EFFECT OF PROTEIN AND FIBERS ON DIGESTIBILITY OF EXTRUDED REFORMED RICE

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EFFECT OF PROTEIN AND FIBERS ON DIGESTIBILITY OF EXTRUDED REFORMED RICE

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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คืนจันทร์ ณ นคร : ผลของโปรตีนและใยอาหารต่อการย่อยของข้าวขึ้นรูปจากกระบวนการเอกซ์ทรูชัน (EFFECT OF PROTEIN AND FIBERS ON DIGESTIBILITY OF EXTRUDED REFORMED RICE) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สุนันทา ทองทา, 108 หน้า.

การศึกษาผลของโปรตีนจากถั่วเหลือง (Soy Protein Isolate: SPI) และใยอาหารต่อคุณสมบัติทางรีโอโลยี ความสามารถในการย่อย และ gastric emptying ของข้าวขึ้นรูปหุงสุก พบว่าข้าวขึ้นรูปที่ถูกผลิตด้วยเครื่องเอกซ์ทรูเดอร์แบบสกรูคู่ที่อุณหภูมิ 90°C มีค่า onset temperature (T_o) และค่า peak temperature (T_p) ของข้าวขึ้นรูปซึ่งถูกแทนที่ด้วยโปรตีนถั่วเหลืองระดับ 20% (20SPI) สูงกว่าข้าวขึ้นรูปที่ถูกแทนที่ด้วย resistant maltodextrin ระดับ 20% (20RMD) และข้าวขึ้นรูปที่ถูกแทนที่ด้วย รำข้าวโพดระดับ 20% (20CB) นอกจากนี้ยังพบว่า peak viscosity ของ 20SPI มีค่าต่ำกว่า 20CB และ 20RMD เมื่อตรวจด้วยเครื่อง Rapid Visco Analyser ซึ่งเป็นผลมาจากการที่ SPI สามารถจับกับน้ำได้ดีและชะลอการเกิดเจลาติไนเซชัน

คุณสมบัติทางรีโอโลยีของข้าว 20SPI หุงสุกพบว่า มีค่า hardness ค่า stickiness and solid-like (G') สูงกว่าตัวอย่างข้าวขึ้นรูปหุงสุกอื่น ๆ จึงทำให้สามารถชะลอการทำงานของแอลฟาอะมิเลส (alpha amylase) ในระหว่างการย่อยในหลอดทดลอง (*in-vitro* digestion) ซึ่งมีผลทำให้ค่า end-point concentration (C_∞) ลดลง นอกจากนี้ยังพบว่าค่าอัตราการย่อยเฟสแรก (first-phase digestion rate, k_1) ของข้าว 20SPI หุงสุกมีค่าสูงกว่าค่าอัตราการย่อยเฟสที่สอง (second-phase digestion rate, k_2) โดยข้าว 20SPI หุงสุกมีค่า end-point ของอัตราการย่อยเฟสที่สอง ($C_{\infty 2}$) และค่าดัชนีน้ำตาลต่ำสุด ในขณะที่ข้าว 20CB หุงสุกมีค่า k_1ของการย่อยต่ำกว่า อาจเนื่องมาจากเจลแป้งที่มีอนุภาคของรำข้าวโพดมีความสมบูรณ์ (integrity) ของเจลสูงสามารถขัดขวางการย่อยของเอนไซม์ในกลุ่มอะมิเลส (amylolytic enzymes) หลังจากนั้นในระหว่างการย่อยความสมบูรณ์ของเจลลดลงซึ่งมีผลทำให้ค่า k_2 and $C_{\infty 2}$ ข้าว 20CB หุงสุกสูงขึ้น ในขณะที่ค่า k ของข้าวขึ้นรูปที่ทดแทนด้วย resistant maltodextrin (RMD) หุงสุกไม่แตกต่างจากค่า k ของข้าวขึ้นรูปหุงสุกตัวอย่างควบคุม ซึ่งเป็นผลมาจากลักษณะโครงสร้างเมทริกซ์ที่เป็นเนื้อเดียวกันของตัวอย่างที่มีความเหมือนกัน การศึกษาผลของ SPI รวมกับใยอาหารต่อการย่อยของข้าวขึ้นรูปหุงสุกพบว่า เมื่อสัดส่วน SPI ที่รวมกับ RMD เพิ่มขึ้นจาก 6% ถึง 14% มีผลทำให้ค่า k และค่า C_∞ เพิ่มขึ้น การเพิ่มขึ้นของสัดส่วน SPI ที่รวมกับรำข้าวโพด (CB) ยังมีผลทำให้ค่า k_1 เพิ่มขึ้นในขณะที่การเพิ่มขึ้นของสัดส่วน CB มีผลทำให้ k_2 เพิ่มขึ้น ดังนั้นการเติม SPI มีผลทำให้ความสมบูรณ์ของข้าวขึ้นรูปลดลงในระหว่างการย่อยในกระเพาะอาหารซึ่งส่งผลให้อัตราการย่อยของเอนไซม์ในกลุ่มอะมิเลสเพิ่มขึ้น

ข้าวขึ้นรูปหุงสุกที่ถูกทดแทนด้วย SPI ใยอาหาร และ SPI รวมกับใยอาหารมีผลทำให้ค่าดัชนีน้ำตาลอยู่ในกลุ่มปานกลาง ในขณะที่ข้าวขึ้นรูปหุงสุกตัวอย่างควบคุมมีค่าดัชนีน้ำตาลอยู่ในกลุ่มสูง นอกจากนี้ยังพบว่าข้าว 20SPI หุงสุกมีค่า lag phase median และ interquartile range box สูงที่สุด ดังนั้นข้าว 20SPI หุงสุกมีระยะเวลาในการถูกบดและผสมในกระเพาะอาหารก่อนการทำให้กระเพาะอาหารว่าง (gastric emptying) นานที่สุด ในขณะที่ข้าว 20CB หุงสุกมีผลทำให้มีความรู้สึกอิ่มนานกว่าข้าวขึ้นรูปหุงสุกตัวอย่างควบคุม

สาขาวิชาเทคโนโลยีอาหาร

ปีการศึกษา 2560

ลายมือชื่อนักศึกษา________

ลายมือชื่ออาจารย์ที่ปรึกษา________

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KUENCHAN NA NAKORN : EFFECT OF PROTEIN AND FIBERS ON DIGESTIBILITY OF EXTRUDED REFORMED RICE. THESIS ADVISOR : ASST. PROF. SUNANTA TONGTA, Ph.D., 108 PP.

EXTRUDED REFORMED RICE/SOY PROTEIN ISOLATE/DIETARY FIBER/DIGESTIBILITY/GASTRIC EMPTYING/RHEOLOGICAL PROPERTIES

The effect of soy protein isolate (SPI) and dietary fibers on rheological properties, digestibility and gastric emptying of cooked extruded rice was investigated. The extruded reformed rice was produced by a twin screw extruder with the barrel temperature of 90°C. The onset temperature (T_o) and peak temperature (T_p) of extruded rice supplemented with 20% soy protein isolate (20SPI) as determined by Differential Scanning Calorimeter was higher than that of extruded rice supplemented with 20% resistant maltodextrin (20RMD) and 20% corn bran (20CB). When using Rapid Visco Analyser, the peak viscosity of 20SPI was lower than that of 20CB and 20RMD. These results also indicate that soy protein isolate has been attributed to competition for moisture and delay of gelatinization.

The 20SPI cooked rice showed higher values of hardness, stickiness and solid-like (G') as analyzed by rheometer compared to other samples which may retard the alpha amylase activity during the *in-vitro* digestion, resulting in the lower end-point (C_∞) of starch amylolysis. The first-phase digestion rate (k_1) of cooked 20SPI was higher than its second-phase digestion rate (k_2). The end-point in the second-phase digestion ($C_{\infty 2}$) and the glycemic index (GI) of cooked 20SPI showed the lowest value. The low value of k_1 was observed in the first-phase of 20CB digestion. It may be due

to the amylolytic enzymes that were impeded, associated with the high integrity of starch gel dispersed by corn bran particles. Then, the integrity of this starch gel was reduced during the amylolysis, which resulted in the higher k_2 and $C_{\infty 2}$. The cooked extruded rice with substituted resistant maltodextrin (RMD) and the control sample exhibited a similar k value due to their similar homogeneous matrix. The effect of the SPI and fibers combination on digestibility of cooked extruded rice was studied. An increase in SPI ratio with the combination of RMD from 6 to 14% exhibited a higher k value and a higher end-point concentration (C_∞). For the combination of SPI and corn bran (CB), an increased k_1 was observed with a higher SPI ratio, while an increase in CB ratio induced the higher k_2. The integrity of cooked extruded rice supplemented with SPI was reduced during the gastric digestion which resulted in the increase of the amylolysis rate.

The cooked extruded rice supplemented with SPI or dietary fibers and the duo combination of them was found to be the medium GI food, while the control was the high GI food. The cooked 20SPI provided the highest lag phase median and interquartile range box, implying that the time of grinding and mixing in the stomach before gastric emptying for the cooked 20SPI was the longest period. The satisfaction of cooked 20CB was significantly higher than that of the control.

School of Food Technology Student's Signature Kuenchan Na Nakorn

Academic Year 2017 Advisor's Signature S. Tongta

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CHAPTER I

INTRODUCTION

1.1 Introduction

Rice is the most widely consumed staple food for a large amount of the world's human population, especially in Asia. Thailand is the leader of the world's rice production and Thai rice exports grow significantly. In 2017, Thailand exported 11.3 million metric tons of rice, following India which exported for the largest amount in the world (Thai Rice Exporters Association, 2018a). In recent years, consumer preferences have shifted towards better-quality rice, particularly towards varieties with good eating quality. The nutritional value of cooked rice is of primary importance for its eating quality. The cooked rice is classified as a high glycemic index (GI) food with values ranging from 64 to 93 (Miller, Pang and Bramall, 1992). The cooked rice is easily available for digestion and absorption. Enzymatic hydrolysis of starch is affected by the amylose content, branch chain length of amylopectin, and the interactions of starch with lipids, proteins and cellulosic material in food (Ai, Hasjim and Jane, 2013; Jane, 2006; Nayak, De J. Berrios and Tang, 2014). Nevertheless, macronutrient and rheology properties of cooked rice are the important factors for a delay of starch digestion. The rheological properties of cooked rice can describe the overall breakdown behavior of cooked rice during digestion (Bornhorst, Ferrua, Rutherfurd, Heldman and Singh, 2013). The rheological properties (deformation and flow) are the important factor which affects transportation, hydrolysis and absorption of hydrolyzed nutrients within the gastrointestinal tract, which are established the relationships with gastric emptying (Wu, Dhital, Williams, Chen and Gidley, 2016). Protein and fiber are macronutrient which is expected to improve the rheology properties of cooked extruded rice and to delay starch digestion.

The reformed rice is made from rice flour using extrusion processing and has the same size and shape as regular rice grain. The extrusion process can produce extruded reformed rice which can incorporate a wide variety of ingredients such as nutrients in order to improve nutritional quality of extruded rice (Zhuang et al., 2010). Several studies have prepared this extruded rice with fortification of protein, vitamin A, minerals, emulsifiers and other cereal (Bett-Garber, Champagne, Ingram and Grimm, 2004; Murphy, Smith, Hauck and O'Connor, 1992; Noguchi, Kugimiya, Haque and Saio, 1982; Pinkaew, Winichagoon, Hurrell and Wegmuller, 2013; Wang et al., 2013; Yoo et al., 2013). Protein and fibers which are important macro nutrients, is feasible to supplement in extruded rice not only to provide a useful alternative in highly nutritious extruded rice but also to modify the rheology properties of cooked extruded rice, consequently potentially delaying gastric emptying. Therefore, the extruded rice with high protein or high fiber is interesting for healthy food products.

Soy protein isolate (SPI) is widely used in the food industry which has excellent processing ability such as gelling, emulsifying ability and water holding capacity. The functionality of soy protein structure provides the formation of a continuous network and rheology properties through hydrophobic, covalent and/or intermolecular hydrogen bonding (Lim and Narsimhan, 2006; Tolstoguzov, 2003). Noguchi et al. (1982) reported that SPI formed fine strings structure in the starch matrix of extruded rice. Protein denaturation and starch fragmentation during extrusion may bring about inter and intra molecular interaction between both polymers (Kumar, Brennan, Mason, Zheng and Brennan, 2017). Since fibers may be insoluble, non-viscous soluble or viscous soluble, they could affect physicochemical or rheology properties of food. Corn bran has been receiving an attention as a source of insoluble dietary fiber. The higher of corn bran ratio decreased the water holding capacity at 100°C, and pasting properties of corn bran-rice flour mixture, which resulted in the lower expansion ratio and softer textural properties of the extruded rice noodles with corn bran addition (Baek, Kim and Lee, 2014). Many previous studies found that insoluble fiber can improve satiety consumption (Clark and Slavin, 2013; Schroeder, Gallaher, Arndt and Marquart, 2009). For non-viscous soluble fiber, resistant maltodextrin, is an interesting ingredient to supplement into food product due to the fact that it is highly soluble, transparency in solution, low viscosity, and high stability under all conditions. There is a potential to use the resistant maltodextrin for the production of corn snack by extrusion. A few studies focused on the rheology of solid meal substituted resistant maltodextrin.

It is clear that combination of foods does influence the glycemic index (GI). The addition of protein to a meal containing carbohydrate can appreciably reduce the glycemic response (Collier and O'Dea, 1983). One of the first food components that was able to reduce the glycemic response was fiber (Bjoerck and Asp, 1994; Haber, Heaton, Murphy and Burroughs, 1977). Insoluble dietary fiber is not digested and the viscosity of some soluble fiber slows digestion. A food’s form can influence satiety, digestive processes and post-absorptive metabolism (Dhillon, Running, Tucker and Mattes, 2016). The effect of rheological properties of the meal on starch digestibility should be concerned especially in solid meal as the rheology properties could change after cooking.

1.2 Research objectives

The objectives of this research are:

1. To investigate the effect of soy protein isolate and fiber type on digestibility of extruded rice.
2. To investigate the effect of duo combination of soy protein isolate and fiber on digestibility of extruded rice.
3. To investigate the effect of rheology propertied on digestibility and gastric emptying of extruded rice.
4. To investigate the effect of soy protein isolate and fibers on digestibility of extruded rice paste.

1.3 Research hypothesis

Enzymatic hydrolysis of starch is affected by the interactions of starch with proteins and fibers in food. Protein may hold water; consequently the gelatinization temperature is increased. Thus, it may delay starch digestion. Dietary fiber resists digestion. The viscosity of fiber can lead to restrict water availability and develop viscosity which may reduce starch digestion. Non-viscosity fiber substitutes the starch content of system; thus, glycemic index can be decreased. As a result, enzymatic digestion may be delayed.

1.4 Scope on this study

This research investigates the effects of protein (soy protein isolated; SPI), insoluble fiber (corn bran; CB) and non-viscous soluble fiber (resistant maltodextrin; RMD) on starch digestibility of extruded rice. The extruded rice were produced by high amylose rice flour, high amylose rice flour supplemented with SPI, CB, RMD and high amylose rice flour supplemented with duo combination of fiber and protein. Physicochemical properties, rheology properties and digestibility were the key properties to investigate for cooked extruded rice. Moreover, Gastric emptying study in human was also included.

1.5 Expected results

Results from this research will lead to develop a fundamental understanding of interactions of starch with proteins and fiber materials in real food system. These macronutrients would delay the starch digestion and gastric emptying. Regarding this essential knowledge, it will contribute a better understanding of the physicochemical properties of extruded rice supplemented with proteins and dietary fiber on their relationship to *in-vitro* digestibility and also lead to slow digestion of low GI rice food. Furthermore, their rheology properties will influence on the digestion rate which is related to the delay gastric emptying. The high protein or high fiber and low GI extruded rice are suitable for diabetics or anyone who wants to control blood glucose response.

CHAPTER II

LITERATURE REVIEW

2.1 Rice

Rice is the most widely consumed staple food for a large amount of the world's human population, especially in Asia. It is a plant belonging to the family Gramineae. The two cultivated species of rice are African rice (*Oryza glaberrima*) and Asian rice (*Oryza sativa*). It is divided to sub-species which are Japonica type, Indica type and Javanica type. Japonica rice is mostly grown in North China, Korea and Japan. Indica rice is mostly grown in South China, Southeast Asia, including Thailand and the United States. But, Javanica rice is not important for commercial rice. Thailand is the leader of the world's rice production and Thai rice exports grow significantly. In 2017, Thailand exported at 11.3 million metric tons of rice, following India which exported for the most of the world (Thai Rice Exporters Association, 2018a). The most of Thai rice exports in 2017, 41% of all rice exports, is white rice (Thai Rice Exporters Association, 2018b). However, white rice is a cheap product. White rice or broken rice can be used to develop a high nutritional and value added product. In recent years, consumer preferences have shifted towards better-quality rice. The nutritional value of rice is of primary importance to eating quality.

2.2 Extrusion

Extrusion is the process of converting a raw material into a product of uniform shape and density by forcing it through a die under controlled conditions (Rauwendaal, 2014). Extrusion can be operated as a continuous process, which is capable of consistent product flow at relatively high throughput rates. The advantages of extrusion include its high productivity, energy efficiency and low cost. In addition, extrusion can yield various types of product. Food ingredients in the extruder undergo many order–disorder transitions, such as starch gelatinization, protein denaturation, and the formation of complexes between lipids and amylose, finally yielding a desirable product. Starch is a primary ingredient of raw materials that are widely used in food extrusion. Extrusion is a thermomechanical process that can break the bonds of starch, leading to starch gelatinization, melting, and degradation. Extrusion can be classified into two categories, cold extrusion and hot extrusion. Both processes pass dough, which is made of principal component (mostly flour), an mixture of additives, and water through a single or twin-screw extruder (Mishra, Mishra and Srinivasa Rao, 2012). Cold extrusion usually describes the extrusion of carbohydrate or protein material without expansion and transforming into glassy pellets. Hot extrusion involves relatively high temperatures above the boiling point of water and results in expansion. It results in fully or partially pre-cooked product. Almost all granules lose their integrity, disintegrate, and melt, and the molecules can re-associate to form new structures during food extrusion. Conversion of starch in the extruder depends on a large number of variables in the machine and raw material parameters. The independent process parameters include screw speed, screw configuration, product moisture content, temperature, total mass flow rate and die configuration. These independent parameters affect such system parameters as residence time distribution, energy requirement for the process, pressure profile along the barrel and pressure drop in the die (Chang and El-Dash, 2003).

2.2.1 Extruded reformed rice

Rice flour is in most of the principal component of the dough for the reformed rice preparation. The rice flour has to be selected to represent an appropriate ratio of amylose to amylopectin required for the desired end product. The high amylose rice starches show higher viscosity and less Newtonian behavior, which can be explained by their higher gelatinization temperature, and greater molecular entanglements between linear polymer chains (Xie et al., 2009), which is required for the reformed rice. The ratio of pregelatinized to ungelatinized flour in the compositions is also important. If proportion of pregelatinized starch is lower than 30%, the reformed rice products will have poor rehydrating characteristics. On the other hand, more than 70% proportion of pregelatinized starch affect the extrusion characteristics and it is difficult to control the size and shape of the reformed rice (Harrow, 1982). The materials for reformed rice are categorized into principal components and additives. The principal components include rice and water. The additives are many micronutrients or macronutrients. Murphy et al. (1992) studied the vitamin A fortification of rice analogue using extrusion and investigated its stability after washing and cooking, and long-term stability. They found that the combinations of saturated oils, tocopherols and ascorbate allowed vitamin A to be more stable. Washing tests demonstrated that all of the vitamin A (100%) was still retained after washing. Cooking tests showed complete water absorption of extruded rice. The effect of various iron sources on the flavor of extruded rice was investigated by Bett-Garber et al. (2004). The extruded rice fortified with iron sources, elemental iron and FeSO4 mixed with zinc, thiamin, and folic acid at different ratios. Their results showed that intensities of water-like, sour taste, hay-like, musty and bean flavours were enhanced by the addition of ferrous sulphate, ferrous sulphate plus multiple fortificants and multiple fortificants without iron at the ratio of 1:50. Astringent mouth-feel was affected by ferrous sulphate and ferrous sulphate plus multiple fortificants. Moreover, the triple-fortified rice grains with iron (Fe), zinc (Zn), and vitamin A was produced by hot extrusion technology (Pinkaew et al., 2013). Their objective was to determine the impact of triple-fortified extruded rice on Zn status of school children in Southern Thailand. It was concluded that Zn fortification of extruded rice grains is efficient and can be used to improve Zn status in school children. Wang et al. (2013) prepared the reformed rice by the formulation optimization of emulsifiers which were glycerol monostearate, soybean lecithin, and sodium-stearoyl lactylate, and thickeners which was gum Arabic, sodium alginate, and sticky rice. The emulsifier addition increased degree of gelatinization, but decreased water soluble carbohydrate, α-amylase sensitivity, water solubility index and adhesive for extrudates, while the thickeners addition increased degree of gelatinization, bulk density, water soluble carbohydrate, α-amylase sensitivity, water absorption index, hydration rate and adhesiveness.

Additionally, the extrusion condition influence on the structure of extruded rice. Zhuang et al. (2010) found out that the structure of extruded rice at barrel temperatures of 30 and 50°C was denser than that of 70°C. However, the bulk density of extrudates with the temperatures of 30 and 50°C was not significantly different. It was also observed that the structure of the extrudates became denser as the feed moisture content increased from 28% to 36%. When the screw speed was lower, a relatively looser structure was formed. With a higher extruder screw speed, retention time was shortened, and degree of gelatinization of feed was lower. In addition, inhomogeneous mixing occurred. Thus, the extruded structure was relatively denser. The microstructure of their extrudates was not difference as indicated from their bulk is similar. Zhuang et al. (2011) made reformed rice from hybrid indica rice (Type 9718) flour with mild water content using a single screw extruder. The effects of feed water content, barrel temperature and screw speed on physicochemical properties of extruded rice were investigated. It was found that both barrel temperature and feed water content were positively correlated with bulk density. Textural study indicated that hardness of all hybrid indica rice extrudates was lower than that of cooked raw rice and parboiled rice. The scanning electron microscope images demonstrated that a granular gel network was formed within the reformed rice.

