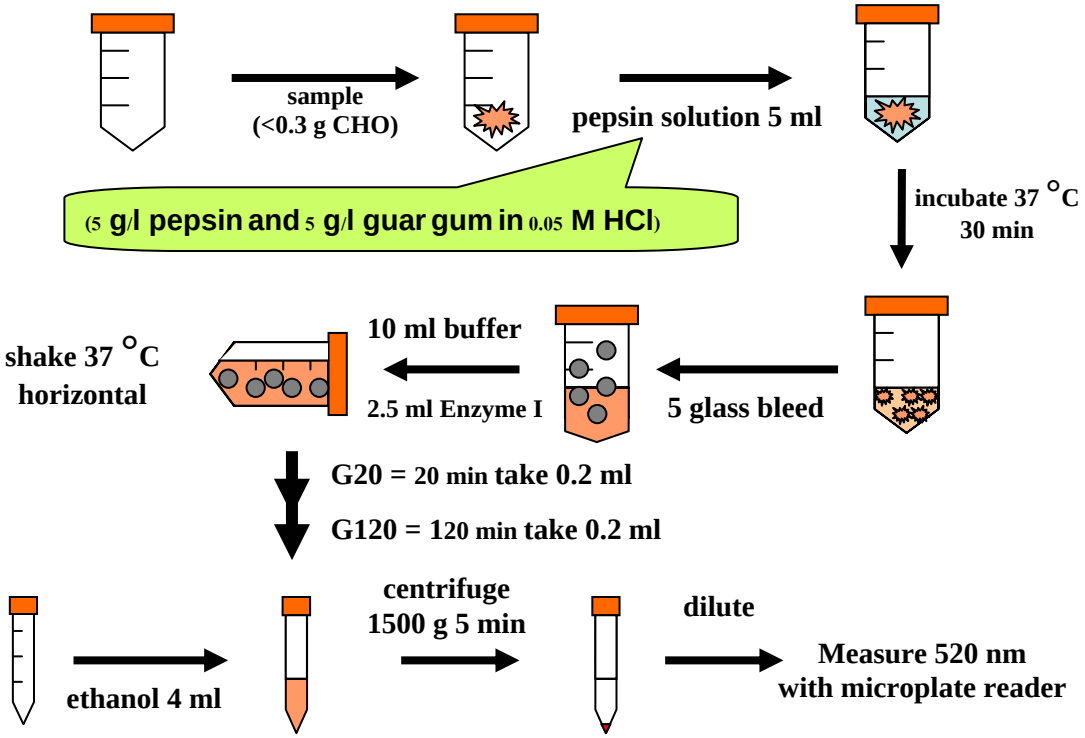
1. Vortex mix or shake vigorously to break up large particles.
2. Place tubes in a boiling water-bath for 30 min.
3. Vortex mix or shake again. Take 1.75 ml portion from 50 ml centrifuge tube to 15 ml centrifuge tube.

4. Cool the tubes in ice-water 15-20 min.
5. Add 0.5 ml 7 M KOH and mix well. Immerse tubes horizontally in the shaking water-bath containing ice-water (0 °C) securing firmly.
6. Incubate, with shaking, for 30 min. prepare in advance a second set on 15 ml centrifuge tubes and into each pipette 2 m 0.5 M acetic acid.
7. Remove the sample tubes from the ice water and immediate take 0.2 ml of the content into the acetic acid and mix well.
8. Add 40 µl diluted amyloglucosidase (50 AGU/ml), mix and incubate for 30 min at 70 °C.
9. Transfer the tubes to boiling water bath for 10 min.
10. Cool to room temperature then dilute with 4 ml water and centrifuge at 1500 g for 5 min to remove the precipitate. Dilute with DI water, add 120 µL glucose-oxidase peroxidase kit and placed in the 37 ºC water bath to incubate 30 min.
11. Add 120 µl 6 M sulfuric acid and measure at 540 nm by microplate reader.