2.3 Starch

Starch is the storage polysaccharide of cereal grains and mainly found in the endosperm. Starch is semi-crystalline polymer as it exists in granules. It is a high molecular weight polymeric carbohydrate composed of D-glucose units. Starch is composed of two major components: amylose and amylopectin. For most common types of cereal endosperm starches, the relative weight percentages of amylose and amylopectin range between 72 and 82% amylopectin, and 18 and 33% amylose. Amylose is a linear chain of α-(1,4)-linked D-glucose units (Figure 2.1a) and some molecules are slightly branched by containing less than 1% of α-(1,6) branching linkage (Hizukuri, Takeda, Yasuda and Suzuki, 1981). Amylose chains turn themselves into a helical conformation with six residues per turn (Zobel, 1988). For this conformation, all hydrophobic groups of the macromolecule face the inner side of the helix and the hydrophilic groups face towards the external side. With this configuration, an internal hydrophobic tunnel is formed and molecules such as iodine and fatty acids can be bound to it (Gallant, Bouchet, Buléon and Pérez, 1992). For rice, amylose contents are classified as waxy (0-2% amylose), very low (5-12%), low (12-20%), intermediate (20-25%) or high (25-33%) (Juliano et al., 1981). Amylopectin is a highly branched component of starch (Figure 2.1b). It is formed through chains mainly of α-(1,4)-linked D-glucose linkages but with 5-6% of α-(1,6) bonds at the branch points (Buléon, Colonna, Planchot and Ball, 1998). The individual chains are specifically classified in terms of their lengths and consequently position within starch granules (Hizukuri, 1985, 1986). Short unit chains are clustered together, and the units of clusters are interconnected by longer chains. The shortest chains are defined to A-chains which are unsubstituted, whereas B-chains are substituted by other chains. This is classical nomenclature by Peat, Whelan and Thomas (1952). The B-chains are further divided according to their positions in the cluster structure model proposed by Hizukuri (1986). B1-chains are short chains, being components of the unit clusters, whereas B2, B3, etc., are long chains that span over two, three, or more clusters, thereby interconnecting them (Pérez and Bertoft, 2010). The semi-crystalline of normal amylose content and waxy rice starches is generally ascribed to amylopectin, as the amylopectin outer side chain branches intertwine and form double helices, which are packed into lamellar crystallites. It is accepted that the crystalline domains of starch granules are composed of A chains and the exterior parts of B chains. Native starch granules are characterised by either an A-type (cereal starches, e.g rice, wheat, maize), a B-type (tuber and root starches, e.g. potato and cassava, retrograded starches of any botanical origin, high amylose cereal starches such as high amylose maize starch), or a C-type (leguminosae starches, e.g. pea, bean and tropical starches of e.g. cassava starch) crystalline pattern. C-type starches are mixtures of A-type and B-type starches (Buléon et al., 1998).

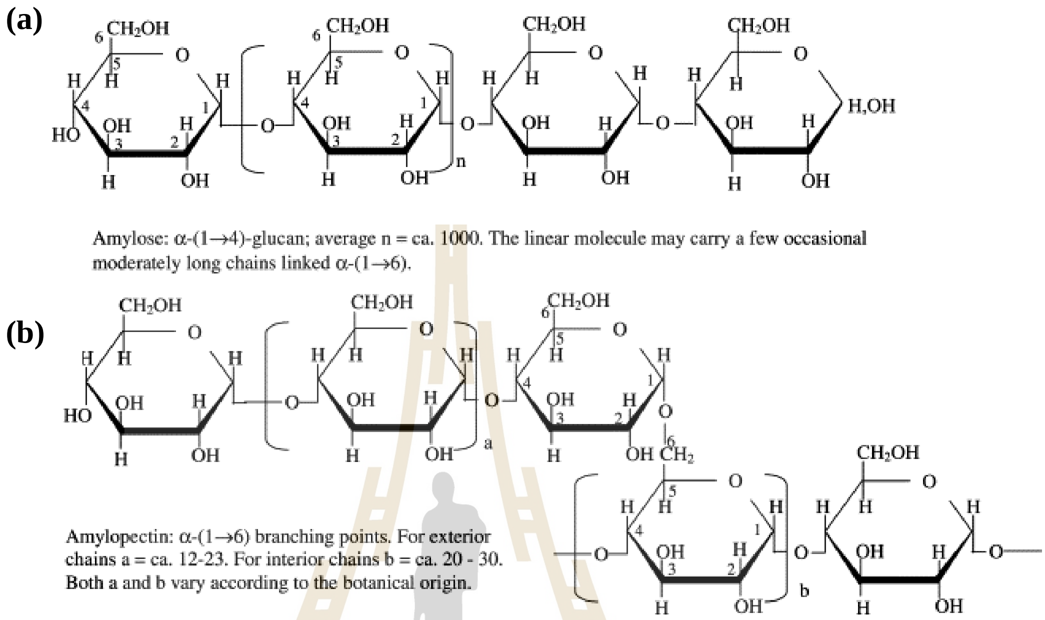


Figure 2.1 Structure of (a) amylose and (b) amylopectin.

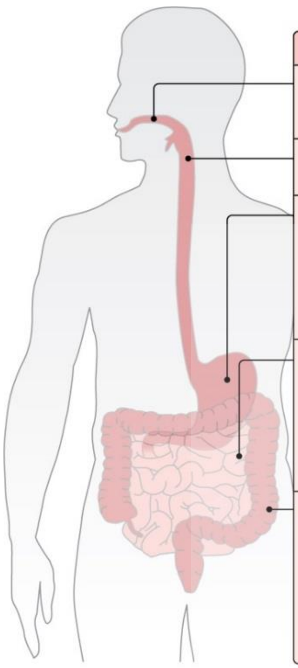
Source: Tester, Karkalas and Qi (2004).

2.4 Starch digestion

Digestion of starch is affected by hydrolyzing enzymes in a complex process which depends on many factors. These include the botanical origin of starch, crystalline areas which tend to be unfavourable for enzyme attack, the source of enzymes, substrate and enzyme concentration, temperature and time, as well as the presence of other substances in the multicomponent matrix in which starch occurs naturally, e.g. proteins and lipids that can also hinder starch–amylase interaction (Tester, Karkalas and Qi, 2004).

Starch is easily digested in the human body after its crystalline structure is destroyed from processing, such as cooking (Ao et al., 2007). The digestion of starch to glucose in the human body requires several enzymatic degradation steps. For human digestion of starch (Figure 2.2), all carbohydrate digestion starts in the mouth with the enzyme, α-amylase, in saliva. Chewing breaks down food, increasing their surface area to volume ratio and exposing starch granules. In the gastrointestinal tract, this is achieved by the action of α-amylase and α-glucosidases (Butler, van der Maarel and Steeneken, 2004). The α-amylase randomly hydrolyzes α-(1,4) glucosidic bonds, generating maltose and short linear oligosaccharides. Salivary α-amylase activity is stopped by the acid in stomach. For small intestine, Pancreatic α-amylase continues breaking down α-(1,4) glucosidic bonds. Intestinal amylase and pancreatic amylase fully break down the starch into maltose, which is then hydrolyzed by maltase into simple sugars called glucose. Amyloglucosidase is exo-enzyme hydrolyzing α-(1,4) and α-(1,6) glucosidic bounds, attacking the terminal glucose residues to yield glucose.

Starch digestion is studies on *in-vivo* and *in-vitro*, and the relationships between the two digestion methods predict physiological responses. *In-vitro* procedures that can be related to *in-vivo* behavior are preferred because of their cost and ethical advantages. Many *in-vitro* studies have been designed to simulate and investigate digestion in the small intestine. The *in-vitro* current of starch digestion was compared with *in-vivo* in pigs (Hasjim, Lavau, Gidley and Gilbert, 2010). *In-vivo* digesta were collected from the small intestine of pigs fed with raw normal maize starch and *in-vitro* digestion was carried out on the same diet fed to the pigs using a method simulating digestion in the mouth, stomach, and small intestine. A qualitative difference was observed between the *in-vitro* and the *in-vivo* digestion. Raw normal maize starch granules were rapidly and almost completely hydrolyzed in the upper part of the small intestine (*in-vivo*). Both amylose and amylopectin were hydrolyzed at a similar rate by amylolytic enzymes in the *in-vivo* and *in-vitro* digestion.



Organ	Physical process	Chemical process
Oral cavity (mouth)	Mastication Goal: Bolus formation	Enzymatic hydrolysis Goal: α-amylase: Starch breakdown Lingual lipase: Lipid breakdown
Esophagus	Peristalsis Goal: Bolus transport	
Stomach	Peristalsis Goal: Continued food breakdown	Enzymatic hydrolysis Goal: Pepsin: Protein breakdown Lipase: Lipid breakdown Acid hydrolysis Goal: Gastric acid (pH ~2): Soften food texture and break down structure
Small intestine	Peristalsis Goal: Digesta transport Segmentation Goal: Mixing to facilitate absorption Diffusion (active/passive) Goal: Absorption of nutrients	Enzymatic hydrolysis Goal: Lipase, phospholipase A: Lipid breakdown Amylase, amyloglucosidase: Starch breakdown Trypsin, chymotrypsin, carboxypeptidase, elastase: Protein breakdown
Large intestine (colon)	Peristalsis Goal: Digesta transport Segmentation Goal: Mixing to facilitate absorption Diffusion (active/passive) Goal: Absorption of fermentation by-products and water	Fermentation Goal: Produces short-chain fatty acids and other by-products

Figure 2.2 Physical and chemical processes in the gastrointestinal tract.

Source: Bornhorst and Singh (2014).

For nutritional purposes, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) according to the rate of glucose release and its absorption in the gastrointestinal tract (Englyst, Kingman and Cummings, 1992). RDS is the portion digested within 20 minutes and SDS is digested between 20 and 120 minutes. SDS is believed to be slowly but completely digested, leading to a slower entry of glucose into the blood stream and lower glycemic response. RS cannot be digested in the small intestine and is left in the colon. The amount of RDS is positively correlated with the glycemic index (GI) of food products (Englyst, Englyst, Hudson, Cole and Cummings, 1999). It has been suggested that the content of RDS and SDS can be used to predict the GI of cereal-based food products. A high SDS content identifies low-GI foods that are rich in slowly released carbohydrates for which health benefits have been proposed (Englyst, Vinoy, Englyst and Lang, 2003).

2.4.1 Glycemic index

Starch and starchy food products can be classified according to their digestibility, which is generally characterized by the rate and the duration of the glycemic response. Predicting and controlling the glucose absorption due to ingestion of starchy food is the great interest in the context of worldwide health concerns (Singh, Dartois and Kaur, 2010). The GI is a ranking of foods based on their actual postprandial blood glucose response compared to a reference food, either glucose or white bread (Jenkins et al., 1981). WHO/FAO define GI as the incremental area under the blood glucose response curve (AUC) of a 50 g available carbohydrate portion of a test food, expressed as a percentage of the response to the same amount of carbohydrate from a standard food consumed by the same subject. The GI value is calculated by dividing the AUC for the test food by the AUC for the reference food and multiplying by 100. GI is a ranking of carbohydrates on a scale from 0 to 100 according to the extent to which they raise blood sugar levels after eating. GI is divided into 3 groups: low GI (≤55), medium GI (56-69) and high GI (≥70) (Nayak et al., 2014). Foods with a high GI are rapidly digested and absorbed, which result in high blood sugar levels. On the other hand, low-GI foods are slowly digested and absorbed, which produce gradual rise in blood sugar and insulin levels.

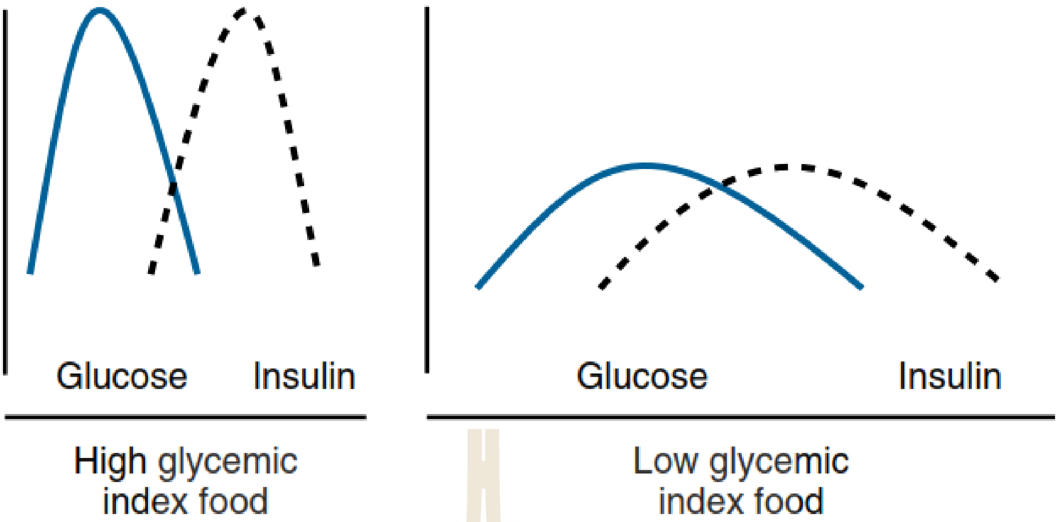


Figure 2.3 The effect of high glycemic index food versus low glycemic food on glucose and insulin.

Source: Onna (2018).

Low-GI foods have proven benefits for health. Low GI diets have been shown to improve glucose in people with diabetes (Type 1 and Type 2). They provide weight control because they help control appetite and delay hunger. Low GI diets also reduce insulin levels and insulin resistance (Figure 2.3). Goni et al. (1997) studied the possible correlation between *in-vitro* and *in-vivo* responses to the same food. They reported that a first-order hydrolytic process was found: $C = C_{\infty} (1-e^{-kt})$ where C is the concentration at t time, C_{∞} is the equilibrium concentration, k is the kinetic constant and t is the chosen time. This is a simple *in-vitro* method that could be used to estimate the metabolic glycemic response to a food. Goni et al. (1997) derived a hydrolysis index (HI) by calculating the area under a hydrolysis curve from plotting the rate of glucose released over a period of 180 min using white-wheat bread as a reference. The good linear correlation is found between HI and the GI following the equation GI = 39.71 +

0.549HI (r = 0.894). The *in-vitro* method has been proposed as an alternative method for classifying carbohydrates. It offers considerable benefits in the speed of testing, the potential to use controlled conditions and the freedom to test novel foods.

The low GI diet has several health benefits for reducing the rate of carbohydrate digestion and absorption which improves blood glucose control. The low GI diet can improve glucose tolerance of diabetics (Miller, 1994). Amano et al. (2007) developed a low GI dietary regimen based on Japanese foods which staple food is white rice (high GI food) in participants with Type 2 diabetes or impaired blood glucose. Although replacing white rice with low GI staple foods could increase fat intake, the participants had lower energy intake without increasing fat intake. Furthermore, an increase in dietary fiber significantly decreases the GI. The GI-based nutrition education is effective in improving blood glucose control in participants with type 2 diabetes. The GI-based nutritional education was the good cause and could be considered a useful tool for improving blood glucose control (Amano et al., 2007).

2.4.2 Factors affecting on starch digestibility

Factors effecting on starch digestion (Figure 2.4) are macronutrient, fiber content, viscosity and structure of food (Riccardi, Rivellese and Giacco, 2008). Enzymatic hydrolysis of starch is affected by the amylose content, branch chain length of amylopectin, and the interactions of starch with lipids, proteins and cellulosic material in food (Ai et al., 2013; Jane, 2006; Nayak et al., 2014).

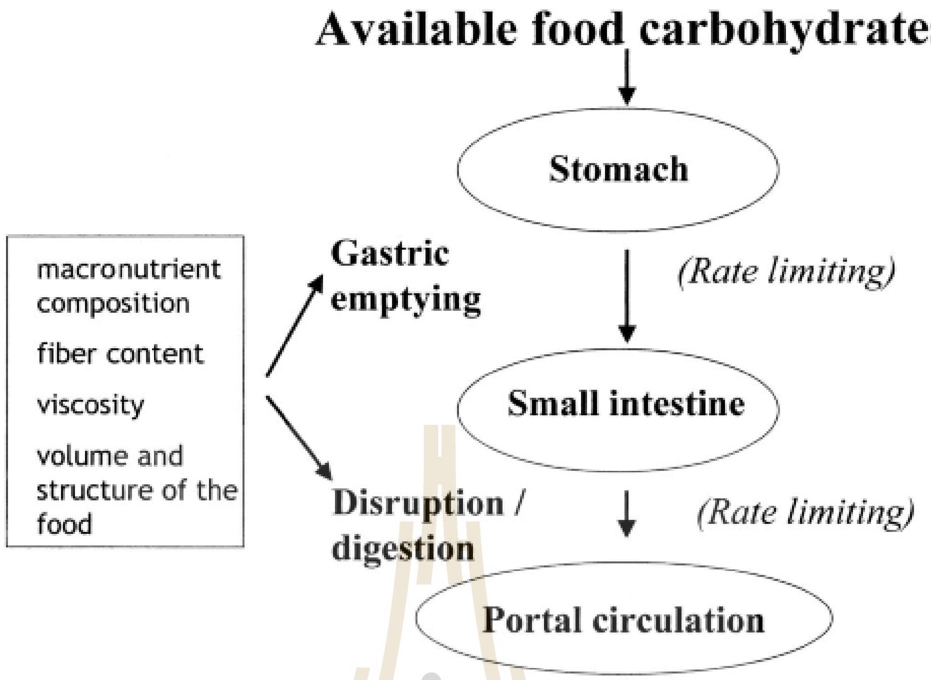


Figure 2.4 Factors influencing the rate of glycemic carbohydrate availability in the gastrointestinal tract.

Source: Riccardi et al. (2008).

2.4.2.1 Amylose

Starches from different amylose content have shown different kinetics digestion and degradation patterns with α-amylase. Starches with lower amylose content will have higher starch digestion rate. Inversely, starches with higher amylose content will be less susceptible to gelatinization, leading to low starch digestion rate (Syahariza, Sar, Hasjim, Tizzotti and Gilbert, 2013). Akerberg et al. (1998) studied the influence of the amylose content on the GI in barley flour-based bread by using barley genotypes varying in amylose content (3-44%). Bread was made from 70% whole-meal barley flour and 30% white wheat flour. It is shown that a barley flour-based bread of low GI and high RS content can be obtained by choosing high-amylose barley and appropriate baking conditions. Frei et al. (2003) studied starch degradability of six Philippine rice cultivars differing in amylose contents. The *in-vitro* enzymatic starch digestion method was applied in order to estimate the GI. The result indicated substantial differences in the estimated GI for each rice cultivar. Waxy rice had more rapid and more complete hydrolysis than high amylose rice. In accordance with studying *in-vitro* digestibility of three types of rice cultivars (Indica, Japonica and Hybrid rice) with difference amylose content (0-26.9%), it showed that increasing amylose in the same type of rice, the contents of RS were increased and estimated GI was decreased. The starch hydrolysis tends to be more rapid and more complete for the waxy and low amylose rice than for the intermediate and high amylose rice (Hu, Zhao, Duan, Linlin and Wu, 2004). Syahariza et al. (2013) studied correlations between starch digestion rate and molecular structural characteristics, including fine structures of the distributions of chain lengths in both amylose and amylopectin. The *in-vitro* digestion rate tended to increase with longer amylose branches and smaller ratios of long amylopectin and long amylose branches to short amylopectin branches. Therefore, amylose content is not the only factor that affects the glycemic index.

2.4.2.2 Dietary fiber

Dietary fiber is non-digestable material (Whistler and BeMiller, 1997) found in the following plant derived food sources: fruits and vegetables, cereals, and legumes. It consists of water-insoluble plant components, primarily cellulose and lignins, water-soluble plant components and non-starch polysaccharides (Whistler et al., 1997). A committee appointed by the American Association of Cereal Chemists proposed the following definition. Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation (Anonymous, 2000).

Dietary fiber can affect the rate of digestion by slowing gastric emptying and restricting digestive enzyme activity. Brennan and Tudorica (2008) evaluated the effect of various dietary fiber on the starch digestibility of fresh pasta. The addition of 2.5-10% dietary fiber into pasta can reduce the GI to be lower. The GI value of pea fiber addition was higher than that of the supplementation of guar gum and locust bean. An increase in the level of fiber supplementation resulted in the decreased in GI value. The dietary fiber could affect physicochemical or texture properties of food, which affects the rate of starch digestion during the *in-vitro* process. The effect of non-starch polysaccharides (NSPs) on starch digestibility of waxy (1.43% amylose) and non-waxy (30.12% amylose) rice starches was investigated. The NSPs included guar gum, xanthan gum, carboxymethyl cellulose (CMC), tapioca fiber and tamarind seed fiber was studied. They were added to waxy and non-waxy rice starches at the levels of 5, 10 and 15 g/100 g dry sample. The mixtures were examined for *in-vitro* starch digestibility, thermal properties and textural properties. Generally, it was found that all NSPs at the concentrations used in this study had little or no effect on starch digestibility. Glycemic response parameters (lower k, HI and GI values than those of the control) slightly decreased in the samples with added NSPs. No obvious effects on thermal properties were obtained. The NSPs contributed to the modification of the gel textures, resulting in higher hardness and adhesiveness values, especially for the guar gum and CMC containing mixtures of the waxy rice starch gel. The non-waxy rice starch mixtures containing guar gum, tapioca fiber and tamarind seed fiber showed higher hardness, while xanthan gum and CMC mixtures exhibited lower hardness (Arranz-Martínez, Srikaeo and González-Sánchez, 2014).

2.4.2.3 Protein

One of the most popular plant protein sources to serve as an ingredient in food formulation is soy protein. Soybean seed contains approximately 40% protein and 20% oil on an average dry matter base. Soy protein isolate (SPI) is widely used in the food industry. Soy proteins are mainly comprised of two storage globulins, 11S glycinin and 7S β-conglycinin (Nishinari, Fang, Guo and Phillips, 2014). The advantages of soybean proteins are: 1) they provide a good balance in amino acid composition because they contain all the essential amino acids, 2) they contain physiologically beneficial components which are shown to lower the cholesterol, and to reduce the risk of hyperlipidemia and cardiovascular diseases, 3) they have excellent processing ability such as gelling, emulsifying ability and water-and oil- holding capacity (Nishinari et al., 2014). Soy contains the largest concentration of isoflavones, a class of phytoestrogens. Phytoestrogens are structurally similar to estradiol and mimic its effects. Soy and phytoestrogens receive increasing attention due to the health benefits associated with their consumption (Cederroth and Nef, 2009). Additionally, a 6-month clinical trial was conducted to compare the effects of isoflavones with that of conjugated estrogens on blood glucose, insulin, and lipid profiles in postmenopausal Taiwanese women. The study revealed that soy isoflavones (100 mg/day) and conjugated estrogen (0.625 mg/day) equally lowered fasting blood glucose and insulin levels in postmenopausal women (Cheng, Shaw, Tsai and Chen, 2004).

The remnants of proteins themselves or complexes with protein remaining indigestible in the intestine were referred to as resistant proteins, which exerted physiological functions similar to dietary fibers and were also better for health (Kato and Iwami, 2002). Seventeen type II (non-insulin-dependent) diabetic subjects were given single breakfast meals consisting of 50 g glucose, or 50 g glucose plus

25 g protein in the form of lean beef, turkey, gelatin, egg white, cottage cheese, fish, or soy. The result showed that high-protein diets decreased both postprandial blood glucose in type 2 diabetes, but the mechanisms which were responsible for these effects were poorly defined (Gannon, Nuttall, Neil and Westphal, 1988). A high-protein diet lowered blood glucose postprandially in persons with type 2 diabetes and improved overall glucose control (Gannon, Nuttall, Saeed, Jordan and Hoover, 2003). von Bibra et al. (2014) reported that a low-glycaemic/high-protein but not a low-fat/high-carbohydrate nutrition modulated diastolic dysfunction in overweight type 2 diabetes patients, improved insulin resistance and might prevent or delay the onset of diabetic cardiomyopathy and the metabolic syndrome.

2.4.2.4 Physicochemical properties

Physicochemical changes in the starch granule occur during cooking such as swelling, softening and gelatinization of starch. These changes improve the texture, nutritional value and digestibility of the starch (Ovando-Martínez, Osorio-Díaz, Whitney, Bello-Pérez and Simsek, 2011).

2.4.2.4.1 Swelling property and pasting properties

Pasting properties reasonably predicted *in-vitro* starch digestion. The very rapidly digested starch (D_o) and rate of digestion (K) significantly are related to the initial, peak, trough, and final viscosities. Gelatinized starch reduces the amount of raw granules and is more susceptible to enzyme digestion that are responsible for decreases in peak and final viscosities and increase in Do and K. (Mahasukhonthachat, Sopade and Gidley, 2010).

Chung, Liu, Lee and Wei (2011) studied swelling factor in rice starch. The swelling factor of Glutinous rapidly increased from 55°C and reached a plateau at 75°C. For non-waxy rice starches, a first increase in swelling factor was observed at 60°C for Arborio and Calrose, and at 65°C for Long-grain. A substantial increase in SF was observed between 85 and 95°C in all non-waxy rice starches. Swelling factor has also been influenced by amylopectin molecular structure. Long-grain rice starch had a smaller proportion of short A chains but a larger proportion of long B3 chains. Longer branch chains in amylopectin may contribute to a stronger crystalline structure, resulting in suppression of swelling. Therefore, a greater amount of amylose and longer amylopectin branch chain length in Long-grain would lead to a reduction in swelling. Swelling factor was positively correlated with rapidly digestible starch (RDS) ($R = 0.942$, $p<0.01$), and negatively correlated with slowly digestible starch (SDS) ($R = -0.935$, $p<0.01$) and resistant starch (RS) ($R = -0.794$, $p<0.05$). When swelling power increased the absorption of enzyme increased. It has lower resistance to enzymatic digestion.

Chung et al. (2011) reported that larger amylose content in rice starch was correlated with lower peak viscosity. The Long-grain rice starch, consisting of a low proportion of short A chains, displayed a high pasting temperature but low peak viscosity. The Long-grain rice starch has higher resistance to enzymatic digestion. Starch consisting of amylopectin with more short A chains (DP 6-12) displayed a lower pasting temperature and low peak viscosity because the short branch chains could not provide strong interaction to maintain the integrity of the swollen granules (Sathaporn and Jane, 2007). The Glutinous rice starch mainly consisted of amylopectin; thus, the starch granule swelled rapidly (lower pasting temperature) and greatly (higher peak viscosity). So, it has low resistance to enzymatic digestion. During the cooling of starch paste, leached amylose molecules rapidly aggregated. The formation of amylose junction zones was responsible for a setback and final viscosity (Jane et al., 1999). With no or less amylose presenting in Glutinous rice starch, final viscosity was substantially low, whereas the highest final viscosity was observed in the Long-grain starch with the highest amylose content. Pasting temperature and final viscosity were negatively correlated with RDS (R = -0.970, -0.975 respectively, $p<0.01$) and positively correlated with SDS (R = 0.968, 0.956 respectively, $p<0.01$) and RS (R = 0.879, $p<0.01$. On the other hand, the peak viscosity showed the opposite trend.

Banana starches from diverse varieties (Macho, Morado, Valery and Enano Gigante) were studied on their physicochemical, structural and digestibility properties. (Agama-Acevedo, Nuñez-Santiago, Alvarez-Ramirez and Bello-Pérez, 2015). Starch of the Valery and Macho varieties showed the highest peak viscosity, while the highest pasting temperature was showed by Macho and Enano Gigante varieties at 79°C and 78°C, respectively. Starch of Valery and Morado varieties presented a slight breakdown and higher setback than Enano Gigante and Macho starches. The pasting profile of Valery and Morado starches indicated that, the swollen granules resisted the shear stress during the cooking step. It was apparent that the high swelling, observed by the peak viscosity, can be involved in these results. When the native banana starches were cooked in a boiling water bath for 20 min, the resistant digestion properties was lost, with an increase in the RDS. Cooking produced a disorganization of the starch components in the semi-crystalline structure of the native banana starches, indicating that the B-type polymorph is critical for the resistance to digestion property. Although the RDS content increased in the gelatinized samples, an amount of SDS of raw and gelatinized starch of Valery increased but that of the Morado variety decreased while that of Macho and Enano varieties was not difference. It is possible that these banana starches which were modified by physical methods (i.e. hydrothermal treatments) increase the SDS fraction. Additionally, the gelatinized banana starches exhibited a higher RS content than that reported in gelatinized cereal and tuber starches. Moreover, the Valery had the highest final viscosity and exhibited the highest SDS; thus, it has higher resistance to enzymatic digestion. But, Enano had the lowest final viscosity and exhibited the lowest SDS, so it had lower resistance to enzymatic digestion.

2.4.2.4.2 Viscosity of food matrix

The addition of viscous dietary fiber to a carbohydrate meal may reduce the glycemic response. By forming viscous solutions, soluble fiber delays the rate of gastric emptying, reduces the mixing and diffusion in the small intestinal, and thus decreases the rate of digestion and glucose absorption by the intestine (Nayak et al., 2014). Fabek, Messerschmidt, Brulport and Goff (2014) studied the effect of six soluble fibers on glucose diffusion by *in-vitro* digestion. Six hydrocolloids, including guar gum, locust bean gum, fenugreek gum, xanthan gum, soluble flaxseed gum extracted from flaxseed hulls and DA-100 variety soy soluble polysaccharides were tested. Hydrocolloids were dissolved with the addition of sodium caseinate and waxy corn starch. The flow behaviour indices showed that all solutions acted as pseudoplastic non-Newtonian fluids, both before and after *in-vitro* digestion. Xanthan gum was able to retain its viscosity compared to the other fibers, across a shear rate range of 30-200 s^{-1} ($p<0.05$). The suspension containing guar gum was also able to retain some viscosity after *in-vitro* digestion. Xanthan gum showed significantly higher apparent viscosities across a range of shear rates, followed by Guar gum. After it proceeded 60 min of digestion, the gums could slow glucose diffusion. All gums demonstrated an ability to suppress the glucose response curve. However, the xanthan gum showed the highest attenuation in glucose diffusion, compared to other gums. Viscosity that may provide a protective barrier for the starch granules could inhibit enzyme hydrolysis. An increase in viscosity may also lead to the reduction the glucose diffusion. Soluble fibers could contribute to a barrier layer at the mucosal membrane, slowing transport of glucose across the mucosa. Investigation of the effect of viscosity on both enzyme activity and glucose release and diffusion may help to understand that in viscosity hinders the postprandial glycemic response.

2.5 Gastric emptying

The blood glucose concentration change is a result of gastric motility. Hyperglycemia has an effect on slowing down the gastric antral action and inhibits motility of the stomach and intestines, whereas hypoglycemia increases gastric emptying rate. The gut hormones contribute to the gastric emptying process along with glucose metabolism (Vermeulen et al., 2011). Gastric emptying determines the rate of appearance of nutrients into the small intestine, so it is a major factor in blood glucose homeostasis by controlling the delivery of carbohydrate to the small intestinal (Horowitz, Edelbroek, Wishart and Straathof, 1993). Several factors influence the rate of gastric emptying of a meal, such as nutrient content, physical properties, composition, and volume. Particularly in the medical literature, the gastric emptying rate is quantified by the lag phase, the time that takes before a meal begins to empty, and a half-emptying time, the time that takes for 50% of the meal to leave the stomach (Bornhorst et al., 2014).

2.5.1 Factors affecting on gastric emptying

The properties of the ingested meal (Figure 2.5) such as calorie content, meal volume, nutrient content, and acid concentration have all been shown that they influence the meal gastric emptying (Bornhorst et al., 2014).

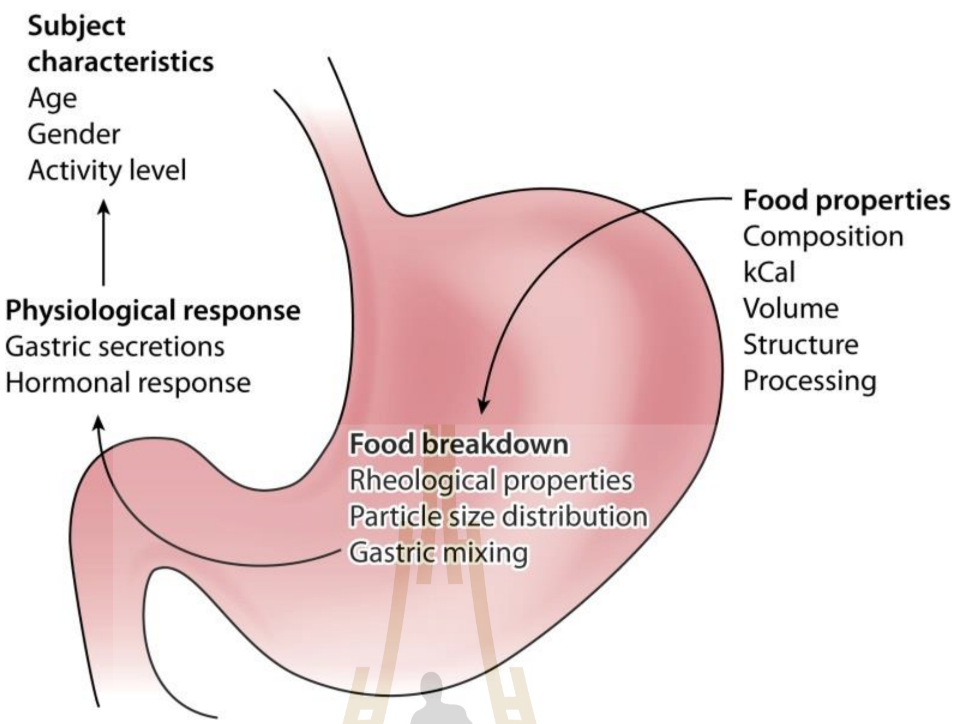


Figure 2.5 Food and subject factors that are interrelated in determining the gastric emptying rate of a meal.

Source: Bornhorst et al. (2014).

Gastric emptying is in part regulated by the ileal brake mechanism through action of the gut hormones (Cisse et al., 2017). The ileal brake is the primary inhibitory feedback mechanism to control transit of a meal through the gastrointestinal tract in order to optimize nutrient digestion and absorption (Van Citters and Lin, 1999). The ileal brake mechanism is triggered when the ileum has more nutrients than it normally would (Maljaars, Peters, Mela and Masclee, 2008). The gastrointestinal processes are regulated primarily by inhibitory feedback arising from the interaction of nutrients with the small intestine and modulated by both stimulation of the vagus nerve and the secretion of gastrointestinal hormones, including glucagon-like peptide-1

(GLP-1), cholecystokinin (CCK), and peptide-YY (PYY) (Marathe, Rayner, Jones and Horowitz, 2013). Activation of the ileal brake results in a dose-dependent delay in gastric emptying and in small intestinal transit for both solid and liquid meals (Maljaars et al., 2008). For most meals, small intestinal-nutrient sensing inhibits ghrelin secretion and stimulates CCK, GLP-1, and PYY secretion (Figure 2.6). On the other hand, ghrelin stimulates gastric emptying but CCK, GLP-1, and PYY inhibit gastric emptying (Steinert et al., 2016).

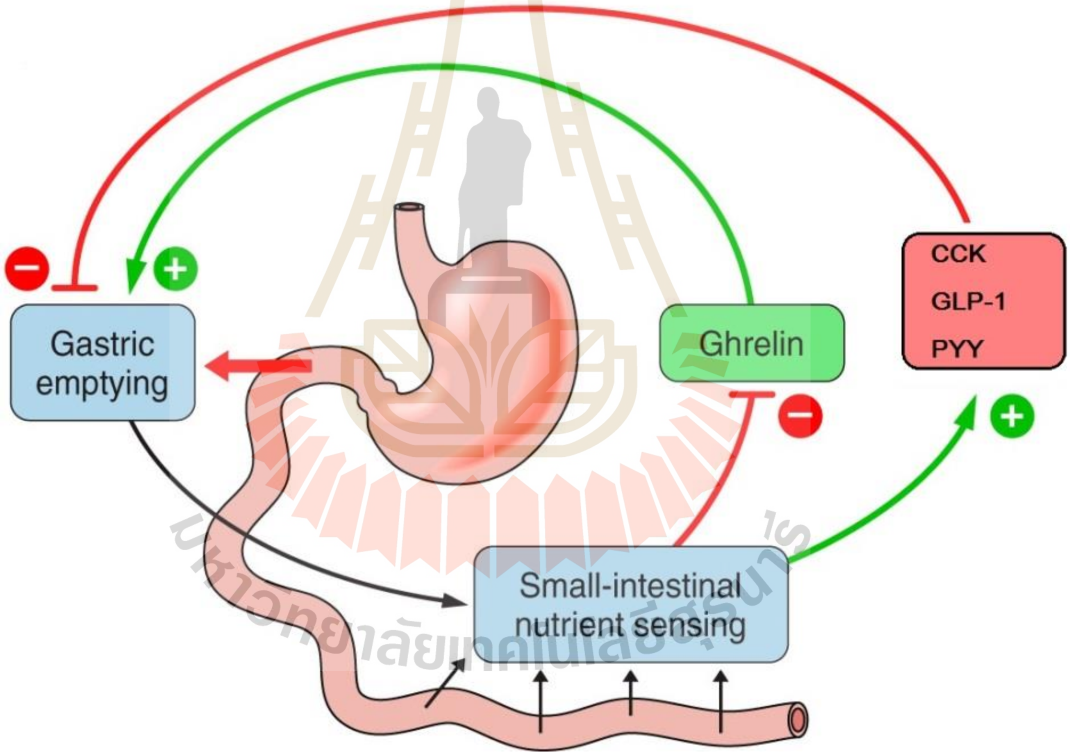


Figure 2.6 The secretory control of ghrelin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide-YY (PYY).

Source: Bornhorst et al. (2014).

Protein and fiber should delay gastric emptying. The physiological effect of ileal brake is caused either by protein or dietary fiber, leading to being slowly digestible of carbohydrate in the small intestine or being fermented in the colon, which causes a satiety effect. Soybean peptone acts directly on small intestinal mucosal cells to stimulate CCK release in a rat (Nishi, Hara and Tomita, 2003). Kashima et al. (2016) showed that a soy protein isolate (SPI) preload of 40 g in liquid meal improved glycemic control in young healthy subjects. Glycemic control appears to be attributed not only to the exaggerated insulin response to SPI preload, but also to non-insulin dependent mechanisms, such as delayed gastric emptying (Kashima et al., 2016). Soy protein can be safely consumed by people with lactose intolerance that often experience gastrointestinal problems after consuming lactose products. Protein induces activation of the ileal brake in humans (Read et al., 1984). It is possible that CCK contributes to this effect because protein is a potent stimulus for the CCK secretion (Liddle, Goldfine, Rosen, Taplitz and Williams, 1985). High protein diets increase CCK and ghrelin expression in the intestine (Babaei et al., 2017). Protein is a strong stimulus for CCK release. CCK levels remained elevated longer after consuming liquid whey, casein, soy and gluten or after consuming whey protein meal when compared to glucose and lactose (Karhunen, Juvonen, Huotari, Purhonen and Herzig, 2008). CCK elicits multiple effects on gastrointestinal system, including the regulation of gastrointestinal mobility, contraction of the gallbladder, pancreatic enzyme secretion, gastric emptying, and gastric acid secretion (Strader and Woods, 2005). This hormone is concentrated in the proximal small intestine and is secreted into the blood on the ingestion of proteins and fats. The physiological actions of CCK include stimulation of pancreatic secretion and gallbladder contraction, regulation of gastric emptying, and induction of satiety. Therefore, in a highly coordinated manner, CCK regulates the ingestion, digestion, and absorption of nutrients. A CCK-releasing factor of intestinal origin has been partially characterized and is responsible for stimulation of CCK secretion after 1) ingestion of protein or fats, 2) instillation of protease inhibitors into the duodenum, or 3) diversion of bile-pancreatic juice from the upper small intestine (Liddle, 1995). Guilloteau et al. (1986) studied the effect of a milk substitute diet containing concentrated soy protein on gastrointestinal hormones. Sixteen calves aged 12-19 weeks were given the substituted milk. They found that postprandial values of CCK were increased 1.4 times. Moreover, high-protein intake induced the greatest release of the PYY and the most pronounced satiety in normal-weight and obese human subjects (Batterham et al., 2006). Karamanlis et al. (2007) studied the effect of protein on gastric emptying. They reported that the gastric half-emptying time was greater for glucose plus protein (50 g glucose + 30 g gelatin) than for either the glucose (50 g glucose) or the protein (30 g gelatin) in 300 mL drink. Ma et al. (2009) reported that the slow gastric emptying was shown after the whey preload in beef soup for type 2 diabetic patients. Whey protein consumed before a carbohydrate meal could stimulate insulin and incretin hormone secretion and slow gastric emptying, leading to a marked reduction in postprandial glycemic in type 2 diabetes. A SPI preload of 40 g in 400 mL liquid meal improved glycemic control in young healthy subjects. Since fibers may be insoluble, non-viscous soluble or viscous soluble, they could affect other properties of food. Thus, types of fiber affect gastric emptying of solid meal. Ray et al. (1983) studied the effect of long-term (2 months) supplementation of diet with 20 g of guar gum and 10 g of wheat bran on metabolic control in 12 obese, poorly controlled noninsulin-dependent diabetic patients. Addition of fiber delayed gastric emptying of liquid and solid food. This effect became statistically significant at 60 and 90 min after intaking of a test meal for liquids and solids, respectively. It was concluded that addition of guar and bran to the diet resulted in long-term improvement of metabolic control in these patients and that delayed gastric emptying. French and Read (1994) determined effects of fiber on delaying gastric emptying and slowing absorption of meals. The 3% guar gum was added to high- and low-fat soups and gastric emptying rate, hunger, and satiety were measured in eight male volunteers. Guar gum delayed the emptying of the low-fat soup but the small delays in the return of hunger. The high-fat soup also emptied more slowly but it had no effect on the return of hunger or the decline in fullness. The delays in the return of hunger and the decline of fullness were far greater when guar gum was added to the fatty soup. These delays were not correlated with the small additional delay in gastric emptying. Resistant starch and corn bran in muffin had the most effect on satiety, whereas polydextrose had little effect and behaved like the low fiber treatment (Willis, Eldridge, Beiseigel, Thomas and Slavin, 2009). An increase in Fibersol-2™ in peach flavored Nestea increased GLP-1 and PYY which affected satiety (Ye, Arumugam, Haugabrooks, Williamson and Hendrich, 2015).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

High amylose rice flour (25.78% amylose) was a gift from General Food Products Co., Ltd. (Nakhon Ratchasima, Thailand). Soy protein isolate (Profam 974) and resistant maltodextrin (Fibersol-2^{TM}) were purchased from the ADM Company (Chicago, USA) and corn bran (NF10085) was supplied by Bunge Milling Company (St. Louis, USA). Alpha-amylase (A3176, Type VI-B, from porcine pancreas, pepsin (P7012, from porcine gastric mucosa), pancreatin (P1750, 4×USP specifications) and amyloglucosidase (A7420, from *Aspergillus niger*) were purchased from Sigma-Aldrich. The Accu-Chek® Performa glucometer and the gold test strip electrodes were purchased from Roche Diagnostics Ltd (Australia).