Calculation:

$$\% \text{ glucose} = \frac{At \times Vt \times C \times D}{As \times Wt} \times 100$$

- At = absorbance of test solution
- Vt = total volume of test solution
- C = concentration (in mg/ml glucose) of standard
- As = absorbance of standard
- Wt = weight (in mg) of sample taken for analysis, which may be corrected for moisture
- D = dilution factor



Measurement of RAG and SAG

APPENDIX C

Determination of Free glucose (FG)

Reagent:

1. Amyloglucosidase (from *Aspergillus niger* 300 U/mL: Sigma # A7095)
2. Invertase (from Bakers yeast: Sigma # I9247)
3. Glucose-oxidase peroxidase (GOD-PAP) (Sigma # G3660)
4. Sodium acetate trihydrate ($CH_3COONa.3H_{B2}O$) (Merck # 106267)
5. Sulfuric acid (H_2SO_4) (Merck # 100731)
6. Calcium chloride ($CaCl_2$) (Merck # 102083)
7. Benzoic acid (0108G USA) (FSN # 505-153-8233)

Sodium acetate buffer (0.1 M)

4. Dissolve 13.6 g sodium acetate trihydrate ($CH_3COONa.3H_2O$) in 250 ml saturated benzoic acid solution, and make to 1 l with water.
5. Adjust to pH 5.2 with 0.1 M acetic acid.
6. To stabilize and activate enzymes, add 4 ml 1 M $CaCl_2$ per liter of buffer.

Procedure:

1. Pipette 25 ml 0.1 M acetate buffer into each sample tube, and shake or vortex-mix vigorously to begin disrupting large particles.
2. Place the tube into a water bath at 100 °C for 30 min, shaking occasionally to prevent aggregation.
3. Remove the tubes to bath at 37 °C, shaking vigorously again.
4. When equilibrated, add 0.2 ml invertase, cap, and immerse horizontally in the shaking water-bath at 37 °C securing firmly.
5. Incubate with shaking, for 30 min.
6. Take the tubes out of the bath and shake vigorously.

7. Remove 1 ml of the contents into a test tube containing 2 ml ethanol, a vortex-mix. (At this stage, continue with the main sample tube and return to the FG portions when convenient.)
8. Centrifuge the portion at 1500 g for 5 min. dilute 1 ml supernatants in 5 ml water (samples) or 20 ml water (standards), mixing well by inversion.
9. Dilute with DI water, add 120 µl glucose-oxidase peroxidase kit and placed in the 37 ºC water bath to incubate 30 min.
10. Add 120 µl 6 M sulfuric acid and measure at 540 nm by microplate reader.

Calculaion

TS = (TG-FG) x 0.9

RDS = (G20 – FG) x 0.9

SDS = (G120 – G20) x 0.9

RS = TS – (RDS + SDS) or (TG – G120) x 0.9

SDI = RDS x 100 /TS

APPENDIX D

Determination of resistant starch (RS)

(International Ireland LTD: code K-RSTAR)

I. Preparation reagen

All reagents should be analytical purity grade.

a. 0.1 M Sodium maleate buffer pH 6.0

Dissolve 23.2 g maleic acid in 1600 ml of distilled water and adjust the pH to 6.0 with 4 M (160 g/l) sodium hydroxide. Add 0.6 g of calcium chloride ($CaCl_2$.2H O) and 0.4 g of sodium azide and adjust the volume to 2 l. Stable at 4 °C for 12 months.

b. 1.2 M Sodium acetate buffer pH 3.8.

Add 69.6 ml of glacial acetic acid to 800 ml of distilled water and adjust to pH 3.8 using 4 M sodium hydroxide. Adjust the volume to 1 l with distilled water. It was stable at room temperature for 12 months.

c. 100 mM Sodium acetate buffer pH 4.5

Add 5.8 ml of glacial acetic acid to 900 ml of distilled water and adjust to pH 4.5 using 4 M sodium hydroxide. Adjust the volume to 1 l with distilled water. Stable at 4 °C for 2 months.

d. 2 M Potassium Hydroxide.

Add 112.2 g KOH to 900 ml of distilled water and dissolve by stirring. Adjust volume to 1 l.

e. 50 % v/v Aqueous IMS

Dilute 500 ml of ethanol (95% or 99%) or industrial methylated spirits (IMS; denatured ethanol; ~ 95% ethanol plus 5% methanol) to 1 l with H O. Store in a well 2 sealed bottle. Stable at room temperature for > 12 months.

f. 3300 U/ml Stock amyloglucosidase (AMG) solution in 50% glycerol.