3.2 Reformed rice extrusion

The reformed rice samples which were extruded rice flour (control) and extruded rice supplemented with 10% and 20% soy protein isolate (10SPI and 20SPI), 20% corn bran (10CB and 20CB), and 20% resistant maltodextrin (10FS and 20RMD) were prepared. The extruded reformed rice with duo combination of protein and fibers at 20% substitution was also produced. The ratios of SPI to fiber (CB or RMD) were 6:14, 10:10, and 14:6. The raw materials were adjusted to 28% moisture content. The raw materials were extruded using a twin screw extruder (APV MPF19:25, APV Baker, Inc., Grand Rapid, MI, USA) with a rice-shaped die. The feed rate was 0.5 kg/h. The barrel temperatures were set at 70, 90, 90, and 70°C. The screw speed was 30 rpm. Finally, the extruded rice was cooled at room temperature for 12 h and sealed in polyethylene bags for further analysis.

3.3 Extruded rice cooking

The ratio of extruded reformed rice, which was not pre-soaked, with water was 1:1.8. The rice was cooked by electric pressure cooker, followed by a 10 min holding period at the warming setting of the cooker.

3.4 Water absorption (WAI) and solubility (WSI) indices of raw materials

Water absorption index (WAI) and water solubility index (WSI) were measured according to Anderson, Conway and Peplinski (1970) with slight modifications A sample (about 1 g) was dispersed in 10 mL distilled water, and then incubated in a shaking waterbath (SW22, JULABO GmbH, Germany) at 95°C and 174 rpm for 30 min. The sample was centrifuged at 2000 g for 15 min. The sediment was weighed and the supernatant was dried in an oven at 105°C to determine solid content. The measurement was conducted in three replications. The indices were calculated as follows:

$$\text{WAI (g/g)} = \frac{\text{weight of water absorbed}}{\text{weight of dry sample}} \tag{1}$$

$$\text{WSI (\%)} = \left[\frac{\text{weight of dissolved solids}}{\text{weight of dry sample}}\right] \times 100 \tag{2}$$

3.5 Physicochemical properties of extruded rice

3.5.1 Pasting properties

The pasting profiles of extruded reformed rice were monitored using a Rapid Visco Analyser (RVA-4, Perten Instruments, Warriewood, Australia). The extruded reformed rice was ground. A sample suspension (10%, w/w, dsb) was equilibrated at 50°C for 1 min, heated at a rate of 6°C/min to 95°C, maintained at 95°C for 5 min, and then cooled to 50°C at a rate of 6°C/min. The paddle rotating speed was at 960 rpm for the first 10 s, followed by 160 rpm for the remainder of the analysis (Reed et al., 2013).

3.5.2 Gelatinization properties

The gelatinization properties of extruded reformed rice were determined using a Differential Scanning Calorimeter (DSC) (DSC1, Mettler Toledo, Switzerland) following the modified method of Lebail, Buleon, Shiftan and Marchessault (2000). The ground samples (7 mg) were weighed into stainless steel pans and distilled water was added to obtain a sample/water ratio of 1:3 (w/w) in the 60 µL stainless steel pans. DSC runs were performed from 25 to 140°C at a heating rate of 3°C/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH) were calculated by STAR^e software version 10.0 (Mettler Toledo, Switzerland)

3.6 Structure of high protein extruded rice

Infrared spectra of native rice flour, soy protein isolate and extruded 20SPI were recorded on a FT-IR spectrometer (Tensor 27, Bruker, UK) equipped using an attenuated total reflectance (ATR) accessory at a resolution of 4 cm^{-1} by 64 scans (Lu, Donner, Yada and Liu, 2016). A half-band width of 15 cm^{-1} and a resolution enhancement factor of 1.5 with Bessel anodization were employed. For studying the interaction of starch/protein in the extruded, the entire spectra were corrected and subjected to a multipoint linear baseline correction using OPUS software.

3.7 Stereo microscopy

The structural properties of the cooked extruded rice were photographed using a stereo microscope (SZX9, Optical Co LTD, Japan). Images of surface and cross sections of the cooked extruded rice were obtained at magnifications of 8x and 20x, respectively.

3.8 Rheological properties of cooked extruded rice

The dynamic viscosity of cooked extruded rice was studied using the modified method of Li, Prakash, Nicholson, Fitzgerald and Gilbert (2016). Dynamic viscoelasticity measurements were carried out in a stress-controlled rheometer AR G2 (TA instruments, USA) with a controlled temperature of 37°C. A four-bladed vane geometry with a diameter of 28 mm and a length of 42 mm and a cup with a diameter of 40 mm were used (TA instruments, USA). After cooking, 25 g of cooked rice were immediately loaded into the cup and gently packed to remove air. The vane was then set down to a distance of 4 mm from the bottom of the cup and was completely immersed in the rice bulk. At the vane temperature of 37°C, the rice was allowed to rest for 5 min before this dynamic test were performed. First, an oscillatory stress sweep test from 0.1 to 1000 Pa at a constant frequency of 10 rad/s and 37°C was conducted to set the upper limit of the linear viscoelastic region (LVR). Second, a frequency sweep over a range of 0.1-100 rad/s at 37°C was performed at the oscillatory stress of 2 Pa, which is within LVR for all rice samples. Viscoelastic parameters, storage or elastic modulus (G'), loss or viscous modulus (G''), and loss tangent (tan δ = G''/G') as a function of angular frequency (ω) were measured.

3.9 Digestibility of cooked extruded rice

3.9.1 Kinetic digestion

The *in-vitro* starch digestion assay was modified by the method of Sopade and Gidley (2009). About 500 mg of the sample was treated with 1 mL of artificial saliva containing porcine α-amylase (250 U per mL of phosphate buffer, pH 6.9) for 10-15 sec before 5 mL of pepsin solution (1 mL per mL of 0.02 M HCl, pH 2) was added and incubated at 37°C for 30 min (gastric digestion) in a shaking water bath (170 rpm). The phosphate buffer consisted of 20 mM sodium phosphate buffer and 6.7 mM sodium chloride. The digesta was neutralized (5 mL, 0.02 M NaOH) before adjusting the pH with 25 mL of 0.2 M sodium acetate buffer (pH 6) prior to adding 5 mL of a mixture of pancreatin (2 mg per mL of acetate buffer) and amyloglucosidase (28 U per mL of acetate buffer) in the acetate buffer. Then, it was incubated in the water bath (37°C, 170 rpm) for up to 240 min, During 0, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240 min, glucose concentration was measured using an Accu-Check® Performa® glucometer.

Starch digestion data of cooked extruded rice were fitted to a first-order equation (Eq. 3) (Goñi, Garcia-Alonso and Saura-Calixto, 1997):

$$C_t = C_\infty(1 - e^{-kt}) \qquad (3)$$

where C_t is the concentration of product at a given time (t), C_∞ is the concentration of product at the end of the reaction, and k is the digestibility rate constant.

However, the interval of starch amylolysis data did not constant. Thus, the different digestion rates at different times were studied using a Logarithm of Slope (LOS) plot in which there is a linear relationship between $ln(dc_t/dt)$ and t as shown in Eq. 4 (Butterworth, Warren, Grassby, Patel and Ellis, 2012; Poulsen, Ruiter, Visser and Lønsmann Iversen, 2003)

$$ln(dc_t/dt) = ln(C_\infty k) - kt \tag{4}$$

k and C_∞ are calculated from the slope $(-k)$ and intercept $\left(ln(C_\infty k)\right)$, respectively. The slope in this study was estimated from the second-order finite-difference formula $ln[(C_{i+1} - C_{i-1})/(t_{i+1} - t_{i-1})]$ as functions of $(t_{i+1} - t_{i-1})/2$ for all except the first and last points which were ignored (Butterworth et al., 2012; Zou, Sissons, Gidley, Gilbert and Warren, 2015).

3.9.2 Glycemic index

The area under hydrolysis curve (AUC) was calculated as the integral of the hydrolysis curve by Eq.(6), and used to obtain the hydrolysis index (HI) by Eq.(7) expressed as the percentage of the ratio of the AUC of the sample from 0 to 240 min to the AUC of white bread

$$D_t = D_0 + D_{\infty-0}\left(1 - exp(-kt)\right) \tag{5}$$

$$AUC = \left[D_\infty t + \frac{D_{\infty-0}}{k} exp(-kt)\right]_{t_1}^{t_2} \tag{6}$$

where D_t = digested starch (g/100 g dry starch) at time t

$D_t = D_0 + D_{\infty-0}$

D_0 = digested starch (g/100 g dry starch) at time t = 0

D_∞ = digested starch (g/100 g dry starch) at time t = ∞

k = rate of digestion, at selected times (g/100 g dry starch per min)

$$HI = \frac{AUC_{sample}}{AUC_{white\ bread}} \tag{7}$$

HI was used in calculating glycemic index (GI) using an Eq.(8) adapted from Goñi et al. (1997)

$$GI = 39.51 + 0.570HI \tag{8}$$

3.10 Gastric emptying and satiety analysis of cooked extruded rice

The Human Research Protection Program IRB of Purdue University approved all aspects of this research. The participants were studied in accordance with Purdue IRB protocol (#1608018056).

3.10.1 Subjects

For subject selection, respondents to the recruitment flyer were asked to come to the testing facility one week before of the start of the study to complete pre-screening and consent forms. Upon passage of the pre-screening, subjects were asked to read and sign the consent form.

Eligible subjects were 18-50 years, BMI 18.5-25 kg/m^2, stable weight for the past 3 months i.e. +/-2.5 kg, regular eating pattern, including breakfast consumption. Subjects were excluded if they had gastrointestinal disease, were smokers, were pre- or post-menopausal women, had celiac disease, were pregnant and lactating women, were following a weight reduction program or had followed one during the last 3 months, had acute or chronic disease, had alcohol consumption >30 units/week, had hypertension, had diabetes, and had previous bariatric surgery.

3.10.2 Test meal

The meal, based on 50 g carbohydrate, included: cooked extruded rice (control), cooked extruded rice supplemented with 20% soy protein isolate (20SPI), cooked extruded rice supplemented with 20% corn bran (20CB), and cooked extruded rice supplemented with 20% resistant maltodextrin (20RMD).

3.10.3 Gastric emptying

The study was a cross-over design human trial consisting of four-hour sessions with a one-week washout period between each treatment arm, where subjects consumed meal containing one of each of the five meals over the five sessions. Subjects were required to come for testing on the same day of each week for five consecutive weeks. Subjects having fasted for at least 10 hours arrived to the testing room before 8 am and were asked to refrain from any heavy physical activity outside their normal routine in that period.

For the gastric emptying breath test, subjects first breathed into two 1.5 L bags for baseline breath measurements. Within 10 min, subjects consumed the meal satiating meal quantities containing 100 mg [^{13}C]-labeled octanoic acid which was used as a tracer to monitor gastric emptying rate. Breath samples were collected in 300 ml bags every 15 min for four hours from the time that they finish consuming the meal (8 am-12 pm). Subjects were instructed to consume all of the meal given to them. No other food or drink was allowed during the remainder of the test session. The meal treatments were given to subjects on the same day of the week with at least a one week in between treatment arms as a washout period. The rate at which the test materials were emptied from the stomach was determined using a $^{13}CO_2$ Urea Breath Analyzer POCone (Otsuka Electronics Co, Ltd, Osaka, Japan).

Calculation of gastric emptying parameters

The breath analyzer provides the change in the $^{13}CO_2$ delta over baseline (DOB, ‰), where the $^{13}CO_2/^{12}CO_2$ ratio of a sample gas was compared to the corresponding ratio of a reference gas (i.e. baseline value). The percent dose ^{13}C recovery per hour (PDR), and cumulative percentage dose recovery over time (CPDR) were calculated using equation 9 and 10, respectively. CO_2 production was assumed to be 300 mmol/(m^2 body surface area x hour), with body surface area calculated using the formula developed by Haycock, Schwartz and Wisotsky (1978).

$$PDR = at^b e^{-ct} \tag{9}$$

where PDR = percentage dose recovery per hour

t = time in hours

a, b, and c = constants

$$CPDR = m(1 - e^{-kt})^{\beta} \tag{10}$$

where CPDR = cumulative percentage dose recovery over time

t = time in hours

k and β = constants

m = total cumulative dose recovery when time is infinite

After calculating PDR and CPDR values from the obtained data set, these functions were modeled using the following equations to discern parameters related to the gastric emptying rate.

Gastric emptying parameters were calculated using the following formulas:

Lag phase (time required for the $^{13}CO_2$ excretion rate to attend its maximal level) (Sanaka and Nakada, 2010)

$$T_{lag} = (\ln \beta)/k \tag{11}$$

Half emptying time (time necessary for half of the ^{13}C dose to be metabolized) (Sanaka et al., 2010)

$$T_{1/2} = \left(-\frac{1}{k}\right) \times \ln\left(1 - 2^{-1/\beta}\right) \tag{12}$$

3.10.4 Satiety analysis

A multi-component satiety scale questionnaire was used to measure the subjective effects of the test materials on perception of hunger, fullness, and satisfaction in subjects. Satiety was evaluated using questions from a previously validated 100 mm visual analogue scale (VAS). VAS ratings were completed in the subject booths at 0 (fasting), 30, 60, 90, 120, 150, 180, 210 and 240 min.

The questions were as follows:

Hungry: How hungry do you feel? Not hungry at all (0 mm) vs I have never been hungrier (100 mm)

Fullness: How full do you feel? Not full at all (0 mm) vs Totally full (100 mm)

Satisfaction: How satisfied do you feel? I am completely empty (0 mm) vs I cannot eat another bite (100 mm)

Differences in the satiety parameters were assessed by calculating total area under the curve (AUC) using the trapezoid rule (Willis et al., 2009).

3.11 Effect protein and fibers on digestibility of extruded rice paste

3.11.1 Extruded rice paste preparation

The extruded rice was ground. A sample suspension (10%, w/w, db) was gelatinized using the RVA by the same condition of pasting properties study (section 3.5.1).

3.11.2 Effect of *in-vitro* digestion on viscosity of extruded rice paste

The viscosity of extruded rice paste during simulated the *in-vitro* digestibility (Sopade et al., 2009) was studied by the modified method of and Bordoloi, Singh and Kaur (2012). After gelatinization, 25 g of extruded rice paste were immediately loaded into the rheometer's cup. The extruded rice paste was digested immediately. Time sweep experiments were carried out to measure viscosity of extruded rice paste during simulated gastric and small intestinal digestion using a dynamic rheometer equipped with a four-bladed vane geometry. The experiment was carried out at 37°C using a multi-step flow procedure. During the first step, the shear rate was maintained at 100 s^{-1} for 20 sec to mix the RVA paste of 1.5 g and 1 mL of porcine α-amylase (250 U per mL of carbonate buffer, pH 7). Before beginning of second step, the shear rate was increased to 300 s^{-1} for 10 sec for a thorough mixing with 5 mL pepsin solution (1 mL per mL of 0.02 M HCl, pH 2). During the second step, a shear rate of 100 s^{-1} was maintained for 30 min. Before the beginning of final step, the mixture was neutralized (5 mL, 0.02 M NaOH), and then the pH was adjusted with 25 mL of 0.2 M sodium acetate buffer (pH 6). After that, 5 mL of the mixture of pancreatin (2 mg per mL of acetate buffer) and amyloglucosidase (28 U per mL of acetate buffer) was added and mixed with the shear rate at 300 s^{-1} for 30 sec. During the final step, a shear rate of 100 s^{-1} was maintained for 90 min.

3.11.3 Kinetic digestion of extruded rice paste

The *in vitro* digestion of extruded rice paste was studied for kinetic digestion of cooked extruded rice similar to the section 3.9.1.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect of protein and fibers on extruded reformed rice properties

4.1.1 Water absorption (WAI) and solubility (WSI) indices of raw materials

Water absorption and water solubility of raw materials (Figure 1), which are rice flour (RF), soy protein isolate (SPI), corn bran (CB), and resistant maltodextrin (RMD), at 90°C refer to the water binding ability of ingredients (SPI, CB, RMD) at extrusion temperature compared with rice flour. The WAI and WSI of SPI were significantly higher than rice flour (Figure 4.1). Heating at higher than 80ºC denatures 11S and 7S proteins, which modifies the quaternary structure and the unfolding of the polypeptide chains of the various soy proteins, produces the high water imbibing capacities at pH 7 which is above the isoelectric point of SPI (pH 5) (Remondetto, Añon and González, 2001; Robinson, 2010). The WSI of RMD was the highest while its WAI was the lowest (Figure 4.1). This was probably due to the RMD is non-viscous soluble fiber which is heat stable (Ohkuma and Wakabayashi, 2008). The WAI of CB was significantly lower than that of SPI and rice flour, respectively, but significantly higher than RMD (Figure 1). The CB is dietary fiber, which is nearly insoluble. Water binding of corn bran is dependent on the arabinose substitution (Doner and Hicks, 1997; Kiszonas, Fuerst and Morris, 2013). Thus, the water accessibility of SPI was higher than rice flour, while that of fibers was lower than rice flour.

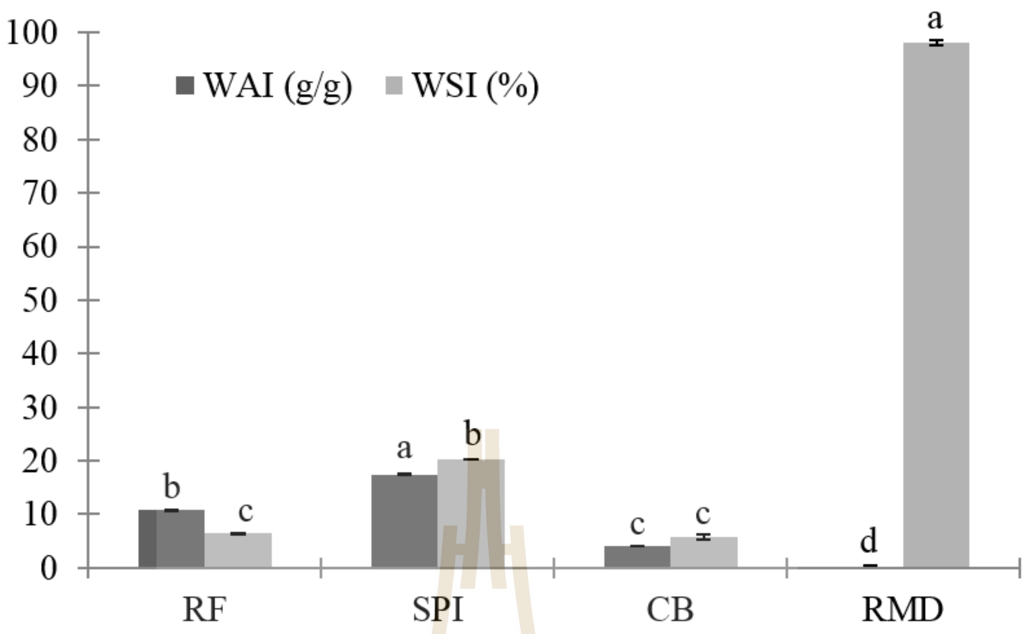


Figure 4.1 Water absorption index (WAI) and solubility index (WSI) at 90°C of rice flour (RF), soy protein isolate (SPI), corn bran (CB) and resistant maltodextrin (RMD).

When rice flour was supplemented with 20% SPI, the WAI of this raw material mixture was the lowest (Figure 4.2). This lowest WAI was probably due to the reduction of starch content, suggesting that the available starch to absorb water is less. As a result of the water accessibility of SPI is more efficient than that of rice flour (Zhu et al., 2010), protein in the blend proportionally restricted starch granule swelling during cooking (Lu et al., 2016). Additionally, the WAI of the mixture of rice flour supplemented with 20% CB was significantly lower than that of rice flour (Figure 4.2). The similar result was reported by Baek et al. (2014) in that the decrease of water holding capacity of rice flour substituted with CB was found after heating at 100°C. The decreased WAI of the CB mixture after heating could be attributed to lower rice flour fraction, which has more efficiency to access water than CB. The WAI of 20%RMD was significantly lower than rice flour. It might be that the rice flour fraction was less, although the RMD did not compete to access water with rice flour. Moreover, The WAI of 20%RMD was significantly higher than that of 20%CB and 20%SPI (Figure 4.2), respectively. Consequently, the water binding capacity of SPI and CB could decrease the available water for starch granule, resulting in the WAI of the mixture decreased, while the WAI of RMD mixture was decreased due to the lower of starch fraction.

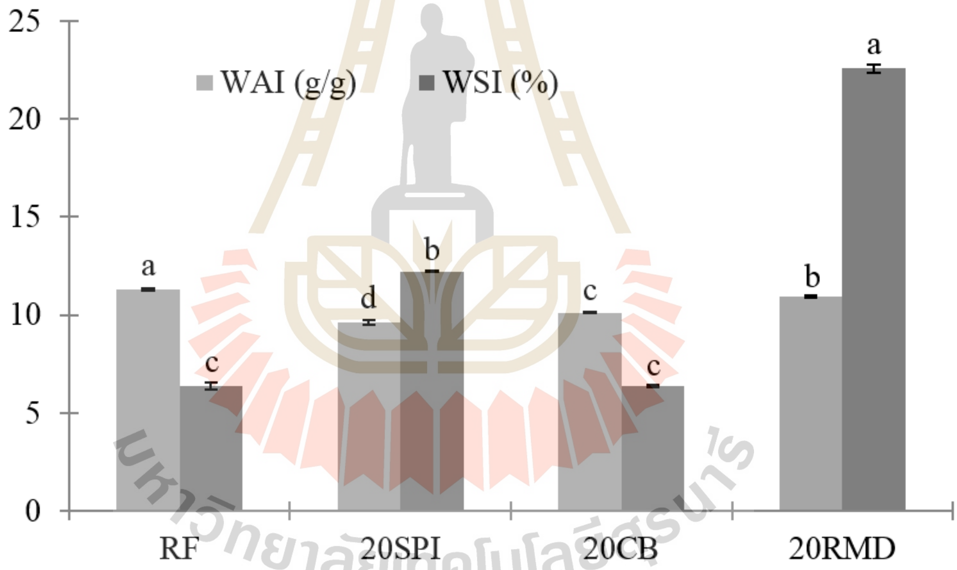


Figure 4.2 Water absorption index (WAI) and solubility index (WSI) at 95°C of rice flour (RF), and rice flour supplemented with 20% soy protein isolate (20SPI), 20% corn bran (20CB), and 20% resistant maltodextrin (20RMD).

4.1.2 Extrusion processing

Extrusion cooking is an essential process which starchy food is worked into a viscous, plastic like dough and cooking before being forced through a die (Wang, Klopfenstein and Ponte, 1993). Extrusion parameters of extruded rice (control), extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER) were shown in Table 4.1. The extrudate of 20SPI that was forced through a die exhibited the highest die pressure. Since SPI absorbed more water than rice flour (Figure 4.1), the addition of soy protein isolate have been attributed to competition for water and delay of gelatinization during extrusion. However, the torque of 20SPI was not significantly different from that of the control, implying that 20% SPI did not significantly affect the apparent viscosity of the mass inside the extruder. The torque and die pressure of 20RMD were the lowest. It might be due to the low water absorption of RMD (Figure 4.1). Therefore, the available water for starch gelatinization of 20RMD was the highest which led to the apparent viscosity reduction of the mass inside the extruder. The die pressure of 20CB was also significantly lower than that of the control. It is possible that corn bran is insoluble fiber and the WAI of CB was significantly lower than that of the RF, thus the available water for starch gelatinization of 20CB was higher than that of the control. Moreover, CB is a hard solid particle with big size was dispersed in the material in barrel, which contributed to the increase of apparent viscosity of the mass inside the extruder and the highest torque.

Table 4.1 Extrusion parameters of extruded rice (control), extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

Sample	**Torque (%)**	**Die pressure (psi)**
Control	54.33±4.04b	541.67±7.63b
20SPI-ER	56.33±1.53b	653.33±25.16a
20CB-ER	80.33±2.52a	370.00±10.00c
20RMD-ER	35.33±1.53c	260.00±5.00d

Note: Mean values with different letters within each column are significantly different ($P<0.05$).

4.1.3 Physicochemical properties of extruded rice

4.1.3.1 Gelatinization properties

The gelatinization properties of native rice flour, extruded rice (control), extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER) were shown in Table 4.2. The native rice flour showed biphasic endotherm (Figure 4.3) which could be due to the fact that the commercial rice flour used contains various rice variety. After extrusion, the weak crystalline region was destroyed as it was observed from the disappearance of the first onset temperature of the control. The gelatinization enthalpy of all extruded rice flour was significantly lower than that of native rice flour. This indicates that the extrusion process destroyed molecular order of rice flour. The decrease of gelatinization enthalpy primarily reflected the loss of molecular (double-helical) order (Cooke and Gidley, 1992). Additionally, the onset temperature (T_o) transition is expected to depend on a complex interplay of factors, and water transport into the granules could be considered to play a very important role (Mira, Persson and Villwock, 2007). The T_o, peak temperature (T_p) and conclusion temperature (T_c) of 20SPI-ER was shifted to higher temperature. It is probable that the high ability to retain water of protein and their interaction with the starch could affect the gelatinization process (Ribotta, Colombo, León and Añón, 2007). These results are similar to other previous studies (Li, Yeh and Fan, 2007; Ribotta et al., 2007; Yu, Jiang and Kopparapu, 2015). The increase of T_p could be related to the interaction among starch, protein and water. As a result of water competition of protein, the availability of water for starch was lower. Therefore, the 20SPI showed the highest of T_o and T_p. The T_o, T_p and, T_c of 20CB-ER showed the lowest value. This may be due to the increased of the available water for starch gelatinization (Acquistucci, Bucci, Magri and Magri, 1997). This might be that the corn bran particle increased the surface area for water accessibility into rice starch granule. The T_o and T_p of 20RMD-ER were not significantly different from those of the control. Thus, resistant maltodextrin did not compete with rice flour for the water due to its lowest WAI (Figure 4.1). Thus, the water for starch gelatinization of 20RMD-ER was not different from that of the control.

Table 4.2 Thermal properties of native rice flour (Native RF), extruded rice (control) and extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), 20% resistant maltodextrins (20RMD-ER).

Sample	T_o (°C)		T_p (°C)	T_c (°C)	Enthalpy (J/g)
Native RF	T_1 60.5±0.05	T_2 70.4±0.04a	75.9±0.10a	80.7±0.23b	5.9±0.23a
Control	n.d.	73.2±0.04b	77.9±0.11c	80.4±0.40b	2.0±0.05b
20SPI	n.d.	74.7±0.20c	79.1±0.03d	81.9±0.02c	1.3±0.11c
20CB	n.d.	70.2±0.01a	76.1±0.05b	79.3±0.09a	1.5±0.16c
20RMD	n.d.	73.0±0.08b	78.0±0.03c	82.3±0.10c	1.1±0.07c

Note: Mean values with different letters within each column are significantly different ($P<0.05$), n.d.: non detected.

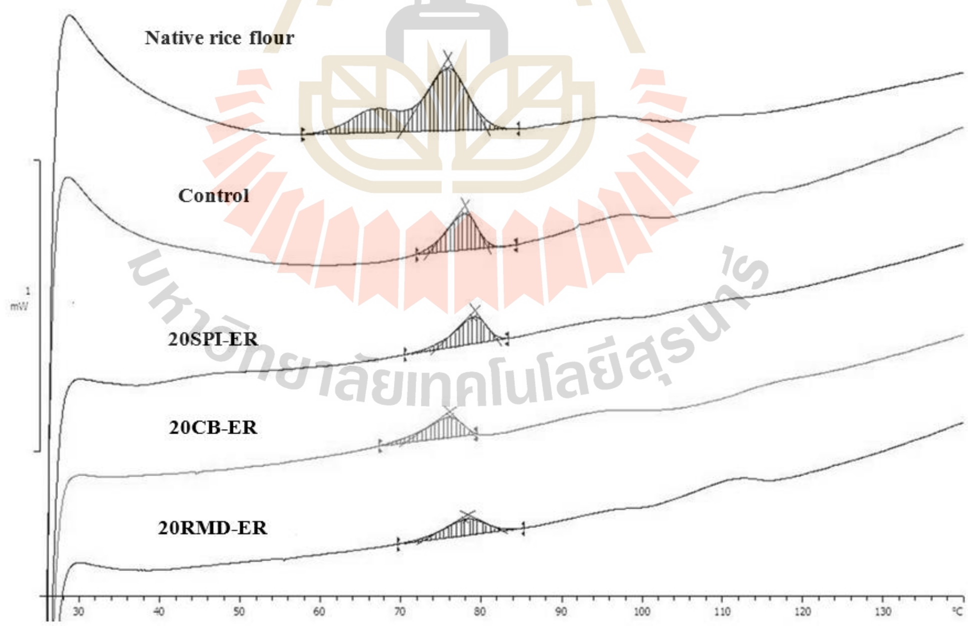


Figure 4.3 DSC thermograms of native rice flour, extruded rice (control) and extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

4.1.3.2 Pasting properties

The pasting profile of extruded rice with 20% substitution of SPI, CB and RMD are showed in Figure 4.4. The extrusion process that promotes starch gelatinization would be expected to decrease peak viscosities because of the lower availability of native starch granules, as shown in DSC results. Peak and final viscosity of extruded rice supplemented with 20SPI-ER exhibited the lowest value. A similar result was reported by Mayachiew, Charunuch and Devahastin (2015) in that the addition of soybean decreased the peak, trough and final viscosity of the soybean-rice powder extruded porridge. This result was related to the higher WAI of SPI. Consequently, the water that was available for starch granule swelling and gelatinization was reduced. However, peak viscosity of 20CB-ER and 20RMD-ER were higher than 20SPI-ER, which related to the WAI of 95°C of those raw material mixtures (Figure 4.2). It might indicating that the swelling of the remaining starch granule of 20CB-ER and 20RMD-ER was higher than that of 20SPI-ER, because the WAI of corn bran and resistant maltodextrin were significantly lower than that of rice flour (Figure 4.1). Therefore, the available water for starch gelatinization of 20CB-ER and 20RMD-ER was higher than that of 20SPI-ER. The final viscosity of 20CB-ER was lower than that of 20RMD-ER, which could be that the re-aggregation of amylose chain was interfered by the corn bran particles which led to the low final viscosity. For the control, the remaining starch granule was completely swollen because starch granule did not compete for water with the other ingredients.

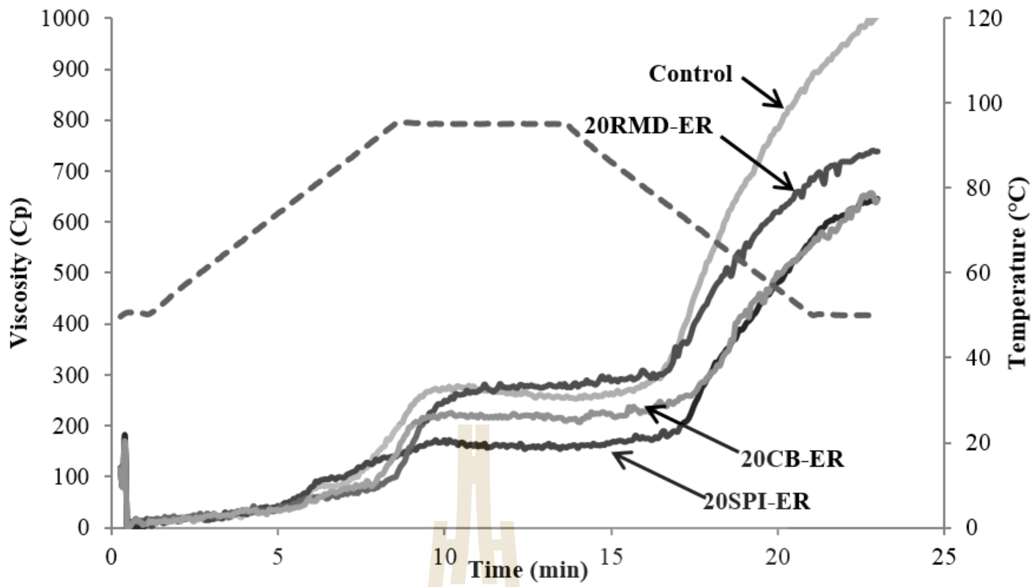


Figure 4.4 Pasting profile of extruded rice (control) and extruded rice at 20% substituted of soy protein isolate (20SPI-ER), corn bran (20CB-ER) and resistant maltodextrin (20RMD-ER).

4.1.3.3 Structure of high protein extruded rice

The chemical interaction of high protein extruded rice was investigated, whereas the chemical interaction between fiber and starch could not be induce by the extrusion process. Fourier transform infrared (FTIR) spectra of rice flour, soy protein isolated and extruded rice supplemented with 20% soy protein isolate (20SPI-ER) was presented in Figure 4.5. Typical wave numbers at 1053, 1047, 1040 and 1022 cm^{-1} are assigned to short-range ordering and amorphous areas in starch (van Soest, Tournois, de Wit and Vliegenthart, 1995). The wave number at 994 cm^{-1} of rice flour, which is mainly due to COH bending vibrations, is especially sensitive to the water content and these vibrations must involve water-starch interaction by, for example, hydrogen bonding (van Soest et al., 1995). The typical protein bands can be clearly distinguished at wavenumbers of 1691, 1647, 1635, 1620, 1536, and 1515 cm^{-1}. Specific amide I and II of protein are assigned in the wavenumber range of 1580-1720 cm^{-1} and 1480-1580 cm^{-1}, respectively (Li, Dobraszczyk, Dias and Gil, 2006). When soy protein isolate was added, the absorbance peak heights of starch fingerprint showed a decreasing order in the wavenumber range of 800-1200 cm^{-1}, while an increasing order of protein fingerprint in the range from 1500-1700 cm^{-1} was observed. The region between 3000 and 3600 cm^{-1} is assigned to OH stretching (Kizil, Irudayaraj and Seetharaman, 2002). The OH stretching region of 20SPI-ER was shifted to be lower, implying that the extrusion induces the hydrogen bond between SPI and starch. A decrease of molecular mobility as a result of starch-protein interaction after extrusion led to a significant shift in the OH stretching band to lower wavenumbers, reflecting an increase in hydrogen bond density and strength (Lu et al., 2016). The stretching vibration of a hydroxyl group involved in a hydrogen bond is expected to have a lower wavenumber than its non-hydrogen bonded counterpart as it has a lower bond force constant due to electron delocalisation in hydrogen bonded structures (Ottenhof, MacNaughtan and Farhat, 2003).

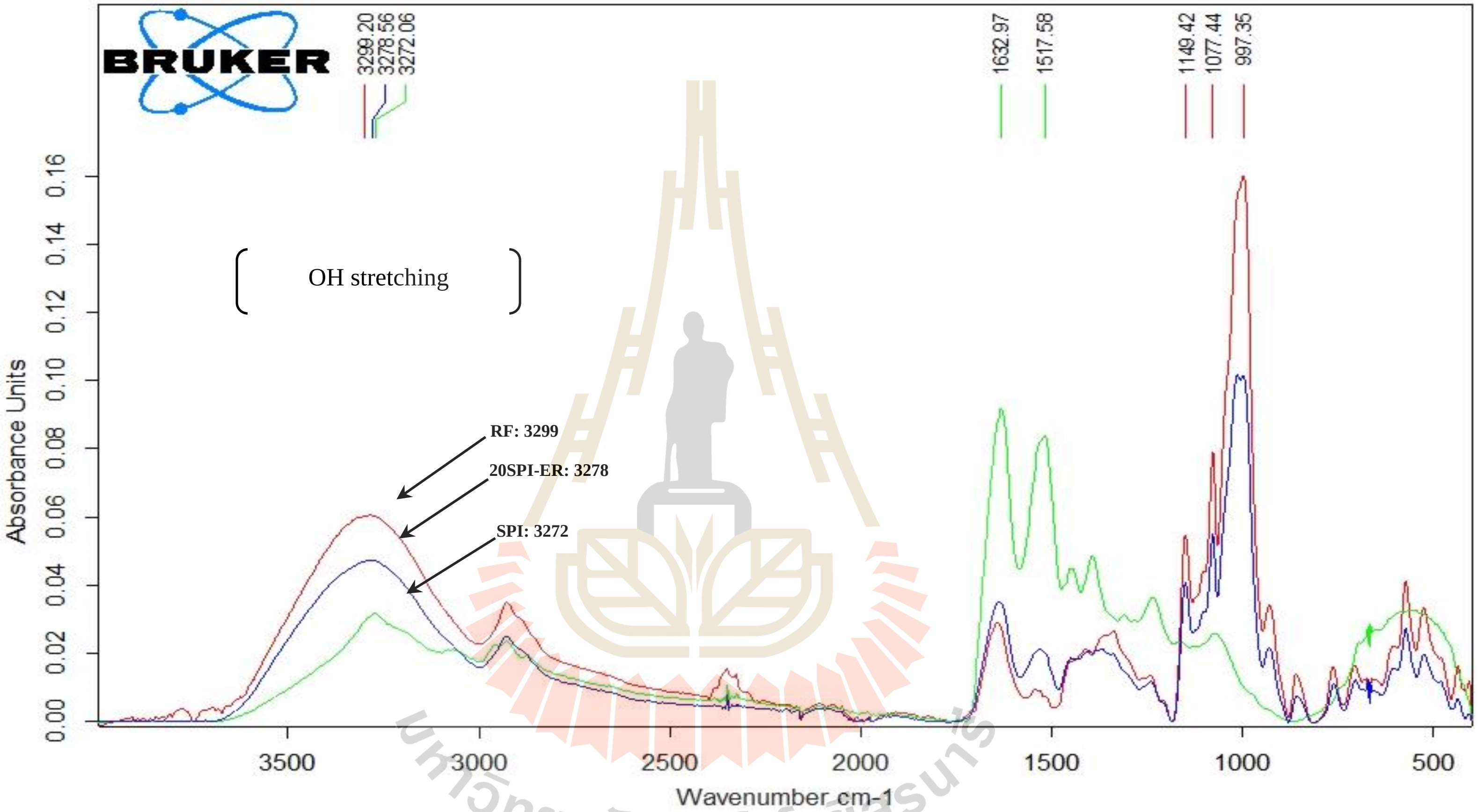


Figure 4.5 Fourier transform infrared (FTIR) spectra of rice flour, soy protein isolated and extruded rice supplemented with 20% soy protein isolate (20SPI-ER).

4.1.3.4 Physical characteristic of cooked extruded rice

The cooked extruded rice showed the similar shape with normal cooked rice (Figure 4.6). The surface of cooked extruded rice supplemented with 20% soy protein isolate (Figure 4.7b) and 20% resistant maltodextrin (Figure 4.7d) was smooth similar to that of the control (Figure 4.7a), whereas the surface of cooked extruded rice supplemented with 20% corn bran was not smooth (Figure 4.7c). The big size of corn bran particles were dispersed in the surface (Figure 4.7c) and incorporated into the starch matrix of this cooked extruded rice grain (Figure 4.8c). However, the inside characteristics of other cooked extruded rice grains were similar to the control (Figure 4.8a, 4.8b, 4.8d). No particles were found in other cooked extruded rice grains and in the control.

Figure 4.6 Cooked extruded rice (a), cooked extruded rice supplemented with 20% soy protein isolate (b), 20% corn bran (c), and 20% resistant maltodextrin (d).

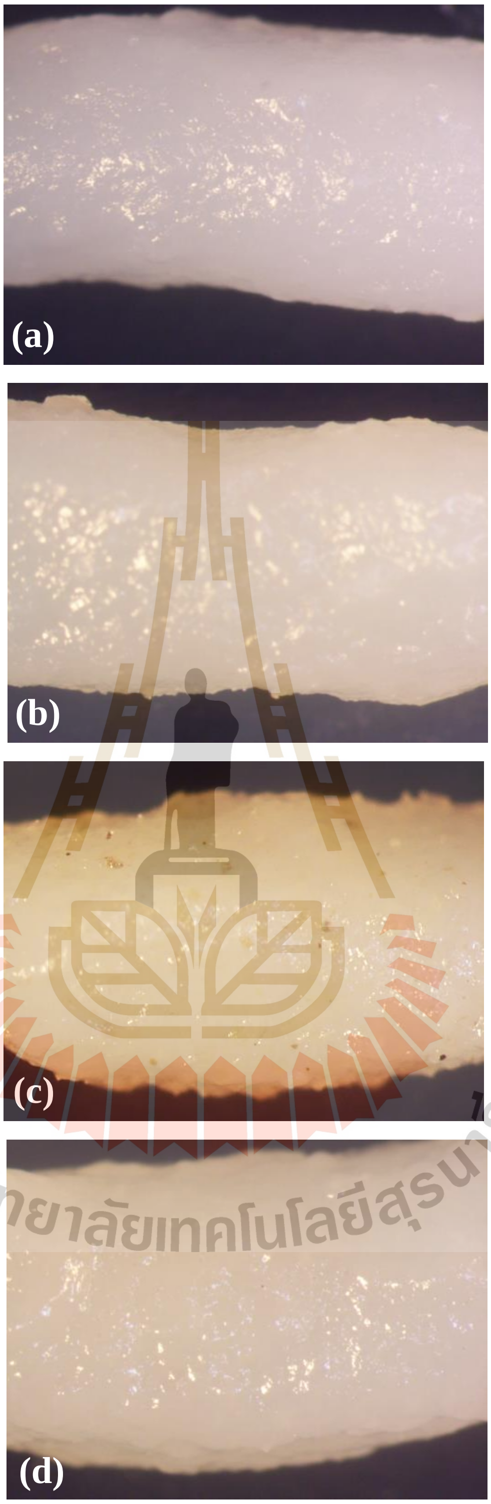


Figure 4.7 Surface of cooked extruded rice (a) and extruded rice at 20% substituted of soy protein isolate (b), corn bran (c) and resistant maltodextrin (d) (magnification 8x).

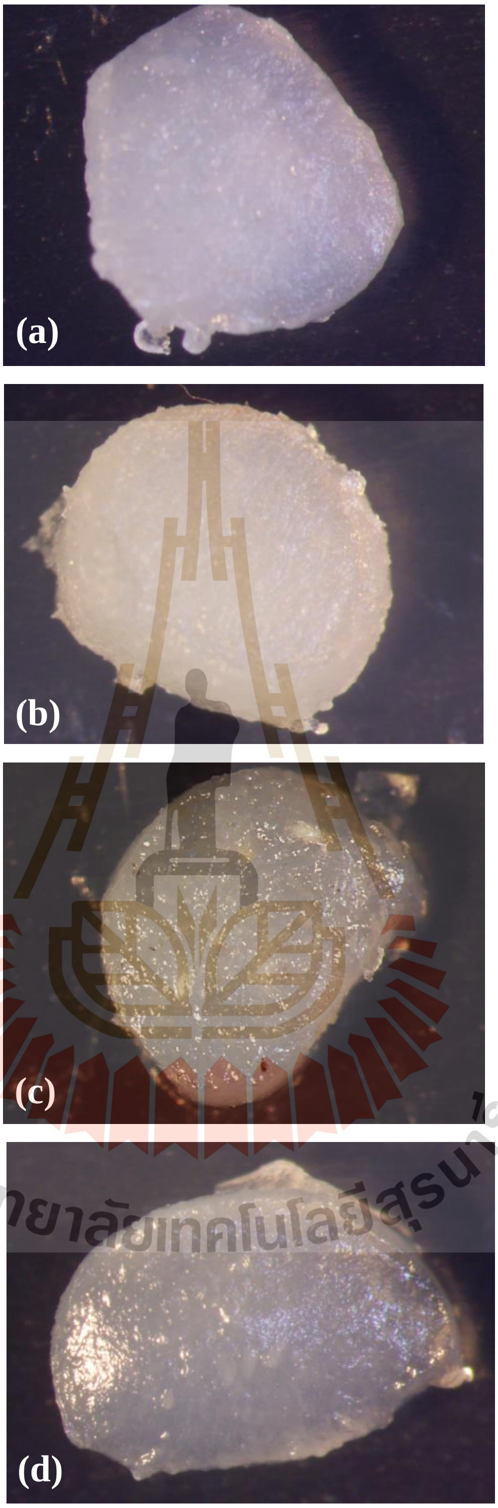


Figure 4.8 Cross section of cooked extruded rice (a) and extruded rice at 20% substituted of soy protein isolate (b), corn bran (c) and resistant maltodextrin (d) (magnification 20x).