Stable for up to 5 years when stored at 4 °C. (One unit of enzyme activity is amount of enzyme required to release 1 micromole of glucose from soluble starch per minute at 40 °C and pH 4.5). AMG solution should be free of detectable levels of free glucose.

g. 300 U/ml amyloglucosidase solution

Dilute 2 ml of concentrated AMG solution [I(f)] to 22 ml with 0.1 M sodium maleate buffer (pH 6.0)[I(a)]. Divide into 5 ml aliquots and store frozen in polypropylene containers between use. Stable to repeated freeze-thaw cycles, and for > 5 y at -20 °C.

h. 10 mg/ml Pancreatic α-amylase plus 3 U/ml AMG.

Immediately before use suspend 1 g pancreatic α-amylase in 100 ml of sodium maleate buffer [I(a)] and stir for 5 min. Add 1.0 ml of 300 U/ml AMG [I(g)] and mix well. Centrifuge at > 1,500 g for 10 min and carefully decant the supernatant. This solution should be used on the day of preparation.

i. Glucose oxidase-peroxidase-aminoantipyrine reagent (GOPOD).

Prepare glucose oxidase-peroxidase-aminoantipyrine reagent (GOPOD) mixture by diluting 50 ml of buffer concentrate to 1.0 l. Use part of this diluted buffer to dissolve the entire contents of the vial containing freeze-dried glucose oxidase-peroxidase-aminoantipyrine mixture. Quantitatively transfer the contents of the vial to 1 l volumetric flask containing diluted buffer. Reagent is stable 2-3 months when stored at 4 °C and > 2 years when stored at -20 °C.

II. Measurement of Resistant Starch

(a) Hydrolysis and solubilisation of non-resistant starch.

i. Accurately weigh a 100 + 5 mg sample directly into each screw cap tube and gently tap tube to ensure that the sample falls to the bottom.

Note: For wet samples such as minced canned beans or food product, the sample size is approximately 0.5 g (weighed accurately). With such materials, the moisture content is usually 60-80%.

ii. Add 4.0 ml of pancreatic -amylase (10 mg/ml) containing AMG (3 U/ml [I (h)] to each tube.

iii. Tightly cap the tubes, mix them on a vortex mixer and attach them horizontally in a shaking water bath, aligned in the direction of motion.

iv. Incubate tubes at 37 °C with continuous shaking (200 strokes/min for exactly 16 hr. (linear motion, a setting of 100 on the water bath is equivalent to 200 strokes/min; 100 forward and 100 reverse).

v. Remove the tubes from the water bath and remove excess surface water with paper towel. Remove the tube caps and treat the contents with 4.0 ml of ethanol (99%) or IMS (99%) with vigorous stirring on a vortex mixer.
vi. Centrifuge the tubes at 1,500 g (approx. 3,000 rpm) for 10 min (non-capped).
vii. Carefully decant supernatants and re-suspend the pellets in 2 ml of 50% ethanol or IMS [I(e)] with vigorous stirring on a vortex mixer. Add a further 6 ml of 50% IMS, mix the tubes and centrifuge again at 1,500 g for 10 min.
ix. Decant the supernatants and repeat this suspension and centrifugation step once more.
x. Carefully decant the supernatants and invert the tubes on absorbent paper to drain excess liquid.
(b) Measurement of Resistant Starch.
i. Add a magnetic stirrer bar (5 x 15 mm) [II(h)] and 2 ml of 2 M KOH [I(d)] to each tube and re-suspended the pellets (and dissolve the RS) by stirring for approx. 20 min in an ice/water bath over a magnetic stirrer.
NOTE:
1. Do not mix on a vortex mixer as this may cause the starch to emulsify.
2. Ensure that the tube contents are vigorously stirring as the KOH solution is added. This will avoid the formation of a lump of starch material that will then be difficult to dissolve.
ii. Add 8 ml of 1.2 M sodium acetate buffer (pH 3.8) [I(b)] to each tube with stirring on the magnetic stirrer. Immediately add 0.1 ml of AMG (3300 U/ml) [I(f)], mix well and place the tubes in a water bath at 50 C.
iii. Incubate the tubes for 30 min with intermittent mixing on a vortex mixer.
iv. **For samples containing > 10% RS content;**
Quantitatively transfer the contents of the tube to a 100 ml volumetric flask (using a water wash bottle).Use an external magnet to retain the stirrer bar in the tube while washing the solution from the tube with the water wash bottle. Adjust to 100 ml with water and mix well. Centrifuge an aliquot of the solution at 1,500 g for 10 min.
v. **For samples containing < 10% RS content;**
Directly centrifuge the tubes at 1,500 g for 10 min (no dilution). For such samples, the final volume in the tube is approximately 10.3 ml (however, this volume will vary

particularly if wet samples are analyzed, and appropriate allowance for volume should be made in the calculations).

vi. Transfer 0.1 ml aliquots (in duplicate) of either the diluted or undiluted supernatants into test tubes, treat with 3.0 ml of GOPOD reagent [I(i)] and incubate at 50 °C for 20 min.

vii. Measure the absorbance of each solution at 510 nm against the reagent blank.