4.2 Rheology properties of cooked extruded rice supplemented with protein and fibers

The rheology properties of cooked extruded rice were displayed in Table 4.3. The n* value of all cooked rice samples was lower than 1 (0.035-0.101), indicating a shear thinning behavior. The consistency coefficient (K*) and loss tangent (tan δ) was used to express the hardness and stickiness of cooked rice (Li et al., 2016). The hardness and stickiness of 20SPI-ER were significantly higher than those of the control, 20CB-ER, and 20RMD-ER. The storage modulus (G') value of 20SPI-ER was also higher than that of the control (Figure 4.9). It is possible that the swelling of starch granule was decreased when 20%SPI was supplemented. Moreover, the SPI substantially changes the viscoelastic properties of the extruded rice to become more elastic than viscous. The swelling of starch granules and the dissolved amylose molecules would cause an increase in G' and this would be attributed to the formation of networks of swollen starch granules associated with protein (Ramírez Ortiz, San Martín-Martínez and Martínez Padilla, 2008). The similar result was reported by Qiu et al. (2015) in that the addition of soy protein isolate (with and without dry heat treatment) slightly increased the apparent viscosity of corn starch and waxy corn starch. The hardness and stickiness (Table 4.3), and G' (Figure 4.9) of the cooked 20CB-ER were higher when compared with those of the control. This might indicate that the added corn bran could provide good compatibility with the starch gel. The insoluble fiber- starch mixed system formed a stronger structure than the single starch system (Sun, Wu, Bu and Xiong, 2015). However, the hardness and stickiness of cooked 20CB-ER was slightly lower than those of cooked 20SPI-ER. It might be that the low gelatinization temperature (T_o, T_p) and the dispersed particle of CB in gel net network resulted that the viscoelastic of cooked 20CB-ER was lower than that of cooked 20SPI-ER. Resistant maltodextrin had a low impact on the *G′* (Figure 4.9) of cooked 20RMD-ER, although the hardness and stickiness of cooked 20RMD-ER were significantly higher than that of the control (Table 4.3). It is possible that the peak and final viscosity of cooked 20RMD-ER were lower than those of the control, however, the T_o, T_p and peak viscosity of cooked 20RMD-ER were not significantly different from those of the control, implying that their starch granule swelling were not significantly different which led to the viscoelasticity of cooked 20RMD was slightly higher than the control. Moreover, RMD cannot form network with starch gel as its short chain polysaccharide has dextrose equivalent (DE) less than 20 and it completely solubilized (Ohkuma et al., 2008).

Table 4.3 Rheology properties of cooked extruded rice (control), cooked extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

Sample	K*	tan δ at 10 rad/s	n*
Control	313.42±1.27c	0.066±0.002c	0.089±0.001a
20SPI-ER	838.21±17.71a	0.085±0.001a	0.052±0.011b
20CB-ER	816.79±27.89a	0.072±0.003b	0.035±0.013b
20RMD-ER	376.88±8.44b	0.069±0.003b	0.090±0.005c

Note: Mean values with different letters within each column are significantly different ($P<0.05$).

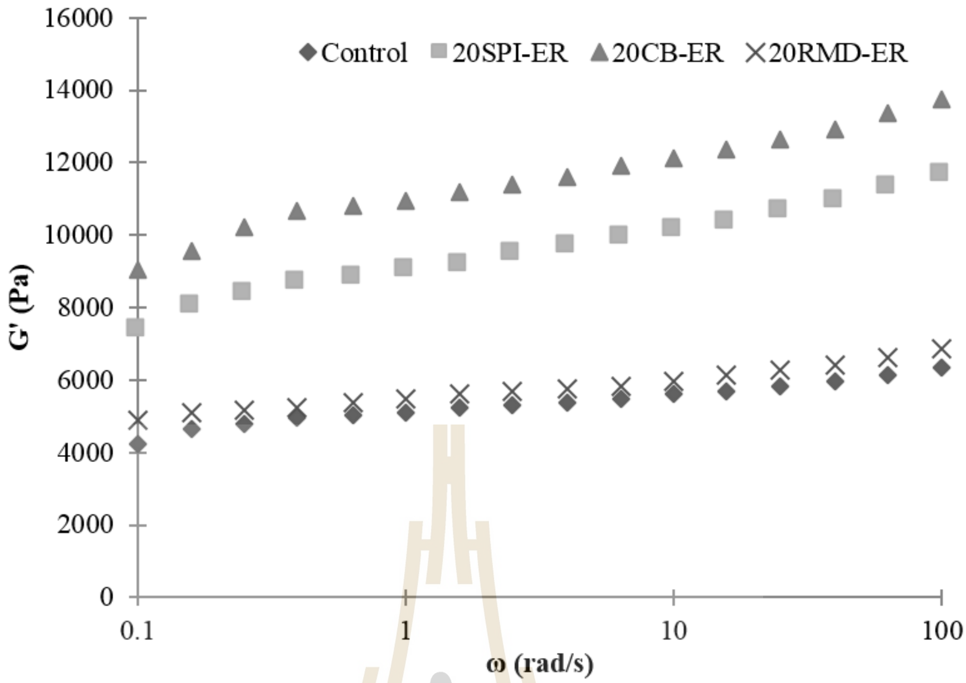


Figure 4.9 Storage modulus of cooked extruded rice (control), cooked extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

4.3 Kinetic digestion of cooked extruded rice supplemented with protein and fibers

The rate of starch digestion is an important factor of the glycemic response. *In-vitro* studies provide the high efficiency of predicting postprandial outcomes. The use of Log of slope (LOS) analysis are very sensitive to alteration of the digestibility rate constant, and it enhances the differences in starch digestibility which are not immediately obvious from conventional digestibility curves (Edwards, Warren, Milligan, Butterworth and Ellis, 2014). LOS plots have the potential of clearly revealing and quantifying differences in rate of processes during starch amylolysis and also enabling the product concentration at the end of amylolysis to be predicted without the need to carry out prolonged digestions. This allows estimations of both the end-point product concentration (C_∞) and the pseudo first-order digestibility rate constant (k) and can also show the changes that occur in digestion rate from rapid to slow as digestion proceeds (Butterworth et al., 2012). In foods containing starch fractions that are digested at different rates, LOS plots reveal two or more distinct linear phases, in which the slope of each distinct phase provides a rate constant, denoted k_1, k_2, enabling the end-point of starch amylolysis (denoted $C_{\infty1}$, $C_{\infty2}$) to be computed for each phase (Edwards et al., 2014). Consequently, the LOS analysis is suitable to demonstrate the digestibility of cooked extruded rice supplemented soy protein and fibers.

4.3.1 Effect of ingredients on digestibility of cooked extruded rice

LOS analysis was applied to obtain the digestibility curves to establish amylolysis followed whether a single-phase or two-phase pseudo-first order process, and to estimate C_∞ and k values of each phase (Table 4.4). The cooked extruded rice (the control) digestion was fitted with a single-phase first order kinetic (Figure 4.10) which the C_∞ was the highest value; resulting in the control was in a high GI food.

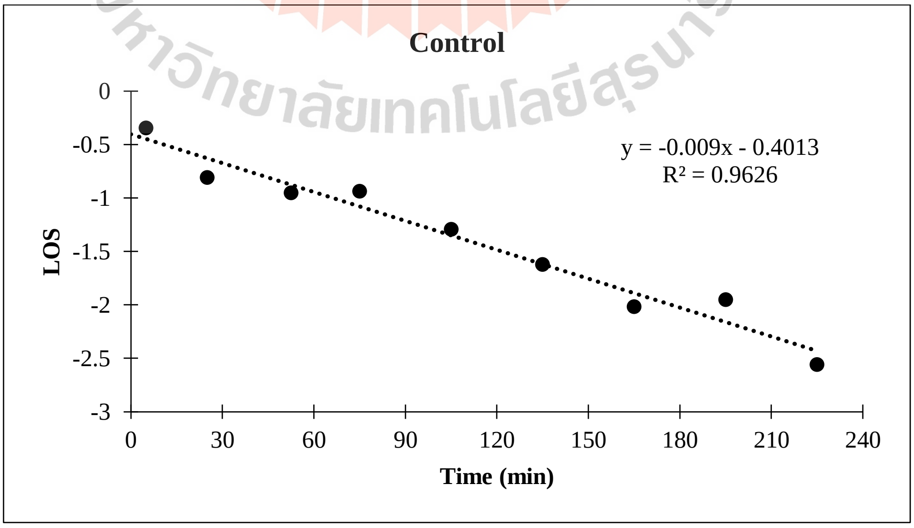


Figure 4.10 LOS plot of cooked extruded rice digestion.

Table 4.4 *In-vitro* digestion parameters and GI of cooked extruded rice (control) and cooked extruded rice supplemented with 10% (10SPI-ER) and 20% soy protein isolate (20SPI-ER), 10% (10CB-ER), 20% corn bran (20CB-ER), and 10% (10RMD-ER) 20% resistant maltodextrin (20RMD-ER).

Sample		**k(×10^{-3})**	**C_∞**	**GI**
Control		9.10±0.64 c	75.08±4.27a	86.24±5.62a
10SPI-ER		15.00±2.29ab	60.85±1.60b	63.13±2.29c
10CB-ER		11.40±2.60bc	70.40±3.55a	66.98±1.36bc
10RMD-ER		12.20±0.84bc	60.09±1.50b	69.66±1.15b
20RMD-ER		11.50±0.56bc	58.68±0.48b	67.99±3.08bc
Sample	**k_1(×10^{-3})**	**k_2(×10^{-3})**	**$C_{\infty 2}$**	**GI**
20SPI-ER	22.90±2.02a	10.00±2.48c	47.64±0.76c	55.72±1.36d
20CB-ER	3.40±0.66b	18.50±3.19a	72.22±3.17a	63.32±2.29c

Note: Mean values with different letters within each column are significantly different ($P<0.05$).

The single-phase process was also found in cooked extruded rice supplemented with 10% soy protein isolate (10SPI-ER), 10% corn bran (10CB-ER), and 10% resistant maltodextrin (10RMD-ER) (Figure 4.11). Both of the fibers at 10% substitution did not significantly affect the digestion rate (k). The C_∞ of cooked 10CB-ER was higher than that of the cooked 10SPI-ER and cooked 10RMD-ER. This might indicates that the big particles of corn bran induce the more surface area for enzyme accessibility of starch which leads to the continuous digestion of starch up to the end-point of amylolysis. The GI value of cooked 10SPI-ER was lower than that of the control and cooked 10RMD-ER. This could be associated with the higher hardness and stickiness of cooked 10SPI-ER.

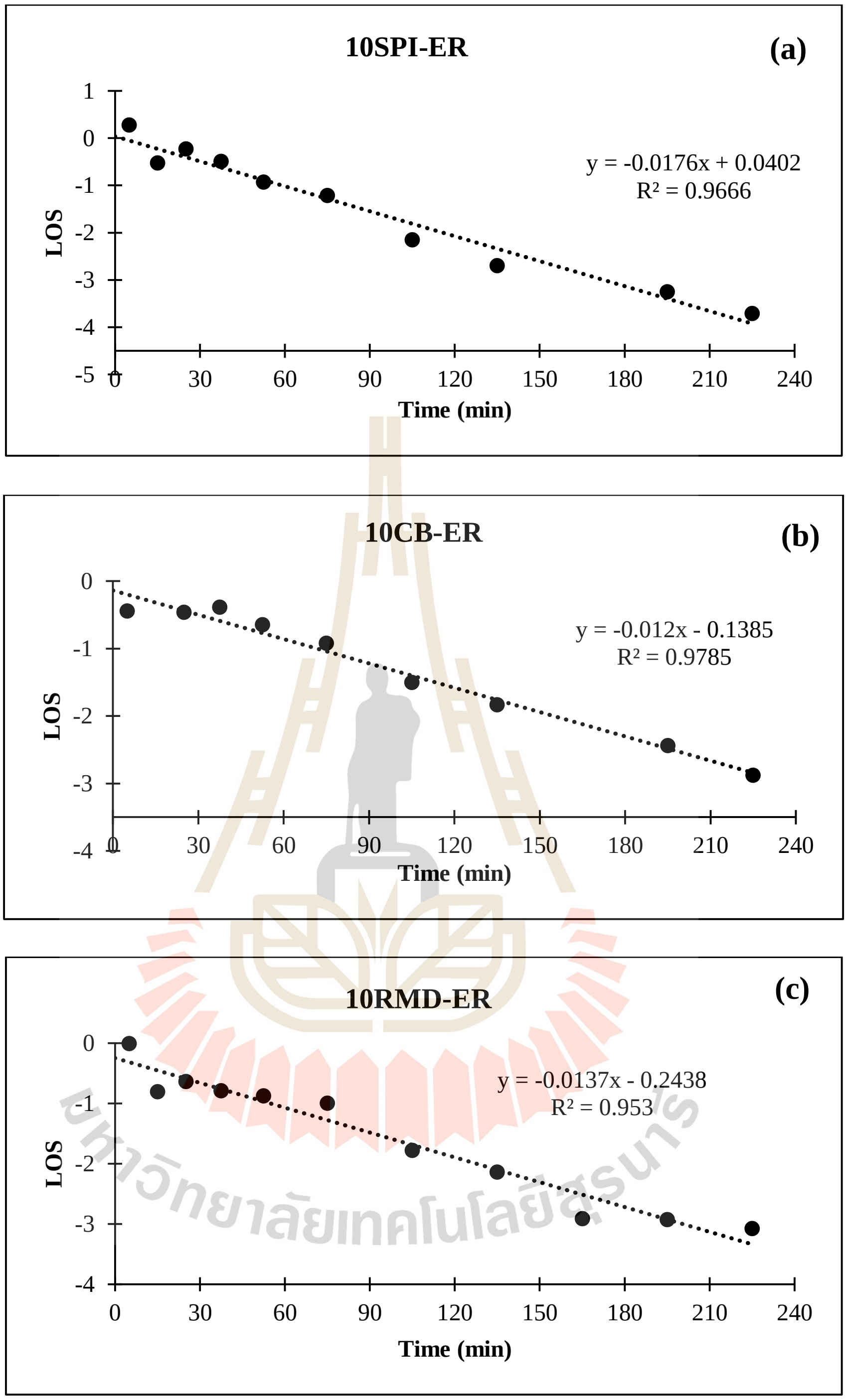


Figure 4.11 LOS plot of the digestion of cooked extruded rice supplemented with (a) 10% soy protein isolate (10SPI-ER), (b) 10% corn bran (10CB-ER), and (c) 10% resistant maltodextrin (10RMD-ER).

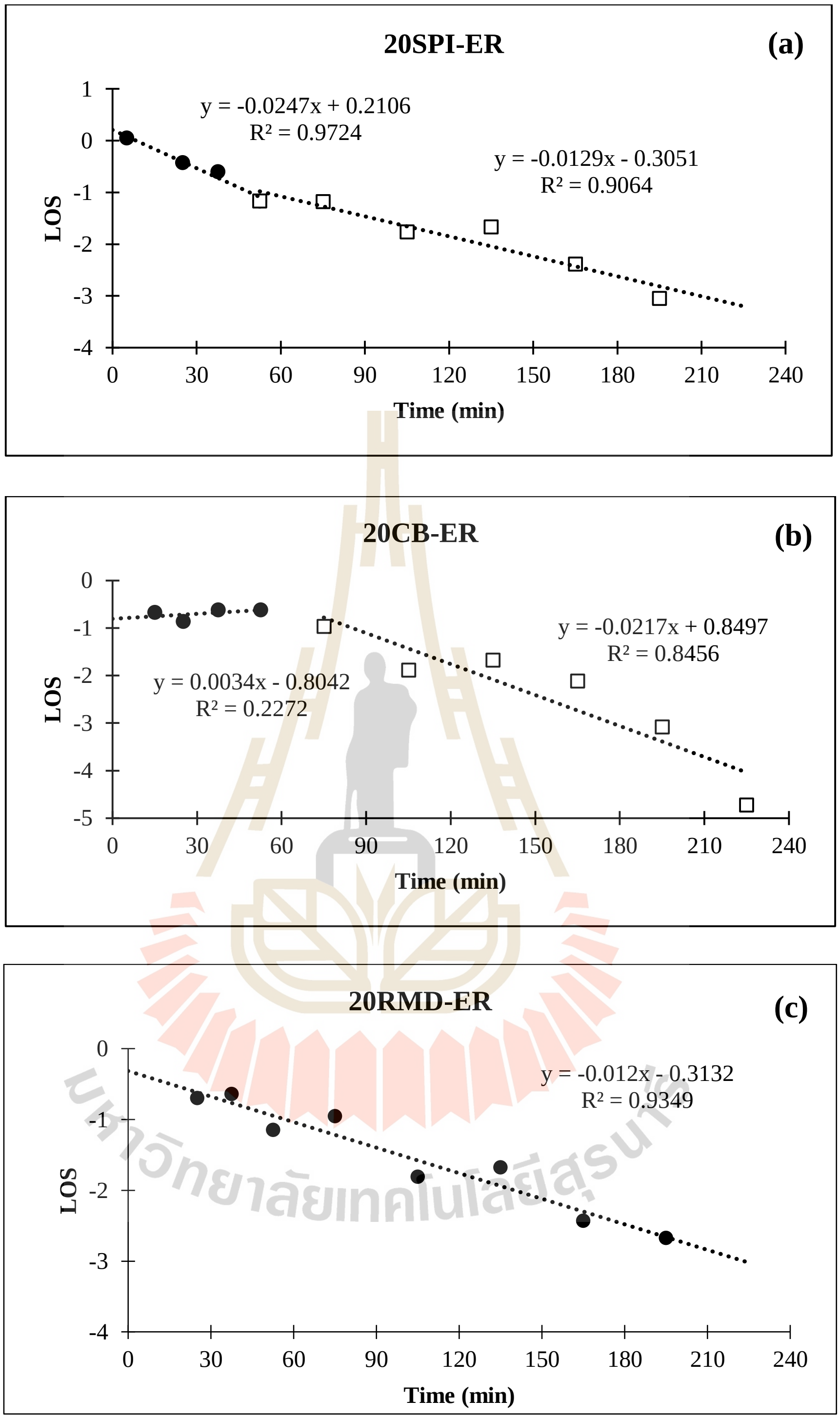


Figure 4.12 LOS plot of the digestion of cooked extruded rice supplemented with (a) 20% soy protein isolate (20SPI-ER), (b) 20% corn bran (20CB-ER), and (c) 20% resistant maltodextrin (20RMD-ER).

The cooked extruded rice supplemented with 20% soy protein isolate (20SPI-ER) and 20% corn bran (20CB-ER) were fitted with two-phase pseudo-first order kinetic (Figure 4.12). The high first-phase digestion rate (k_1) of cooked 20SPI-ER was observed, implying that the integrity of cooked 20SPI-ER was reduced by gastric digestion step. After that, the high hardness and stickiness of cooked 20SPI-ER could decrease the enzyme permeability throughout the particle which led to the low second-phase digestion rate (k_2) compared with the k_1, and the end-point in the second-phase digestion ($C_{\infty 2}$) and GI were lower than that of the control. In contrast, a low k_1 of cooked 20CB-ER was observed, due to the high integrity of starch gel of first-phase and the high hardness and stickiness of cooked 20CB-ER might be the cause of the low enzyme accessibility and lower digestion rate. After 60 min, the integrity of starch gel of cooked 20CB-ER reduced during the digestion, resulting in the increase of enzyme accessibility, which caused the high k_2. The GI value of cooked 20CB-ER was higher than that of cooked 20SPI-ER. Many previous studies reported that dietary fiber can reduce the integrity of starch network (Li et al., 2018; Tudorică, Kuri and Brennan, 2002). Tudorică et al. (2002) reported that the reduction in pasta firmness may be associated with the role of fiber supplements in disrupting the protein starch matrix within the pasta microstructure. Rice bran fiber showed a negative effect on starch structure by damaging the integrity of the starch network of rice pasta (Li et al., 2018). However, the C_{∞} of cooked 10CB-ER and $C_{\infty 2}$ of cooked 20CB-ER were not significantly different from the C_{∞} of the control, implying that the integrity of cooked extruded rice supplemented with CB was not different from that of the control when the corn bran particle was leached out during the amylolysis. The cooked 20RMD-ER showed the similar single-phase pseudo-first order kinetic to cooked 10RMD-ER and the control, which is possible that their homogeneous matrix during the digestion were similar. In addition, the *G'* of cooked supplemented with RMD were not different from that of the control. Moreover, the increase of fibers supplementation level did not have a significant effect on the end-point of starch amylolysis and on the GI value of cooked extruded rice supplemented with both fibers. Nevertheless, the GI value cooked extruded rice supplemented with soy protein isolate or dietary fibers was the medium GI food.

4.3.2 Effect of duo combination of soy protein isolate with fibers on digestibility of cooked extruded rice

The digestibility of extruded reformed rice with duo combination of protein and fibers at 20% substitution with various ratios was investigated. The single-phase pseudo-first order kinetic of cooked extruded rice supplemented with 20% duo combination of soy protein isolated (SPI) and resistant maltodextrin (RMD) was observed (Table 4.5). It implies that this duo combination cooked rice had the homogeneous matrix during digestion. The higher SPI ratio increased the k and the C_∞ of this duo combination cooked rice due to that the SPI was digested by gastric digestion process, resulting in the decrease of the integrity of gel. Additionally, cooked extruded rice supplemented with 20% duo combination of SPI with corn bran (CB) were digested following the two-phase pseudo-first order kinetic (Table 4.5) that was similar to the kinetic of cooked 20SPI or 20CB. When SPI ratio increased, the k_1 of the duo combination of SPI with CB was increased. These results demonstrate that an increase in SPI fraction induced the low integrity of cooked extruded rice in the first-phase by gastric digestion process, which contributed to a higher amylolysis rate of the first-phase. Furthermore, the increase of SPI ratio and decrease of CB ratio increased the integrity and dense of the cooked extruded rice which caused a decrease in the $C_{\infty 2}$. The increase of SPI fraction induced the high hardness and stickiness of cooked extruded rice, which contributed to the lower enzyme accessibility in the second-phase. In contrast, when the corn bran ratio increased, it showed that k_1 was lower than k_2. These results suggesting that the corn bran particles that dispersed in the cooked extruded rice leached out after the first-phase amylolysis which reduced integrity of cooked extruded rice and increased the enzyme accessibility of the second-phase. The lower fraction of corn bran particle has interfered the structural integrity and homogeneity of the cooked extruded rice, which was associated with the low end-point of starch amylolysis. Furthermore, the GI values of cooked extruded rice supplemented with 20% of duo combination of soy protein isolate and dietary fibers were found to be the medium group.