Prepare reagent blank solutions

Mixing 0.1 ml of 0.1 M sodium acetate buffer (pH 4.5) [I(c)] and 3.0 ml GOPOD reagent.

Prepare glucose standards (in quadruplicate)

Mixing 0.1 ml of 1 mg/ml glucose and 3.0 ml GOPOD reagent.

III. Calculations

Calculate resistant starch in test samples as follows

Resistant Starch (g/100g sample) (samples containing > 10% RS):

= ΔE x F x 100/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 90.

Resistant Starch (g/100g sample) (samples containing < 10% RS):

= ΔE x F x 10.3/0.1 x 1/1000 x 100/W x 162/180

= ΔE x F/W x 9.27.

where:

ΔE = absorbance (reaction) read against the reagent blank.

F = conversion from absorbance to micrograms (the absorbance obtained for 100 μg of glucose in the GOPOD reaction is determined and F = 100 (μg of glucose) divided by the GOPOD absorbance for this 100 μg of glucose.

100/0.1 = volume correction (0.1 ml taken from 100 ml).

1/1000 = conversion from micrograms to milligrams.

W = dry weight of sample analyzed

= "as is" weight x (100-moisture content)/100

100/W = factor to present RS as a percentage of sample weight.

162/180 = factor to convert from free glucose as determined to qanhydro-glucose as occurs in starch.

10.3/0.1 = volume correction (0.1 ml taken from 10.3 ml) for samples containing 0-10% RS where the incubation solution is not diluted and the final volume is ~ 10.3 ml.

APPENDIX E
แบบสอบถามการประเมินผลทางประสาทสัมผัส

ผลิตภัณฑ์ เพื่อสุขภาพ

ชื่อ นาย/นาง/น.ส. _____นามสกุล_____อายุ _____ปี รหัสผลิตภัณฑ์_____

วันที่ เดือน พ.ศ. 2550 เวลา_____ น.

ตอนที่ 1 ก่อนชิม กรุณาให้คะแนนความชอบของท่าน โดยการมอง แล้วขีดเครื่องหมาย (√) ลงในช่องว่างให้ตรงกับความรู้สึกของท่านมากที่สุด

ความชอบลักษณะโดยทั่วไป หมายถึงความชอบของท่านเมื่อเห็นผลิตภัณฑ์

( ) ชอบมากที่สุด

( ) ชอบมาก

( ) ชอบปานกลาง

( ) ชอบเล็กน้อย

( ) อยู่ระหว่างชอบกับไม่ชอบ

( ) ไม่ชอบเล็กน้อย

( ) ไม่ชอบปานกลาง

( ) ไม่ชอบมาก

( ) ไม่ชอบมากที่สุด

ตอนที่ 2 หลังชิม กรุณาให้คะแนนโดยขีดเครื่องหมาย (√) ลงในช่องว่างให้ตรงกับความรู้สึกของท่านมากที่สุด

1. ความชอบผลิตภัณฑ์โดยรวม	2. สี	3. ความหวาน	4. เนื้อสัมผัส
( ) ชอบมากที่สุด	( ) เข้มเกินไปมาก	( ) หวานเกินไปมาก	( ) แข็งเกินไปมาก
( ) ชอบมาก	( ) เข้มเกินไป	( ) หวานเกินไป	( ) แข็งเกินไป
( ) ชอบปานกลาง	( ) กำลังดี	( ) กำลังดี	( ) กำลังดี
( ) ชอบเล็กน้อย	( ) เข้มน้อยเกินไป	( ) หวานน้อยเกินไป	( ) นุ่มเกินไป
( ) อยู่ระหว่างชอบกับไม่ชอบ	( ) เข้มน้อยเกินไปมาก	( ) หวานน้อยเกินไปมาก	( ) นุ่มเกินไปมาก
( ) ไม่ชอบเล็กน้อย			
( ) ไม่ชอบปานกลาง			
( ) ไม่ชอบมาก			
( ) ไม่ชอบมากที่สุด			

ข้อเสนอแนะ

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APPENDIX F

Determination of moisture

Hot-air-oven method; AOAC 2000, 952.45

Principle:

A well homogeneous sample is dried in an oven (usually at 100 ± 5 °C) until constant weight is obtained. The loss of weight is taken as a measure of moisture content in the sample. Acid washed sand is used to mix with the wet sample prior to dry in order to increase area for rapid of complete evaporation of water from the wet sample.

Procedure:

1. Weigh approximate 20 g of acid washed sand into a porcelain dish containing a small glass stirring rod and dry in hot air oven at 100 ± 5 °C for 30 min.
2. Remove the sand dish and cool in the desicator.
3. Weigh sand dish (= a g) and then approximately 5 g sample. Reweigh (= b g)
4. Add small amount of distilled water to disperse the sample evenly and evaporate the water as mush possible on the boiling water bath. The sample dish should be frequently mixed until dries.
5. Transfer the sample dish to hot air oven and dry the sample at 100 ± 5 °C for 2 hr.
6. Remove the sample dish and cool in desicator and weigh (= c g)
7. Return the sample dish to the hot air oven and dry until a constant weight is obtained. Reweigh every 30 min.
8. The different weight between each interval time should not be more than 1-3 mg

Calculation:

$$\% \text{ moisture} = \frac{(b-a)}{(b-c)} \times 100 \text{ (w/w)}$$

APPENDIX G

Determination of crude fat

(Soxhlet extraction; AOAC 2000, 945.16)

Principal:

Fats or lipids are characterized by their considerable solubility in organic solvents not in water and their physical properties which reflect their hydrophobic, hydrocarbon nature. Fats can be conveniently determined in food extracting the dried ground sample with light petroleum or equivalent solvents in a continuous extraction apparatus called a soxhlet. The selected method for extraction depends on the type of sample. Extraction of lipids from milk is relatively simple compared to the extraction of lipids from plant or animal tissue. In order to ensure that all the free fat is extracted, acid hydrolysis is introduced to our routine analysis before a petroleum ether extraction. The hydrolysis liberates the lipid portion of lipopolysaccharide, lipoprotein, and other lipid conjugates.

Reagent:

1. 4 M hydrochloric acid (Merck # 1.00317)
2. Petroleum ether (B.P. 35-60 C) (J.B. Baker # 9268-05)

Instruments:

1. Erlenmeyer flask 250 mL
2. Filter paper # 595
3. Soxhlet extractor
4. Thimble
5. Flat bottom flask

Procedure:

1. Weigh 5 g of sample into an Erlenmeyer flask.
2. Add 50 mL of 4 M hydrochloric acid, and then mix well.
3. Connect to an air condenser and reflux with gentle boiling for 1 hr.

4. Sample the filtrated by filter paper (Whatman # 595) and washed with warm water until the filtrate became free from acid by using pH paper
5. Dry the filter paper containing digested sample in an oven at 60 C for 1-2 hr. Then transfer it into an extraction thimble.
6. Dry the flat bottom flask in an over at 100 c for 30 min and weigh.
7. Add 160 mL of petroleum ether into a pre-weighed round flat bottom flask, then connect with soxhlet extractor. Extract the petroleum ether in the flask on a steam bath in a fume hood.
8. Dry the flask in an oven at 60 C until constant weight is obtained.

Calculation:

$$\% \text{ Crude fat} = \frac{\text{average weight of fat (g)}}{\text{Weight of sample}} \times 100$$

APPENDIX H

Determination of crude protein

Micro-Kjeldahl method; AOAC 2000, 981.10

Principle:

The foodstuff is oxidized by heating with concentrated sulfuric acid in the presence of catalyst. Sodium or potassium sulfate is frequently added to raise the boiling point of sulfuric acid. In this digestion process, nitrogen in the sample is converted to ammonium sulfate. After making alkali with concentrated sodium hydroxide, the ammonia is distilled and trapped with know amount of standard acid. The uncreated acid is then determinate by titration with standard acid solution. The amount of acid used indicates the ammonia liberated from the foodstuff. Nitrogen content is hence determinate. To obtain protein content, the amount of the nitrogen in the sample is multiplied by the converting factor, 6.25 being usually used since most protein contain 16 % nitrogen.