Table 4.5 *In-vitro* digestion parameters and GI of cooked extruded rice and cooked extruded rice supplemented with 20% substituted of protein and fiber.

Sample		**k(×10^{-3})**	**C_{∞}**	**GI**
Control		9.10±0.64c	72.08±4.27a	86.24±5.62a
6SPI14RMD-ER		10.80±0.10c	52.40±1.36c	56.62±1.39bc
10SPI10RMD-ER		17.5±1.90b	60.38±0.28b	55.60±1.50bc
14SPI6RMD-ER		18.2±1.02b	60.73±0.66b	53.53±1.06c
Sample	**k_1(×10^{-3})**	**k_2(×10^{-3})**	**$C_{\infty 2}$**	**GI**
6SPI14CB-ER	15.20±0.06b	21.70±0.20a	73.10±1.13a	58.62±1.77b
10SPI10CB-ER	16.60±0.20b	19.10±0.40ab	73.74±1.35a	56.77±1.13bc
14SPI6CB-ER	21.70±1.20a	19.23±3.10ab	61.57±1.88b	54.09±0.26bc

Note: Mean values with different letters within each column are significantly different ($P<0.05$), 6% soy protein isolate + 14% corn bran (6SPI14CB-ER), 10% soy protein isolate + 10% corn bran (10SPI10CB-ER), 14% soy protein isolate + 6% corn bran (14SPI6CB-ER), 6% soy protein isolate + 14% resistant maltodextrin (6SPI14RMD-ER), 10% soy protein isolate + 10% resistant maltodextrin (10SPI10RMD-ER), 14% soy protein isolate + 6% resistant maltodextrin (14SPI6RMD-ER).

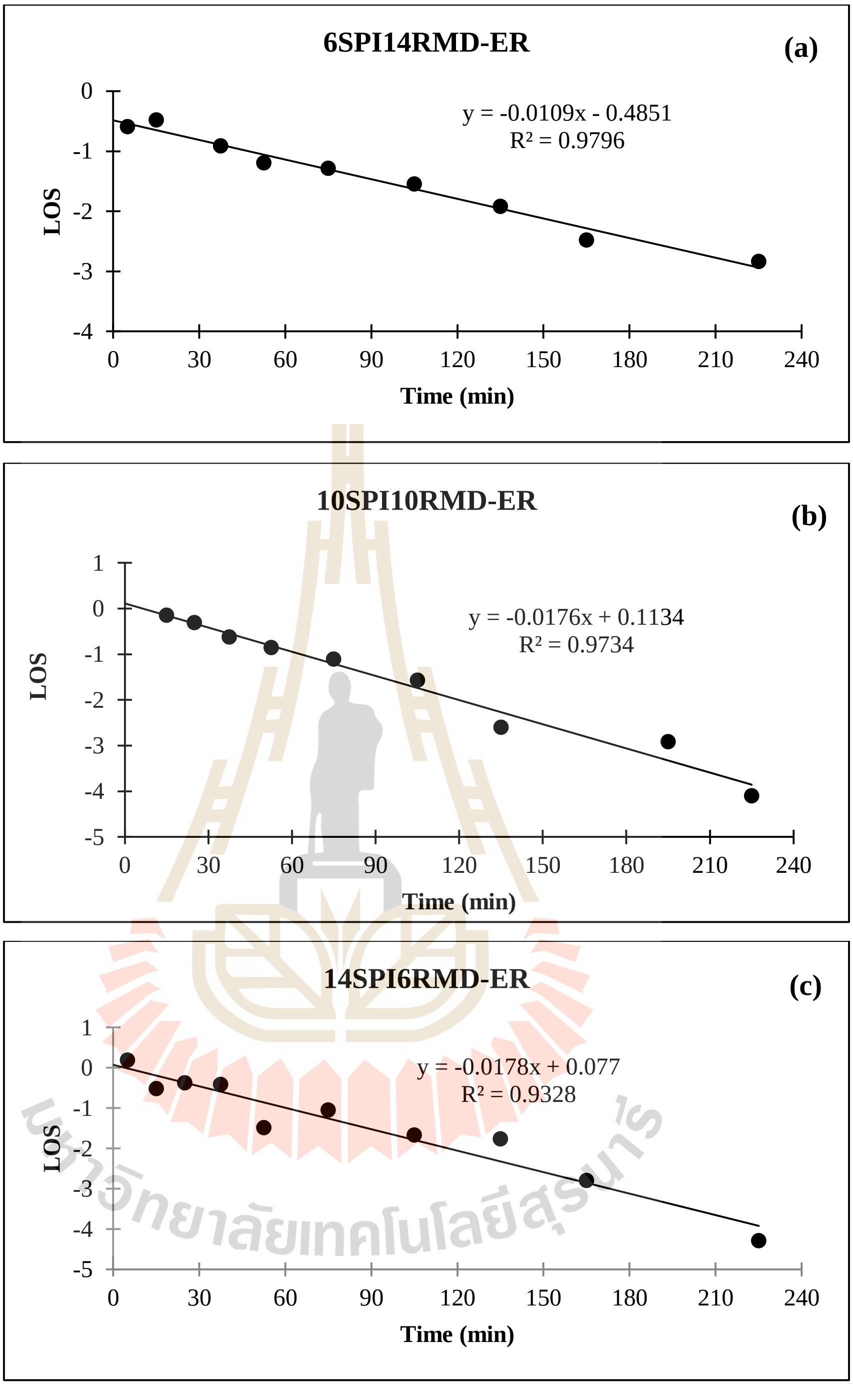


Figure 4.13 LOS plot of the digestion of cooked extruded rice supplemented with 20% substituted of protein and resistant maltodextrin: (a) 6% soy protein isolate + 14% resistant maltodextrin (6SPI14RMD-ER), (b) 10% soy protein isolate + 10% resistant maltodextrin (10SPI10RMD-ER), (c) 14% soy protein isolate + 6% resistant maltodextrin (14SPI6RMD-ER).

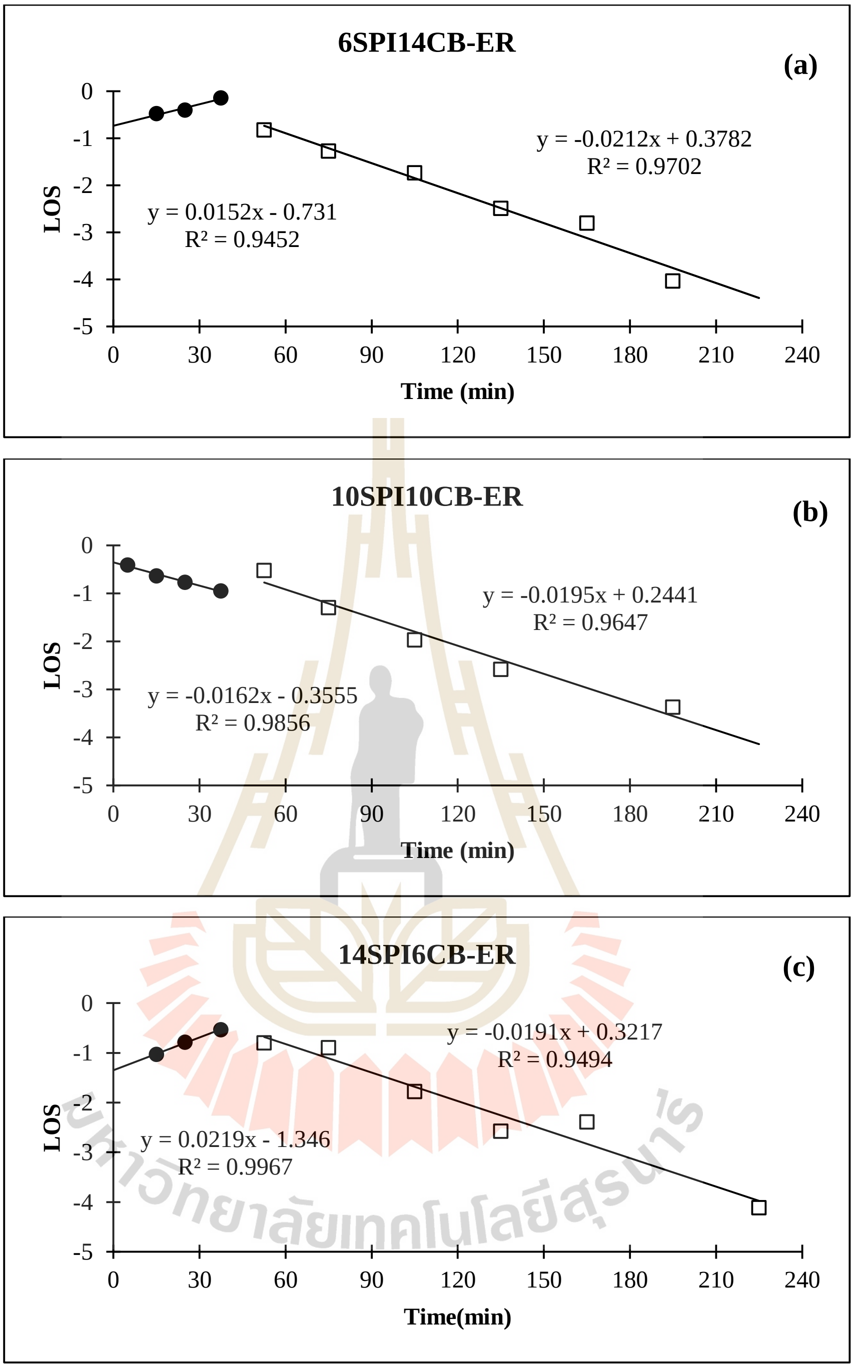


Figure 4.14 LOS plot of the digestion of cooked extruded rice supplemented with 20% substituted of protein and resistant maltodextrin: (a) 6% soy protein isolate + 14% corn bran (6SPI14CB-ER), (b) 10% soy protein isolate + 10% corn bran (10SPI10CB-ER), (c) 14% soy protein isolate + 6% corn bran (14SPI6CB-ER).

4.4 Gastric emptying and satiety analysis of cooked extruded rice

4.4.1 Gastric emptying

Sixteen healthy subjects completed all 5 visits. Mean age for the women was 28.31±2.62 and 30.20±2.96 for men. Mean body mass index was 24.28±3.97 and 24.54±1.56 for women and men, respectively. The half-emptying time and lag phase of extruded rice meal were shown in Figure 4.15a and 4.15b, respectively. The half-emptying time median and lag phase median of cooked 20SPI-ER were not significantly different from those of the control ($P = 0.327$ and $P = 0.366$, respectively). The lag phase median and interquartile range box (50% of data) of cooked 20SPI-ER showed the highest value (Figure 4.15b). It suggests that 20% soy protein isolate substitution might not be sufficient to significantly delay gastric emptying; however, the cooked 20SPI-ER showed the longest period in the stomach (Figure 4.15b). Since the cooked 20SPI-ER demonstrated the highest hardness and stickiness, these would be contributed to slow gastric emptying (Kim et al., 2004). In addition, protein induced activation of the ileal brake in humans and it is a potent stimulus for the cholecystokinin (CCK) secretion (Liddle et al., 1985; Read et al., 1984). The previous study reported that the high-protein intake produced a greater peptide-YY (PYY) released in human subjects (Batterham et al., 2006). Therefore, soy protein isolate has a potential on delay gastric emptying when compared with the control and the other added fiber samples. When the half-emptying time median and lag phase median of cooked 20CB-ER were compared to those of the control, no significant difference was found ($P = 0.152$ and $P = 0.546$, respectively). The half-emptying time median and lag phase median of cooked 20RMD-ER were significantly lower from those of the control ($P = 0.004$ and $P = 0.016$, respectively) and 20SPI ($P = 0.049$ and $P = 0.002$, respectively), implying that cooked 20RMD-ER was faster gastric emptying than the control and cooked

20SPI-ER. As compared to cooked 20SPI-ER, the texture of cooked 20RMD-ER was less hard and low sticky, consequently, it brought about a faster gastric empting rate. Moreover, the lower starch fraction of 20RMD increased the gastric emptying rate.

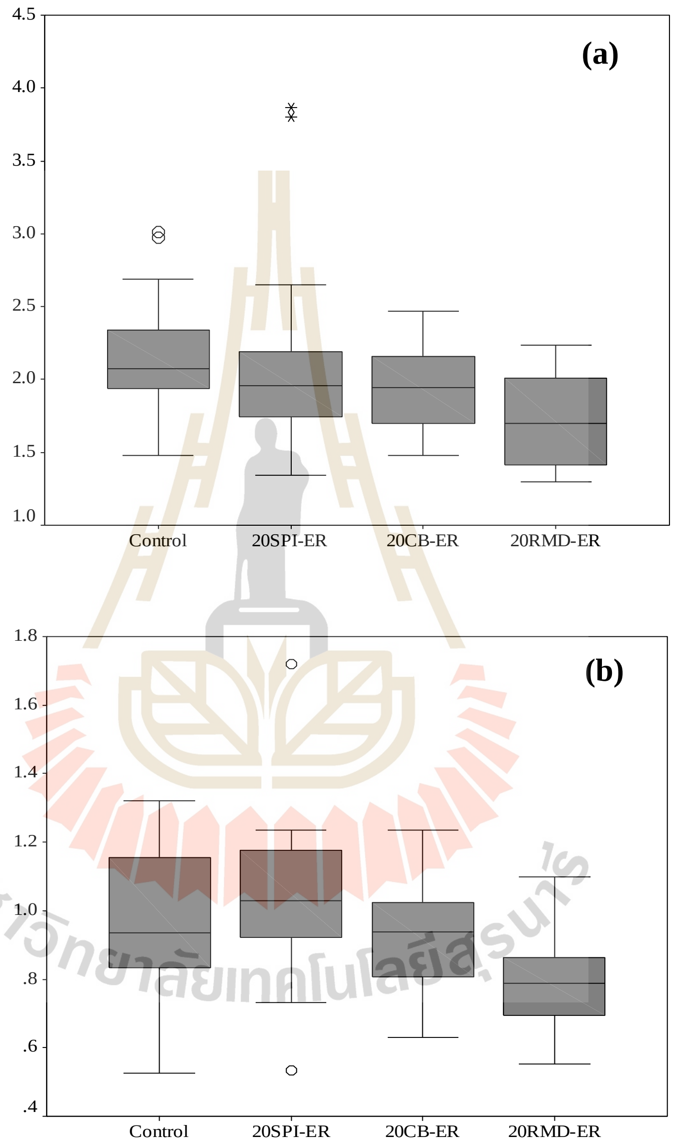


Figure 4.15 Half-emptying time boxplot (a) and Lag phase boxplot (b) of cooked extruded rice (control), cooked extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

4.4.2 Satiety analysis

Regarding the satiety study, the AUC analysis of satisfaction of 20CB was significantly higher than that of the control (Figure 4.16c). The high hardness and high solid-like of cooked 20CB-ER and hard particle of corn bran induce the increased of satiation and satiety. Dietary fiber has different physiological effects which impact on satiation that marks the end of eating and satiety that inhibits of hunger, because of their properties of adding bulk (satiation) and producing viscosity (satiety) (Slavin and Green, 2007). These results are in accordance with the study of Samra and Anderson (2007) in that satiety was improved after consumption of insoluble fiber. Willis et al. (2009) compared a low-fiber muffin and four high-fiber muffins containing 4 types of fibers (corn bran, barley β-glucan+oat fiber, resistant starch, and polydextrose) on satiety. They found that resistant starch and corn bran demonstrated longer duration of satiety than the treatments of low-fiber, barley β-glucan+oat fiber, and polydextrose. Many studies supported the concept that higher dietary fiber intake promotes satiety, decreases hunger, and helps provide a feeling of fullness (Clark et al., 2013; Levine et al., 1989; Rebello, O'Neil and Greenway, 2016). The AUC analysis of the hunger mean (Figure 4.16a) and fullness mean (Figure 4.16b) of cooked extruded rice with the addition of soy protein or dietary fibers were not significantly different from that of the control.

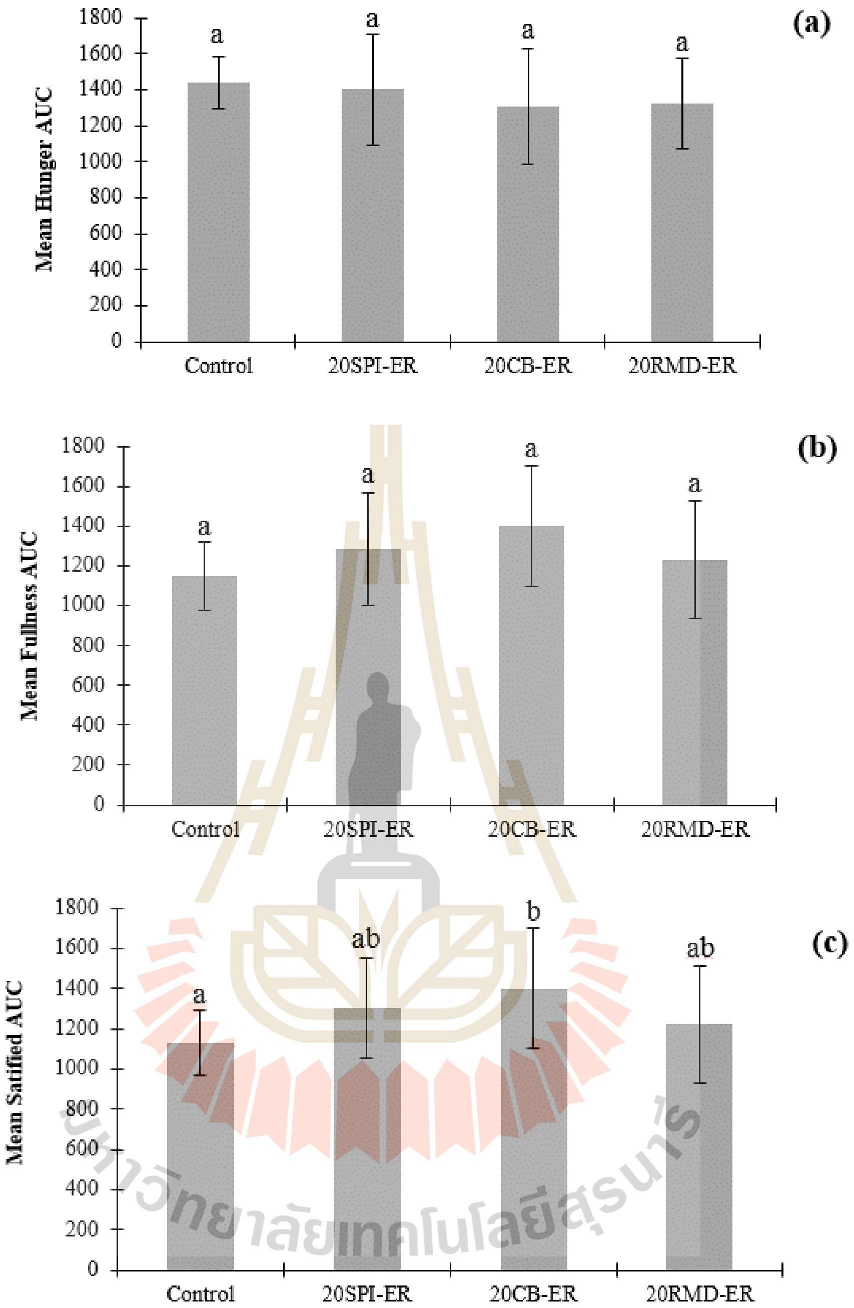


Figure 4.16 Mean area under curve (AUC) for hunger (a), fullness (b), and satisfaction (c) after consuming each test meal: cooked extruded rice (control), cooked extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

4.5 Effect of protein and fibers on digestibility of extruded rice paste

A food physical form has the effect on digestibility of food products. The extruded rice paste refers to homogenous liquid meal which is even less complicated than solid meal. The protein and fibers fortification could induce the high viscosity and complexed structure of the liquid meal.

4.5.1 Effect of *in-vitro* digestion on viscosity of extruded rice paste

The extruded rice paste refers to the liquid-like and the continuous phase food. The viscosity of extruded rice paste depends on the components and the interaction between the dispersed phase and the matrix. The viscosity during the *in-vitro* digestion of the control paste was significantly higher than that of the 20SPI-ER, 20CB-ER and 20RMD-ER paste (Figure 4.17). The lowest viscosity of 20SPI-ER paste during the gastric digestion was observed. This implies that pepsin digestion step induce disintegration of structure of 20SPI-ER paste. However, the viscosity of 20SPI-ER paste during the amylolysis was higher than that of 20CB-ER and 20RMD-ER paste, respectively. It suggested that the network of 20SPI-ER paste was stronger than that of the 20CB-ER and 20RMD-ER paste, as fibers can only disperse in the starch paste. The viscosity of 20RMD paste during the amylolysis was the lowest, since the RMD had the highest WSI and it cannot form the network with the starch paste.