Reagents:

1. Concentrated sulfuric acid (Merck # 1.00731)
2. Catalyst; Potassium sulfate + selenium (IV) dioxide (1050 + 1.5 w/w) (Potassium sulfate: Codex # 363607; Selenium (IV) dioxide: Merck # 8.00953)
3. 50 % Sodium hydroxide (Merck # 1.06498)
4. Hydrochloric acid (standardized) (Merck # 1.00317)
5. Indicator solution: 0.1 % methylene blue + 0.2 5 methyl red (methylene blue: Merck #133449; methyl red # 1.06076)
6. 2% Boric acid (Merck # 1/00165)
7. Bushi 420 digester with Bushi 412 scrubber
8. Buchi 323 distillation unit

Procedure:

1. Weigh accurately 0.5-3 g (0.002-0.2 g N) of homogenized sample and transfer to digestion tube.
2. Add about 9 g of catalyst mixture and one glass bead.
3. Add 25 mL conc. Sulfuric acid and place the digestion tube in the digester.
4. Digest for additional 1.5 hr after the solution becomes clear or unit oxidation is complete and cool at room temperature.
5. Place 500 mL Erlenmeyer flask containing 100 mL of 25 % boric acid few drops of indicator in the distillation with a trip of the condenser extending below the surface of the acid solution.
6. Connect the digestion tube to the distillation unit.
7. Add 120 mL of water and 80 mL of 50 % sodium hydroxide to the digested tube by pressing a start button. The ammonia distillate is let through the splash head and condensed into a flask containing boric acid solution.
8. Distilled for 4 min, then lower the receiver flask so that the tio of the condenset above the solution, wash down the deliver tube with water and allow the washing to drain into the flask.
9. Titrate the ammonia in the flask back to the original purplish color with standardized 0.1 M HCl.
10. The procedure of a blank follow exactly the same method as the sample.

Calculation:

$$\% N = \frac{\text{titration (sample-blank) x N of NaOH x 1.4007 x 100}}{\text{Weight of sample x 1000}}$$

% protein = % N x appropriate converting factor

APPENDIX I

Determination of Total Dietary fiber

(Enzymatic Gravimatric Method; AOAC 2000, 991.43)

Principle:

Total dietary fiber (TDF) was determined by modification of the enzymatic gravimatic method of modified AOAC (2000) method. The dried samples are gelatinized with heat stable α-amylase and then the sample was enzymatically digested with protease and amyloglucosidase to remove protein and remain starch. 95% ethanol was added to precipitate the soluble dietary fiber. The total residue is filtered washed with 78% ethanol, 95% ethanol and acetone, respectively. After dried and weighed, protein and ash in the residue were measured and substracted from the total residue. Then the total dietary fiber content of the food sample is obtained. Step in the measurement of total dietary fiber are summarized in Figure 9

Reagent:

1. 0.08 M Phosphate buffer pH 6.0: Weight 1.400g of sodium phosphate diabasis anhydrous (Na_2HPO_4) and 9.68 g sodium phosphate monobasic monohydrate (NaH_2PO_4) into 700 ml. deionised water and dilute to 1 l with deionised water. Check pH with pH meter.
2. Alpha-amylase enzyme. Store in refrigerator
3. Protease enzyme. Store in refrigerator
4. Amyloglucosidase enzyme Store in refrigerator
5. 0.275 N Sodium hydroxide solution: Dissolve 11.0 g NaOH in 1000 ml deionised water.
6. 0.325 N Hydrochloric acid solution : Dilute stock solution of know titer
7. Celite 545, Acid washed
8. 78%, 95% ethanol and acetone (v/v)

Determination

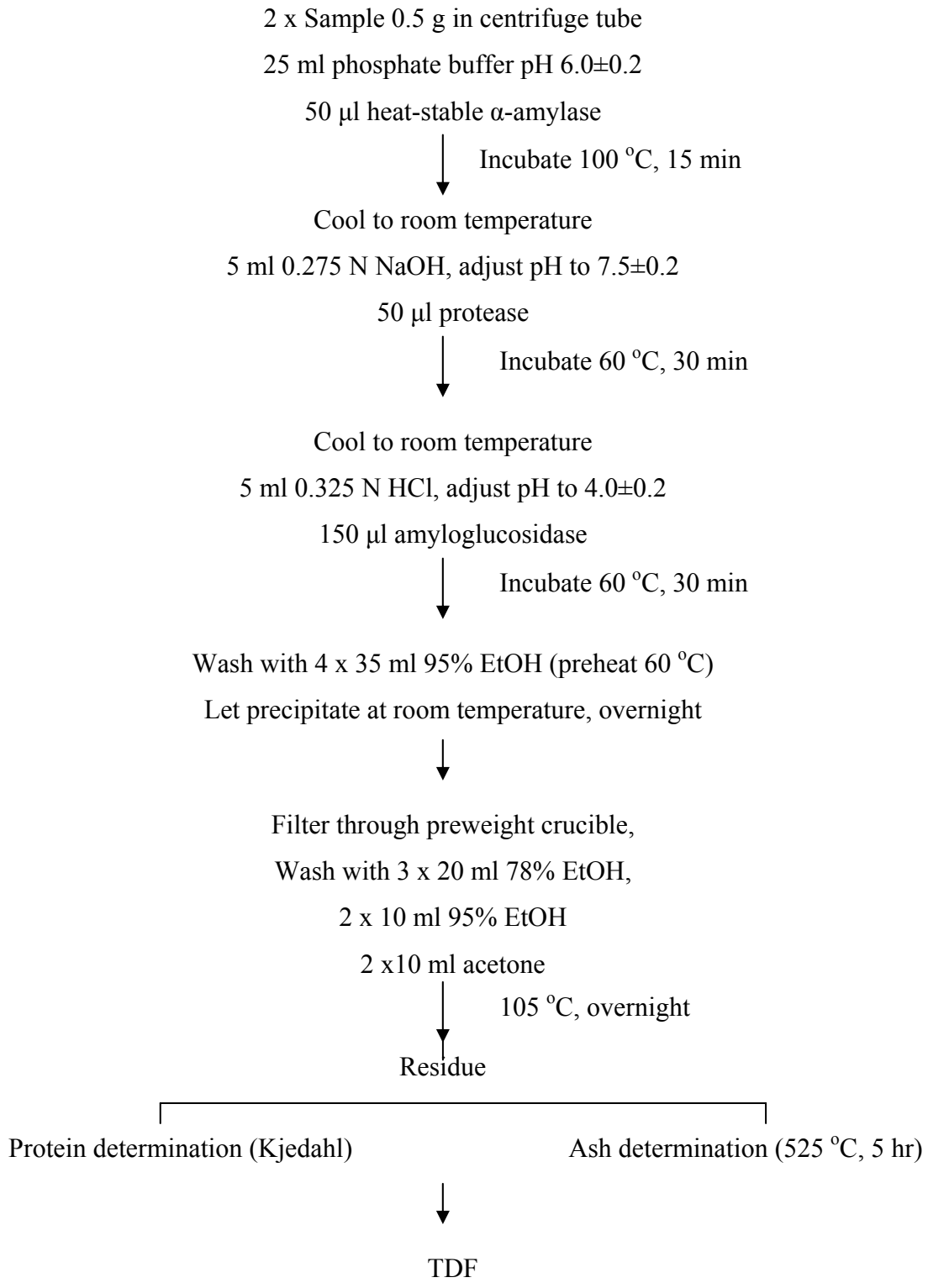

Figure 9 measurement of total dietary fiber

Calculation:

Blank = residue – protein – ash

$$TDF(g\ \%) = \frac{(residue - protein - ash - blank) \times 100}{Weight\ of\ sample}$$

APPENDIX J

RAG and SAG of orange juices and sugar cane syrups[1] (per 100 ml wet weight).

NAME	RAG (g/100ml)	SAG (g/100ml)
25 % Orange juice	3.95	1.92
Fresh orange juice	2.54	0.24
100 % Orange juice	1.55	0.75
12.5 % w/w sugar cane syrup	11.67	-1.84
25 % w/w sugar cane syrup	23.82	4.84
50 % w/w sugar cane syrup	53.22	7.80

[1]Values are a mean of duplicate analysis.

APPENDIX K

Nutritive analysis of rice products replacement with rice flour (g/100 g wet weight) [1, 2]

Type of product	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Energy (Kcal)
Steamed bun	35.18	1.11 (0.50)	7.07 (3.52)	6.32 (1.62)	50.32	286.44
Bread stick	1.11	2.51 (0.83)	13.28 (2.49)	13.24 (0.77)	69.86	451.72
Red bean filled bun	29.47	1.78 (0.96)	9.49 (0.30)	6.97 (0.85)	52.29	309.85
Bread	37.65	1.46 (2.82)	10.17 (0.52)	9.22 (4.62)	41.5	289.66

[1]Result are mean (SD) of the analysis in duplicate.
[2]Factor of 4, 4 and 9 were used to calculate the energy from protein, carbohydrate and fat respectively