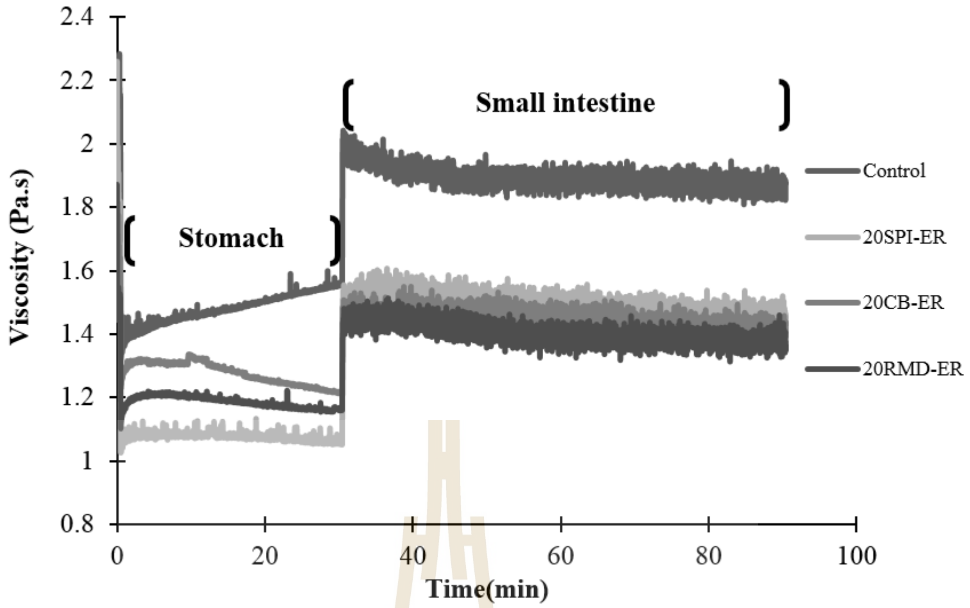


Figure 4.17 Effect of *in-vitro* digestion on viscosity of extruded rice paste (the control), and extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER) paste.

4.5.2 Kinetic digestion of extruded rice paste

The *in-vitro* digestion parameters of the control paste, and the paste of 20SPI-ER, 20CB-ER, and 20RMD-ER were presented on Table 4.6. The k value of the 20RMD-ER paste was significantly higher than that of the control which resulted from that the viscosity of 20RMD-ER paste during the digestion (Figure 4.17) was lower than that of the control paste. The low viscosity of 20RMD paste could increase the enzyme accessibility. On the other hand, the k values of the 20SPI-ER and 20CB-ER paste were significantly lower than that of the control paste. It seems that soy protein isolate and corn bran particles can retard the enzyme penetration to the starch paste.

Thus, the macronutrient is able to delay amylolysis rate of extruded rice paste, which was more efficient than the viscosity. Moreover, the **C_∞** of extruded rice supplemented with 20% macronutrients paste was decreased because the starch fraction was replaced with macronutrients.

Table 4.6 *In-vitro* digestion parameters of extruded rice paste (the control), and extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER) paste.

Sample	k($\times 10^{-2}$)	C_∞
Control	2.91±0.52b	71.80±1.00a
20SPI-ER	2.23±0.12c	63.95±2.87b
20CB-ER	2.07±0.11c	63.63±0.71 b
20RMD-ER	3.87±0.09a	62.65±0.69b

Note: Mean values with different letters within each column are significantly different ($P<0.05$).

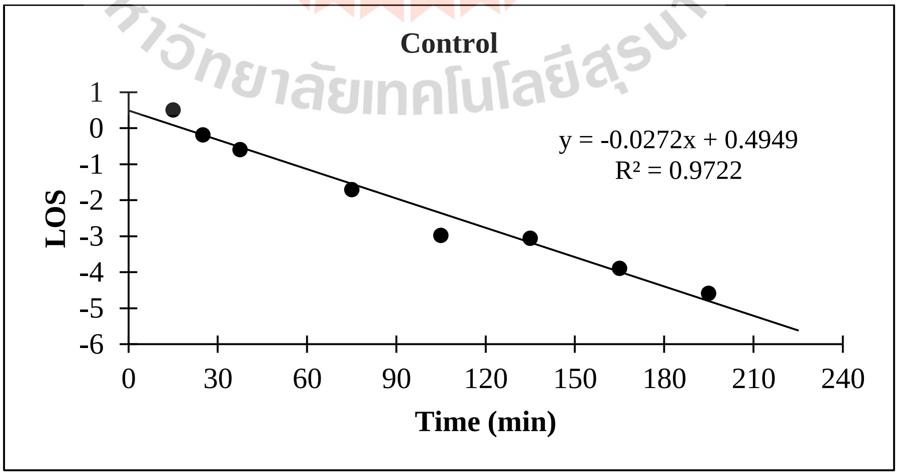


Figure 4.18 LOS plot of the digestion of cooked extruded rice paste.

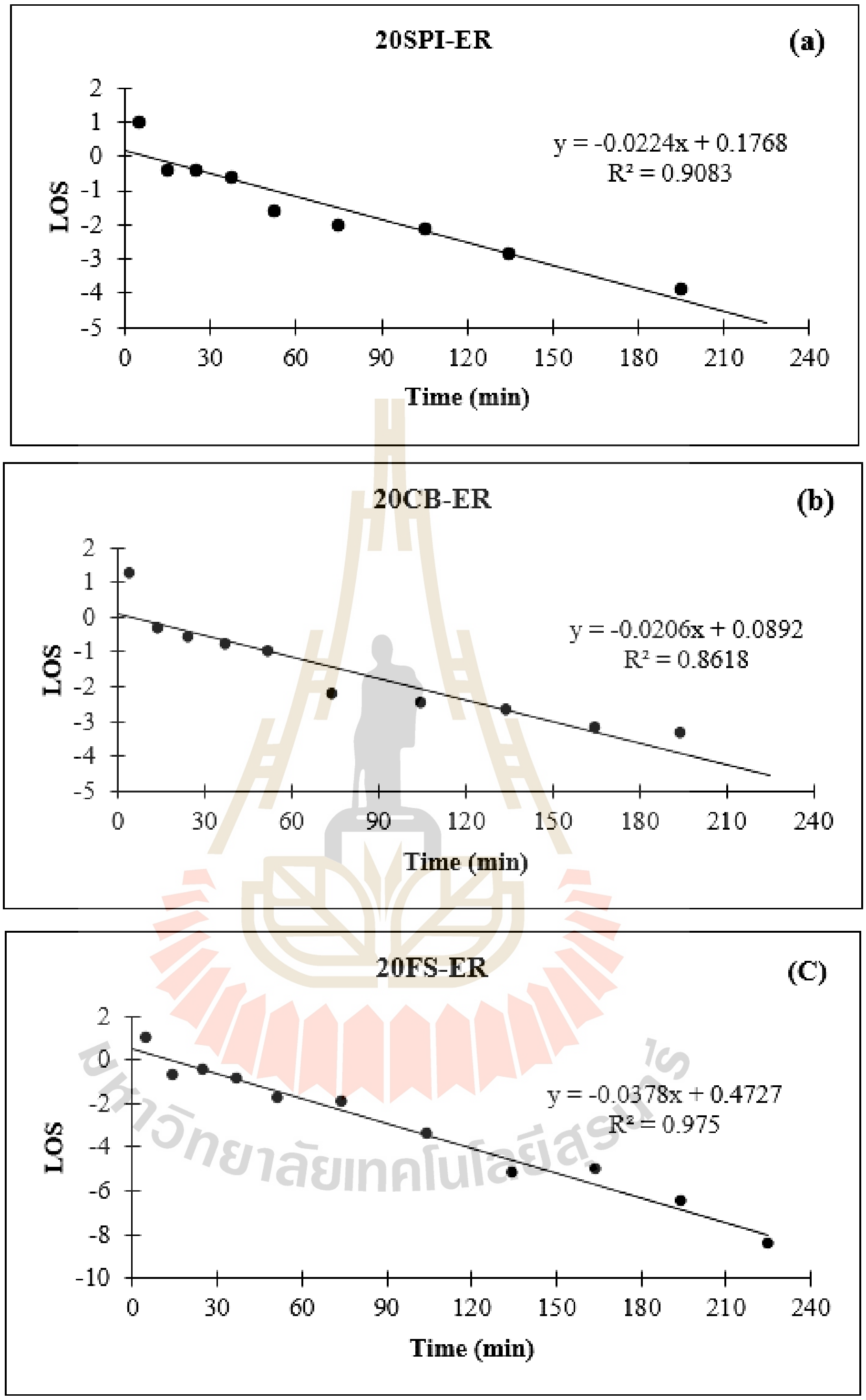


Figure 4.19 LOS plot of the digestion of cooked extruded rice supplemented with (a) 20% soy protein isolate (20SPI-ER), (b) 20% corn bran (20CB-ER), and (c) 20% resistant maltodextrin (20RMD-ER) paste.

CHAPTER V

SUMMARY

Soy protein isolate (SPI) fortification in extruded reformed rice could delay the starch gelatinization which subsequently increased the hardness, stickiness and *G’* of cooked 20SPI, resulting in decreasing the end-point of starch amylolysis. The integrity of cooked extruded rice supplemented with SPI was reduced during the gastric digestion which resulted in the higher amylolysis rate as indicated from cooked rice containing SPI. The time of grinding and mixing in the stomach before gastric emptying for the cooked 20SPI was the longest period. Therefore, SPI has the potential to decrease the glucose released and delay gastric emptying of cooked extruded rice.

The corn bran (CB) addition brought about the faster starch gelatinization of extruded rice, as the CB particle increased the surface area for water accessibility in the starch granule. The high hardness, stickiness and *G’* of the cooked 20CB contributed the high integrity of starch gel in the first-phase digestion, which decreased enzyme accessibility. However, the digestion process causes the reduction of the cooked 20CB integrity, resulting in improving the amylolysis rate of second-phase. The high hardness and solid-like of cooked 20CB induced satiation and satiety.

The resistant maltodextrin (RMD) could be interfere the starch granule swelling which decreased the peak and final viscosity of 20RMD, inducing the slightly increase in hardness, stickiness and *G’* of cooked 20RMD. The homogeneous matrix of cooked extruded rice with RMD leads to the single-phase pseudo-first order kinetic. The high

RMD ratio reduced the disintegration of cooked extruded rice by gastric digestion step when compared with the high SPI ratio, resulting in the decreasing of digestion rate and the end-point concentration.

The rheological characteristics of foods influence the digestion of starch by affecting the availability of enzyme. For the solid food that high viscoelastic matrix food, the high hardness, stickiness and *G'* of cooked rice which induce by soy protein isolate and fiber, delaying the rate of enzyme access to starch. Moreover, liquid meal of extruded rice paste induces the higher digestion rate when compared with the cooked extruded rice. However, the high viscosity of extruded paste with SPI adding induced the low amylolysis rate when comparted with fiber adding. SPI and CB particles can interfere the enzyme penetration into the starch, thus, this digestion rate and the end-point glucose concentration of extruded rice paste was decreased.

Overall, SPI and fiber substitution reduce the end-point of starch amylolysis and produce the medium GI extruded rice. High integrity of starch gel can be produced by SPI addition, which leads to its end-point concentration was lower than the cooked extruded rice supplemented with fibers.

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APPENDIX

Appendix A

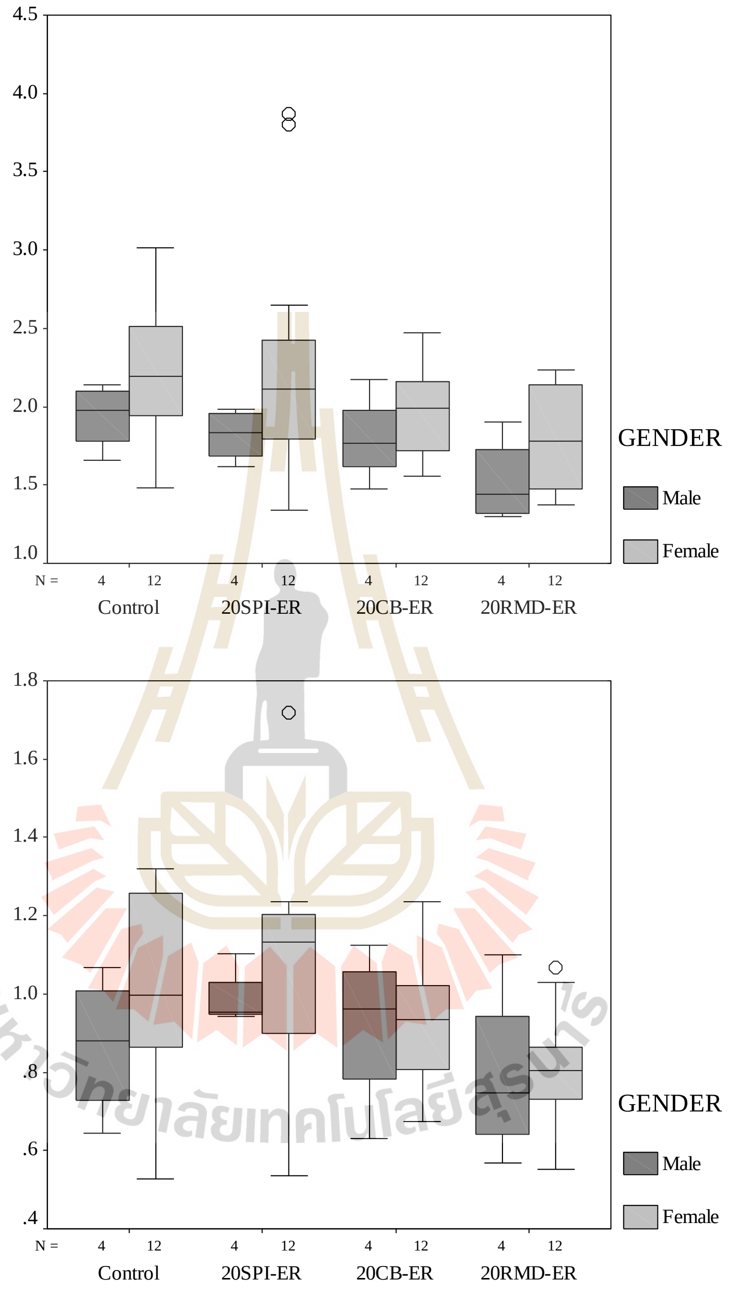

Figure A1 Effect of gender (female = 12, male = 4) on half-emptying time boxplot (a) and lag phase boxplot (b) of cooked extruded rice (control), cooked extruded rice supplemented with 20% soy protein isolate (20SPI-ER), 20% corn bran (20CB-ER), and 20% resistant maltodextrin (20RMD-ER).

Appendix B

Appendix B1

PRE-SCREENING CONSENT FORM

Effects of extruded rice in combination with protein and fibers on gastric emptying

Principal Investigator: Bruce Hamaker, PhD.

Department of Food Science, Nelson Hall Purdue University

West Lafayette, IN 47907

(765) 494-0625

<u>Purpose of research</u>

This study focuses on the potential use of formed (extruded) rice with soy protein and/or commercial soluble dietary fibers to increase satiety or fullness effect. The meals will include: normal cooked rice, cooked extruded rice, cooked 20% soy protein isolate extruded rice, cooked 20% corn bran extruded rice, cooked 20% dietary fiber (Fibersol-2™) extruded rice, and cooked 10% soy protein isolate + 10% Fibersol-2™ extruded rice.

<u>Pre-screening activities</u>

SUBJECT PRE-SCREENING

Height (in): ____________

Weight (lb): ___________

Birthdate (MM/DD/YY): ___________

Gender: ☐ Male ☐ Female

Please answer the following questions;

1. Have you had stable weight for the past 3 months i.e. +/- 2.5 kg?
2. Do you have a regular eating pattern, including breakfast consumption?
3. Have you ever had any of the following (circle your response):
    - Gastrointestinal disease
    - Celiac disease
    - Acute or chronic disease
    - Hypertension
    - Diabetes
    - Previous bariatric surgery
    - None of these apply to me
4. How would you currently describe your weight management goal?

☐ Lose weight ☐ Maintain weight ☐ Gain weight

5. Please answer the following questions:

• Do you currently smoke?	Yes	No
• Do you consume alcohol more than 30 units per week?	Yes	No
• Are you pre- or post-menopausal?	Yes	No
• Are you pregnant or lactating?	Yes	No

6. How often do you consume rice?

☐ Never ☐ 1-2 days per week ☐ 3-4 days per week

☐ 5-6 days per week ☐ Every day

Risks to the individual

There are no study specific risks. There is a minor risk associated with a breach of confidentiality pertaining to the information gathered from the subjects. However, all information and records of subjects will be locked in a secure location on the Purdue campus. Any record saved electronically will also not contain names of subjects and the computer will require password security to log in and gain access to that information.

Contact information:

If you have any questions about this research project, you can contact Bruce Hamaker (765-494-5668). If I have concerns about the treatment of research participants, you can contact the Committee on the Use of Human Research Subjects at Purdue University, 610 Purdue Mall, Hovde Hall Room 307, West Lafayette, IN 47907-2040. The phone number for the Committee's secretary is (765) 494-5942. The email address is irb@purdue.edu.

I consent to answer the following pre-screening form for the study "Effects of extruded rice in combination with protein and fibers on gastric emptying". I understand that this does not obligate me to participate in the study, and that by filling out the form that I will necessarily be a participant in the study.

______________________________ __________________

Participant's Signature Date

____

Participant's Name

______________________________ __________________

Researcher's Signature Date

Appendix B2

Satiety Questionnaire

Subject Number:________________________________Date______________

Sample Number:_______________________

Time:________________________________

☐ Before eat ☐ After eat

☐ 30 min ☐ 60 min ☐ 90 min ☐ 120 min

☐ 150 min ☐ 180 min ☐ 210 min ☐ 240 min

1. **How hungry do you feel?**

0 ____ 10

0 = I am not hungry at all 10 = I have never been more hungry

2. **How full do you feel?**

0 ____ 10

0 = Not at all full 10 = Totally full

3. **How satisfied do you feel?**

0 ____ 10

0 = I am completely empty 10 = I cannot eat another bite

Appendix C

C1. Total starch determination (modified by the method of AOAC, 1990)

1. Mill cereal, plant or food product to pass a 0.5 mm screen.
2. Add milled sample (~ 100 mg; weighed accurately) to a glass test tube (16 × 120 mm). Tap the tube to ensure that all of the sample drops to the bottom of the tube.
3. Add 0.2 mL of aqueous ethanol (80% v/v) to wet the sample and aid dispersion. Stir the tube on a vortex mixer.
4. Immediately add 3 mL of thermostable α-amylase (300 U per mL of 100 mM sodium acetate buffer, pH 5.0). Incubate the tube in a boiling water bath for 6 min (Stir the tube vigorously after 2, 4 and 6 min).
5. Place the tube in a bath at 50°C; add 0.1 mL of amyloglucosidase (330 U on starch). Stir the tube on a vortex mixer and incubate at 50°C for 30 min.
6. Transfer the entire contents of the test tube to a 100 mL volumetric flask (with a funnel to assist transfer). Use a wash bottle to rinse the tube contents thoroughly. Adjust to volume with distilled water. Mix thoroughly. Centrifuge an aliquot of this solution at 3,000 rpm for 10 min. Use the clear for glucose determination by PGO enzyme (Sigma, P7119)

$$Starch\,(\%) = \Delta A \times \frac{F}{W} \times FV \times 0.9$$

where

ΔA	=	absorbance (reaction)
F	=	100 µg of D-glucose / absorbance for 100 µg of glucose
W	=	the weight in milligrams (dry basis) of the flour

C2. Glucose content

The glucose content was determined by PGO enzyme (Sigma, P7119)

Reagents

1. The PGO Enzymes Solution is prepared by adding the contents of 1 capsule of PGO Enzymes to 100 ml of water in an amber bottle. Invert bottle several times with gentle shaking to dissolve.
2. The o-Dianisidine Solution is prepared by dissolving 50 mg of o-dianisidine dihydrochloride in 20 ml of water.
3. The PGO Enzymes Reaction Solution is prepared by mixing 100 ml of the PGO Enzyme Solution and 1.6 ml of the o-Dianisidine Solution. Mix by inverting several times or with mild shaking.

Procedure

1. Add 0.5 ml of water to the blank tube.
2. Add 0.5 ml of glucose standard solution (40, 80, 120, 160, 200 µg/mL) to the standard tube.
3. Add 0.5 ml of sample to each tube.
4. Add 5.0 ml of the PGO Enzymes Reaction Solution to each tube and mix each tube thoroughly.
5. Incubate all tubes at 37°C for 30±5 minutes. Avoid exposure to direct sunlight or bright daylight.
6. The mixture was measured the absorbance against reagent blank at 540 nm.
7. The glucose content was calculated by the glucose standard equation.

CURRICULUM VITAE

Kuenchan Na Nakorn was born in Pattani, Thailand. She received her bachelor's degree in science (Food Science and Nutrition) from Prince of Songkhla University in 2005 and received her master's degree in science (Food Technology) from Prince of Songkhla University in 2009.

While studying for her master's degree, she got a scholarship from the TRF-Master Research Grants from The Thailand Research Fund. She gave an oral presentation on "Effect of amylose content on structural and pasting properties of pregelatinized rice starches" in Proceeding of 9th National Grad Research Conference (Burapha University, Chonburi, Thailand, 14-15 March 2007). She published the article entitled "Crystallinity and rheological properties of pregelatinized rice starches differing in amylose content" in 2008 (Starch/Stärke 61:101-108).

In 2012, she was awarded a scholarship under the Strategic Scholarships Fellowships Frontier Research Networks (specific for Southern Region) from the Office of the Higher Education Commission to undertake a Ph.D. program at Suranaree University of Technology. During her Ph.D. study, she had an opportunity to conduct her research at Purdue University, US. She gave a poster presentation on "*In-vitro* digestibility of imitated rice supplemented with soluble fiber and soy protein isolate" at Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University (May 2006). She gave an oral presentation on "Gastric emptying of extruded rice as affected by protein and fibers" in the 5nd SUT International Agricultural Colloquium 2017 (Suranaree University of Technology University, Thailand, 15-16 August, 2017).