APPENDIX L

CHO content of snack products (g/100g as eaten)*

NAME		RAG	SAG	TG	FG	RDS	SDS	TS	RS	TDF	SDI£
Steamed bun	control	45.64 [b] (2.18)	3.52 [a] (2.13)	49.88 [b] (3.17)	4.75 [b] (0.25)	36.80 [b] (1.96)	3.17 [a] (1.92)	40.62 [a] (2.86)	0.07 [a]	1.6	90.90 [b] (6.45)
	modified	35.27 [a] (1.23)	4.33 [a] (1.86)	44.42 [a] (1.57)	1.88 [a] (0.21)	30.06 [a] (1.11)	3.90 [a] (1.67)	38.29 [a] (1.41)	0.15 [b]	1.4	78.57 [a] (2.84)
Bread stick	control	62.34 [b] (1.65)	9.82 [a] (1.20)	71.78 [a] (4.58)	1.93 [a] (0.22)	54.37 [b] (1.49)	8.84 [a] (1.08)	62.86 [a] (4.13)	0.26 [a]	2.9	86.73 [b] (5.67)
	modified	40.32 [a] (1.33)	7.31 [a] (2.17)	68.53 [a] (2.09)	2.88 [b] (0.38)	33.69 [a] (1.20)	6.58 [a] (1.95)	59.09 [a] (1.88)	0.45 [b]	3.4	57.06 [a] (1.83)
Red bean filled bun	control	29.48 [b] (1.39)	4.60 [a] (3.72)	38.86 [a] (1.98)	9.08 [b] (0.08)	18.36 [a] (1.25)	4.14 [b] (3.35)	26.80 [a] (1.78)	0.37 [a]	2.60	68.65 [a] (4.56)
	modified	26.80 [a] (0.93)	2.05 [a] (0.72)	38.37 [a] (3.40)	2.88 [a] (0.01)	21.53 [b] (0.84)	1.84 [a] (0.65)	31.94 [b] (3.06)	0.52 [b]	2.19	67.82 [a] (6.46)
Bread	control	45.54 [b] (2.32)	3.52 [a] (3.51)	45.96 [b] (1.44)	1.69 [a] (0.23)	39.46 [b] (2.09)	3.17 [a] (3.16)	39.84 [b] (1.30)	0.13 [a]	2.7	99.12 [b] (3.28)
	modified	33.07 [a] (0.41)	3.76 [a] (2.08)	37.85 [a] (1.42)	3.28 [b] (0.10)	26.81 [a] (0.37)	3.38 [a] (1.87)	31.11 [a] (1.28)	0.42 [b]	4.3	86.27 [a] (3.59)

[1]Values within the same column with difference superscripts are significant different by one way ANOVA and Duncan's multiple comparison test at $p < 0.05$.

*TG, total glucose; FG, free glucose; including that from sucrose; TS, total starch; RS, resistant starch; RDS, rapidly available starch; SDS, slowly digestible starch (calculated as the sum of glucose from RDS and FG).

£SDI, starch digestible index

[1]Values within the same column with difference superscripts are significant different by one way ANOVA and Duncan's multiple comparison test at $p < 0.05$.

[2]RS content were determined by Megazyme RS kit

PPENDIX M

RAG and SAG of steamed bun during storage (g/100g as eaten) [1]

Steamed Bun	RAG (g/100g)	SAG (g/100g)
Control	45.64 [c] (2.18)	3.05 [ab] (2.13)
0 month	35.27 [a] (1.23)	3.58 [bc] (1.86)
1 month	40.38 [b] (2.01)	5.70 [c] (1.84)
2 month	40.82 [b] (1.66)	0.67 [a] (0.98)
3 month	39.67 [b] (1.74)	1.53 [a] (1.05)

[1]Values within the same column with difference superscripts are significant different by one way ANOVA and Duncan's multiple comparison test at $p < 0.05$.

BIOGRAPHY

NAME	Miss Narumol Ratanakonpun
DATE OF BIRTH	Dec 7, 1982
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTION ATTENDED	Kasetsart University, 2000-2004 Bachelor of Science Mahidol University, 2005-2007 Master of Science (Nutrition)
HOME-ADDRESS	100/178 Bangkloysainoi Road, Meung, Nonthaburi 11110 Tel. : 025712930 Mobile : 0813435687 E-Mail : koy0724@hotmail.com.